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The Official Journal of the American Association of Traditional Chinese Veterinary Medicine, the American Academy of Veterinary Acupuncture, the International Veterinary Acupuncture Society and the World Association of Traditional Chinese Veterinary Medicine

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The Official Journal of the American Association of Traditional Chinese Veterinary Medicine (AATCVM), the American Academy of Veterinary Acupuncture (AAVA), the International Veterinary Acupuncture Society (IVAS) and the World Association of Traditional Chinese Veterinary Medicine (WATCVM).

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The WATCVM supports the education, research, and practice of Traditional Chinese Veterinary Medicine worldwide.

Animal Use and Care: All research studies involving animals must have been performed in compliance with guidelines outlined in the *Animal Welfare Act, US Public Health Service Policy on the Humane Care and Use of Laboratory Animals, NRC Guide for the Care and Use of Laboratory Animals,* or *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Fass 1999) or with equivalent guidelines. A manuscript containing information that suggests that animals were subjected to adverse, stressful, or harsh conditions or treatments will not be considered for publication. Manuscripts describing studies that involved the use of animals, including studies that involved the use of privately owned animals (e.g. animals owned by clients, staff members, students, or private entities) must include a statement that the study protocol was reviewed and approved by an appropriate oversight entity (e.g. an animal care and use committee or institutional review board) or was performed in compliance with institutional guidelines for research on animals. Manuscripts describing prospective studies that involved privately owned animals must include a statement indicating that owner consent was obtained.

Conflict of Interest: Authors are expected to acknowledge all sources of funding or financial support and to disclose to the Editor-in-Chief any financial interests (including ownership, employment, consultancy arrangements, and service as an officer or board member) they have with companies that manufacture products that are the subject of their research or with companies that manufacture competing products.

AJTCVM Statement of Informed Consent: Animal owners (clients) and their animals have a right to privacy that should not be infringed upon without informed consent. Identifying information, including animals' names, initials or medical record numbers, should not be published in written descriptions or photographs unless the information is essential for scientific purposes and the animal owner gives written informed consent for publication. Clients must consent to all photographs of animals included in a manuscript. Pedigrees should not be published without signed consent from the involved breeders. Identifying details should be omitted if they are not essential. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning and editors should so note the changes. Authors should identify individuals who provide writing assistance and disclose the funding source for this assistance.

About the Cover

In 2017 the covers of AJTCVM will boast the color gold to honor the Earth Element.

Steaming Chinese Dumplings/Potstickers

In 2017 the AJTCVM covers honor the Earth Element. When honouring the Earth Element, we are reminded of the importance of good nourishing food for the formation of *Gu Qi* by the *Zang-fu* organ, Spleen. Cooking and eating are vital parts of our everyday lives and we are reminded about the special qualities of the different foods. The cover picture depicts the preparation of Chinese dumplings/potstickers. These irresistible dumplings filled with vegetables (usually cabbage) and a small amount of meat are steamed on one side and pan-fried on the other. According to legend, the Chinese have been enjoying potstickers since the Song Dynasty (960-1280 BCE) when they were invented by a chef in China's Imperial Court. He accidently burnt a batch of dumplings when they were left on the stove too long. There was no time to prepare a new batch so the chef served the dumplings which were burnt on the bottom only with a steamed top; announcing they were his own special creation. Fortunately the court members loved them!

Errata Corrige

The editors apologize for an error in the Figure 1 flowchart in "A Randomized Controlled Study Comparing *Da Xiang Lian Wan* to Metronidazole in the Treatment of Stress Colitis in Shelter/Rescue Dogs". The arrows from the second diamond should be "Yes" to "Exclude from the study" and "No" to proceed to the next diamond. AJTCVM 12 (1) 2017: page 47.

The American Journal of Traditional Chinese Veterinary Medicine (AJTCVM)

An Internationally Peer-Reviewed Journal of Veterinary Acupuncture, Chinese Herbal Medicine, Tui-na and Food Therapy

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Editorial

Call to Authors and Would-Be Authors! Let's Take a Look at "Instructions to Authors"

Judith E Saik, DVM, DACVP

The life blood of this journal or any scientific journal is the authors. It is truly amazing to see the level of dedication to science and creativity in designing studies by our authors. They look at medical issues that challenge veterinarians in their daily care of sick animals and all of this is usually done on a very small budget. Papers submitted by authors have no lack of important core information but the mechanics of putting a paper together can be challenging. Each scientific journal has different format styles, organization and different content requirements for papers which can further confuse an author. An excellent source that can greatly assist an author in picking their way through the myriad of details is "Instructions to Authors" which is found on the website of most journals. At AJTCVM, our "Instructions to Authors" can be found on the AATCVM website (www.aatcvm.org). Although available on the home page of the website, the editors thought it would be helpful to authors to dedicate the editorial space this time to publishing our "Instructions to Authors". The guide has been divided into 2 parts due to its length with the first part covering general manuscript information and the second part (placed in the back of this issue) providing an overview of article types. Hopefully this will make accessing AJTCVM manuscript preparation easier for those of you who have been struck by the muse and maybe inspire those of you who haven't considered publishing a paper to give it a try!

AJTCVM INSTRUCTIONS TO AUTHORS Part I

The American Journal of Traditional Chinese Veterinary Medicine is a biannual international peer-reviewed scientific journal. It publishes original research in areas of veterinary interest in acupuncture, Chinese herbal medicine, *Tui-na* and Food Therapy. It is the official publication of the American Association of Traditional Chinese Veterinary Medicine, the American Association of Veterinary Acupuncture, the International Veterinary Acupuncture Society and the World Association of Traditional Chinese Veterinary Medicine.

GENERAL MANUSCRIPT INFORMATION

- Only manuscripts reporting some aspect of Traditional Chinese Veterinary Medicine (TCVM) or Traditional Chinese Medicine (TCM) will be considered for publication in the American Journal of Traditional Chinese Veterinary Medicine (AJTCVM).
- All manuscripts submitted *must not have been published elsewhere in English*.
- Manuscripts submitted to AJTCVM *must not be submitted elsewhere*, while under consideration for acceptance by the AJTCVM.
- Manuscripts should be submitted electronically, attached to an email to Dr. Judith E Saik, AJTCVM Editor-in-Chief addressed to: saikj@ajtcvm.org.
- Manuscripts must be submitted in the format required by the AJTCVM and manuscripts that are not correctly written will be returned to the primary author for re-formatting and re-submission after following the instructions below.
- The Editor-in-Chief reserves the right to reject manuscripts without a TCVM focus or in a format or style unsuitable for the AJTCVM.
- The Editor-in-Chief reserves the right to edit manuscripts to fit the standard format and style of the AJTCVM and scientific journals in general and it is common for several editing requests to be made to authors until the manuscript is in an acceptable standard format to be sent to reviewers.

- Scientific writing is very different than creative writing and conversational speaking and the editing and re-writing process can be frustrating for some authors. The AJTCVM has a standard style for all articles, which may be different than an author has previously experienced. Many authors have learned excellent scientific writing skills from the painful editing and rewriting process, when they remained open-minded and patient.
- The Editor-in-Chief will assist new authors, as much as time permits.
- All manuscripts will be sent to 2-3 TCVM experts for review and approval as described in the **Peer Review Process** section below.
- Associate Editors proof all manuscripts that have been accepted by the reviewers and may request additional information or changes from the authors.
- If a clinical study manuscript is being prepared, please review the **Reporting Guidelines for Randomized Controlled Clinical Trials in TCVM**, adapted from the CONSORT and REFLECT statements on the AJTCVM website, to ensure all points under each category have been addressed in the manuscript.

ANIMAL USE AND CARE REQUIREMENTS

- A manuscript containing information that suggests that animals were subjected to adverse, stressful or harsh conditions or treatments *will not* be considered for publication by AJTCVM.
- All research studies involving animals must have been performed in compliance with guidelines outlined in the Animal Welfare Act (*http://awic.nal.usda.gov*), United States Public Health Service Policy on the Humane Care and Use of Laboratory Animals (*http://grants.nih.gov/grants/olaw/references/phspol.htm*), the Guide for the Care and Use of Laboratory Animals (*http://oacu.od.nih.gov/regs/guide/guide4.htm*), the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (*https://www.aaalac.org/about/Ag_Guide_3rd_ed.pdf*) or equivalent guidelines from other countries.
- Authors from countries other than the United States may be asked to submit the animal use and care guidelines from their country.
- Manuscripts describing studies that involved the use of animals, including studies that involved the use of privately owned animals (animals owned by clients, staff members, students or private entities), must include a statement that the study protocol was reviewed and approved by an appropriate oversight committee (an animal care and use committee or institutional review board) or was performed in compliance with institutional guidelines for research on animals.
- Manuscripts describing prospective studies that involved privately owned animals must include a statement indicating that owner consent was obtained.

ELECTRONIC MANUSCRIPT FORMAT REQUIREMENTS

- All manuscripts must be submitted in an electronic format using ***Microsoft Word*** (version 1997 or newer).
- The manuscript must be typed using *Times New Roman, 10 pt font, single spaced with one inch and justified margins*.
- The body of the text should be *single spaced* with *indented paragraphs* (first line by 0.5) with *no extra line spacing between paragraphs.*
- Number pages consecutively, but *do not* number individual lines.
- Do not use any accessory programs such as automatic footnotes or automatic references programs in the text, as these programs may interfere with the publishing program used.
- Tables and figures *should not* be embedded within the manuscript, but simply referred to in the appropriate place in the text and placed after the references in order of appearance in the text (see **Overview of Basic AJTCVM Format** section below).

BASIC FORMAT PRIOR TO THE TEXT OF ALL MANUSCRIPTS

Title:

- No title page is necessary.
- The title should be *at the top of the page, centered, bolded* and *in title case letters* (Example: **First Letter of Major Words Capitalized).**
- If the study was randomized, controlled and/or blinded, please put this in the title.

Authors' Names and Credentials:

- List each author's complete first, middle initial (optional) and last names (in that order) including their credentials *centered below the title and not bolded.*
- List only AVMA recognized credentials such as DVM, VMD, MS, PhD, MD (etc.) and AVMA recognized specialties such as DACVIM, DACVS, DACVA and DACT (etc.).
- CVA and other certification titles for veterinary acupuncture, Chinese herbal medicine, *Tui-na* (etc.) that are not currently recognized by the AVMA, should not be included as credentials for publication.

Communicating Author's email:

- *Centered below the author list, provide a current email and street address for the corresponding author*; this is for internal communications use only

and will not be published.

Affiliations and Locations of the author:

- Below the email address, in the left margin put the word "From:" *un-bolded and with a colon* and include the affiliation (group or hospital) of each author with the name of the institution or private practice and the town and state followed by the author(s)' last name(s) in parentheses. Example: From: The Department of Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA (Ziegler, Bryon, Memon) or From: The Holistic Veterinary Care Center, Kalamazoo, MI (Jones) and Town and Country Animal Clinic (www.tcac.com), Battle Creek, MI (Smith, Thompson).
- *Do not* include street addresses
- *A website address* may be included after the clinic name at the primary author's request.

Study Funding Source(s):

- Ignore if not applicable to the study.
- Authors are expected to acknowledge all sources of funding or financial support (Example: Funded by a grant from the American Association of Traditional Chinese Veterinary Medicine).
- Authors must disclose any financial interests they have with any companies that manufacture products that are the subject of their research or that manufacture competing products.
- Company ownership, employment, consultancy arrangements and service as an officer or board member should be disclosed.
- Delete this section if not applicable.

Abstract:

- Below title and authors; **ABSTRACT** should be *left aligned and bolded.*
- On the next line *(single spaced) not indented*, write a *250 word summary* of pertinent information from the manuscript.
- If the study was randomized, controlled and blinded, make sure this is included in the abstract.
- Make sure it is clear whether the study was of a naturally occurring or induced disease, if applicable to the article type.
- The abstract *should not* contain information *not found* in the manuscript.
- An abstract that contains only introductory information and then states what will be presented *will not be accepted.*
- The abstract should be a *summary of what is presented* in the paper; information from various sections of the paper may be used to form the abstract.
- The statistical tests used and *p*-value used to determine significance should be provided.
- A concluding sentence should be written, addressing the value of the study.

Key words:

- After a double space, the heading **Key words:** (*bolded, first letter capitalized, followed by a colon*) should be placed *in the left margin* followed by a list of 5-6 non-capitalized key words, separated by commas.

Abbreviations:

- The title **ABBREVIATIONS** should be *capitalized and centered (no colon)* and placed 2 spaces below the **Key words**.
- Make a list (one abbreviation and definition per line) of all the important abbreviations repeatedly used in the text
- An abbreviation should be left aligned, bolded, then the tab key pressed to form an equal space and the definition provided. Example:

ABBREVIATIONS	
CHM	Chinese herbal medicine
Aqua-AP	Aqua-acupuncture

- When first introduced in the abstract *and* text, the words should be completely written with the abbreviation in parentheses [e.g. Chinese herbal medicine (CHM)].

BASIC FORMAT AND STYLE OF THE TEXT OF ALL MANUSCRIPTS

General information about Names, *Pin-yin* and other Terms:

- Describe the animal care and use and state that it complied with one of the guidelines described above.
- The animal's name should not appear in the text or title.
- In general use the term acupoint instead of point unless written as "acupuncture point" or with a common description (Example: Back *Shu* Association point, Influential point and *Yuan* (Source) point etc.).
- All Chinese *Pin-yin* terms throughout the manuscript should be *italicized* and *capitalized* (Example: *Qi, Yin, Yang, Jing, Shen, Zang-fu* etc.).
- Capitalize all TCVM pathogens (Example: Wind, Cold, Heat, Summer Heat, Heat Toxin, Damp and Phlegm).
- Capitalize the Six Roots: Exterior, Interior, Excess, Deficiency, Hot and Cold.
- Capitalize the Five Elements (Example: Five Elements, Wood, Fire, Earth, Metal and Water).
- Capitalize the words: Stagnation, Stasis, Blood and Body Fluids.
- *Do not capitalize blood* when using it in the

conventional sense (Example: blood vessel, blood lead assay; *Do not use* the "Find" function under the "Edit" tab on the Microsoft toolbar and simply change all the words "blood" to "Blood", as some will be incorrect.

- When referring to *Zang-fu* organs capitalize the first letter of the organ (Example: Spleen, Kidney, Stomach, Lung, Heart etc.)
- *Do not capitalize the organ name* if using it in the conventional sense (Example: heart failure, lung lobe, kidney disease).
- Use the following terms as shown: Upper Burner (*Shang Jiao*) instead of Upper *Jiao*, Middle Burner (*Zhong Jiao*) instead of Middle *Jiao*, Lower Burner (*Xia Jiao*) instead of Lower *Jiao*, *Yuan* (Source) point, *Yuan* (Source) *Qi*, Back *Shu* Association point, Front *Mu* Alarm point.

Acupuncture and Acupuncture Points:

- List all acupoints used and their indications and actions and depth of insertion (usually present in Table form).
- Provide needle gauge, length and type (indicate manufacturer as a foot note).
- Indicate the technique used (Example: dry needles non-manipulated, dry needles manipulated, electroacupuncture, aqua-acupuncture, moxibustion, laser and others).
- If dry needles were manipulated, provide a detailed description of the manipulation technique, so others can accurately repeat the method.
- If electro-acupuncture was used, provide equipment information (indicate manufacturer as a foot note) and the frequencies used the duration of treatment at each frequency.
- If aqua-acupuncture was used, provide details of the hypodermic needle size, depth inserted, primary substance (indicate manufacturer as a foot note), substance concentration, dilution substance (indicate manufacturer as a foot note) and amount injected at each site; if different amounts for different acupoints, then list in a Table.
- If moxibustion was used, provide a complete description of the herb (indicate manufacturer as a foot note), technique and duration at each acupoint; if different durations for different acupoints, then list in a Table.
- If laser or ultrasonic stimulation of acupoints was used, provide equipment information (indicate manufacturer as a foot note), the frequencies used, the duration of each frequency and total duration of treatment at each acupoint; if different durations are used for different acupoints, then list in a Table.
- If some other treatment of acupuncture points was used provide a detailed description of the technique.
- *Clearly indicate the duration of each treatment, frequency of treatment, total numbers of treatments and total time period over which treatments were given*; if different times between different treatments list in a Table to clarify.
- After the first use of the type of acupuncture with the abbreviation in parentheses in the text, these standard abbreviations should be used (Example: dry needle acupuncture (DNAP), Electro-acupuncture (EAP), Aqua-acupuncture (Aqua-AP) and moxibustion (Moxa).
- Laser or ultrasonic treatment of acupuncture points should be written out without an abbreviation.
- No specific dates of treatment should appear anywhere in the manuscript (Example: *do not write* "on May 5, 2010… and again on May 15, 2010"… instead write "the second treatment was administered 10 days later".
- All transpositional acupuncture points should be referred to using the *letters, a dash and the appropriate number* (Example: LU-1, SP-6, HT-7).
- The following standard abbreviations should be used for AJTCVM: LU (Lung), LI (Large Intestine), ST (Stomach), SP (Spleen), HT (Heart), SI (Small Intestine), BL (Bladder), KID (Kidney), PC (Pericardium), TH (Triple Heater), GB (Gallbladder), LIV (Liver), CV (Conception Vessel) and GV (Governing Vessel).
- The terms *Du Mai* and *Ren Mai* should be changed to GV and CV Channels respectively.
- Other Extraordinary Channel names should be written with the *Pin-yin* name followed by Channel (e.g. *Chong* Channel, *Dai* Channel, *Yang-Qiao* Channel, *Yin-Qiao* Channel, *Yang-Wei* Channel and *Yin-Wei* Channel.
- No *Pin-yin* names for transpositional acupoints should be used.
- When classical acupoints and transpositional acupoints are the same, the transpositional acupoint abbreviation should be used.
- Classical acupoints and other miscellaneous acupoints should be written using their *Pin-yin* names presented in italics, with only the first word capitalized with dashes between the words (Example: *Da-feng-men.*
- A brief description of the location of all classical points should be given in parentheses after the name. Example: *Da-feng-men* (midline at the level of the cranial ear bases).
- The reason for selection of each acupoint, as it pertains to the diagnosis, should be provided.
- A Table of *all the acupoints used, insertion depth and attributes, indications and actions of each* should be created (see Table section below for the standard Table format).

Herbal Medicine Formulas and Individual Herbs

- Please use the term 'herbal medicine' instead of 'herbal therapy'.
- The country of origin of the herbal medicine should be indicated (Example: Chinese herbal medicine, Korean herbal medicine and Western herbal medicine etc.).
- Do not write "Traditional Chinese herbal medicine" simply write "Chinese herbal medicine".
- All herbal medicine formulas should be written as *Pin-yin* names, in italics, with the first letter of each word capitalized, the words *not separated by dashes* and the common name in parentheses. Example: *Si Jun Zi Tang* (Four Gentlemen Decoction).
- If an herbal formula has no *Pin-yin* name, then write the English name with the classical formula, from which it was modified, in parentheses. Example: Liver Happy (classical antecedent *Chai Hu Shu Gan Wan*).
- A superscript small case letter should be placed next to the formula name as a footnote and the manufacturer's name listed at the end of the text under that footnote letter (see footnotes below).
- The *form of the herb* (top dressing, capsule, teapills, granule or biscuits) administered, *dosage* (amount per kg of body weight), *number of doses per day*, *whether given before, after or with meals* and total duration of treatment should be provided for all herbal medicines.
- The rationale for selection of each herbal medicine should be provided.
- Single herbs should be listed as the italicized *Pin-yin* name followed by the English name in parentheses Example: *Ren Shen* (Ginseng) and their effects as they pertain to the case should be described.
- Pharmaceutical or Latin names *should be used* and authors will be asked to change to the shortened English name.
- A table for each herbal formula should be created which contains individual herb *Pin-yin* and English names, percentage of each ingredient in the formula if possible and their actions (See **Tables** section below).

Tui-na

- List all *Tui-na* techniques used by *Pin-yin* name capitalizing only the first letter of the first word with dashes between each word with the definition in parentheses. Example: *Yi-zhi-chan* (single thumb), *Nie-fa* (pinching).
- Clearly indicate locations, duration and frequency of *Tui-na* treatments and include total number of treatments and total time period over which treatments were performed.
- Create a Table of *Tui-na* techniques used and the actions and indications of each type of treatment (See **Tables** section below).
- Indicate the type, locations, duration and frequency of *Tui-na* techniques recommended for at-home treatment by the caretaker**.**
- Please provide information about actual in-home *Tui-na* treatments if available; if not say "information about actual in-home *Tui-na* treatments was not available".

FOOTNOTE FORMAT

- *A bold line* should be placed between text and the foot notes (Example: _____**)**.
- The footnote title **FOOTNOTES** (*bolded, capitalized and left aligned*) should be placed beneath the line.
- Manufacturers of herbal medicine and any equipment described in the paper should appear as a superscript small letter ([a,b,c] etc.) in the text *at the end of the appropriate word* and listed (one footnote per line) in the footnotes section *with no periods at the end of the line*

FOOTNOTES

[a.] WQ-6F Electronic Acupunctoscope, Donghua Electronic Equipment Factory, Beijing, China

[b.] KINGLI needles, Kingli Medical Appliance Co., LTD, Wuxi, China

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- *Do not* use any automatic footnote programs.
- A bold line should then be placed between the end of the footnotes and the next section.

ACKNOWLEDGEMENTS

- Ignore if not applicable to the study.
- The section title is *bolded, capitalized, left aligned* (Example: **ACKNOWLEDGEMENTS)**.
- If applicable, any source of assistance should be listed below the footnotes; caretakers of animals in the report are not acknowledged.
- Financial sources of assistance are only listed under **Funding Source(s)** (See **Basic Format Prior to the Text of All Manuscripts** above).

REFERENCES- GENERAL CONSIDERATIONS

- References are required for all papers submitted.
- The section title should be *bolded, capitalized, left aligned* (Example: **REFERENCES** two spaces below the **Footnotes** or **Acknowledgement** sections and before the **Tables)**.
- References should be numbered consecutively as they appear in the text using the *automatic numbering system* found under the *Format* section of the ***Microsoft Word*** toolbar.
- *Do not use an automatic referencing program* that inserts references with superscript numbers or letters.
- All references *must* include appropriate page numbers.

- References should be indicated in the text as superscript numbers *at the end of the sentence after the period*. (Example: Horses with severe anhidrosis may die from becoming over-heated.[1])
- *No reference numbers should be placed within the sentence*, even if they refer to different items in the sentence (Example: Write as follows: In two studies of the treatment for back pain in sport horses, Adams evaluated acupuncture and Thompson evaluated Chinese herbal medicine.[1,2])
- *Do not list the same reference more than once* and use *ibid*, but simply list the pages in the order to which they are referred in the text (Example: Schwartz C. Four Paws Five Directions. Berkley, CA: Celestial Arts 1996:35-39,1-3,15,12.)
- Proceedings or course notes should not be used as references as many readers will not have access to them. If such information is available to anyone on a website, then that can be used, but the web address must be provided.

REFERENCE FORMATS

- **Please *note the following closely* in the examples below:**
  - List last name and initials of first name with *no commas or periods in the name*.
  - If multiple authors, *list three names with commas between*, then use the term "et al." to indicate other authors (Example: Smith RL, Jones TJ, Fry LM et al).
  - *Do not insert an "and"* between any author names.
  - Use *sentence case for the title of paper or chapter* and *title case for book names*.
  - *Note below the placement of periods, semicolons, spaces and colons* as these are standard AJTCVM format.
  - Always place a period at the end of the reference.

Journal Article:

- Xie H, Collahan P, Ott E. Evaluation of electroacupuncture treatment of horses with signs of chronic thoracolumbar pain. J Am Vet Med Assoc 2005; 227(5):281-286. (Note: Always provide the volume and number in the example the volume is 227 and number is 5)

Book:

- Schwartz C. Four Paws Five Directions. Berkley, CA: Celestial Arts 1996:35-39,1-3,15.

Book Chapter:

- Schoen A. Acupuncture for musculoskeletal disorders. Veterinary Acupuncture 2nd Ed, Schoen A (ed). St Louis, Mo: Mosby 2001:161-165.

TABLE FORMAT AND STYLE

- Tables are needed to show experimental data, acupoints, herbal medicine formula ingredients and *Tui-na* descriptions.
- All tables should be typed in *New Times Roman 10 point font, single spaced, contain as few rows and columns* as necessary to report the data and be saved as ***Microsoft Word*** *(Excel format is not acceptable)*.
- The title for all tables should be at the top of the table with a *single space between the title and the actual table*.
- The word "Table" and "number" *should be bolded followed by a colon* and the actual title should be *un-bolded in sentence format* (only the first letter capitalized) with no period at the end (Example: **Table 2:** Ingredients of the Chinese herbal medicine *Sheng Mai Yin*[a] and their actions (the superscript "a" refers to the manufacturer of the herbal medicine).
- Number tables consecutively, as cited in the text.
- Insert references to the Tables in the text *at the end* of the sentence *before the period* (e.g. The acupoints initially stimulated were BL-21, BL-20, BL-23 and KID-3 (Table 1).
- *Do not say* (*See* Table 1).
- Give each column a short or abbreviated *centered* heading.
- All cells containing *numbers or short words* should be *centered* and *all others* should be *left aligned*.
- Do not write complete sentences in the cells, only list items separated by a comma and *do not* put a period at the end of the list.
- Use the *pleural* under actions (Example: Clears Heat *not* Clear Heat).
- Explain abbreviations immediately under the table (Example: EA=electro-acupuncture or Moxa= moxibustion).
- Manufacturers should be shown for all herbal formulas as a superscript letter and a footnote as shown above and below.
- References for any information in the Table should be shown by the appropriate superscript number as shown above and below.

TABLE TEMPLATES AND EXAMPLES

- A standard Table format is used for all articles in AJTCVM
- *Cut and paste the table examples below into your article, preserve the section titles, but delete the example information and replace with information pertinent to the article and use as a template to create the standard AJTCVM table formats.*
- The superscript "1", after the title **Attributes, Indications and Actions** or **Actions,** indicates the reference supplying the information.

Table 1: Acupoints used to treat quadriparesis in a 5-year-old pregnant alpaca with the needle depth, indications and actions for each acupoint

Acupoint	Approximate Needle Depth (inches)	Attributes, Indications and Actions
BL-18	0.50	Back-*shu* Association point for LIV to tonify liver *Qi*, supports the sinews, strengthens the back, relieves back pain
BL-23	0.50	Back-*shu* Association point for KID, strengthens the hind quarters and back, relieves back pain
BL-62	0.50	Confluent point with *Yang-Qiao Mai* (*Yang* Stepping Vessel), ataxia, "wobbler" syndrome
BL-11	0.30	Influential point for bone, arthritis, cervical stiffness, thoracic limb stiffness

Table 2: Ingredients of the Chinese herbal medicine *Sheng Mai Yin*[a] and their actions

Pin Yin Name	English Name	Amount %	Actions[1]
Ren Shen	Ginseng	37.5%	Replenishes the *Yuan* (Source) *Qi*, tonifies Spleen and Lung, promotes Body Fluids, calms *Shen*
Mai Men Dong	Ophiopogon	37.5%	Nourishes *Yin*, clears false Heat, promotes Body Fluids, relieves thirst
Wu Wei Zi	Schisandra	35%	Restrains dissipation of Lung *Qi*, promotes Body Fluids, preserves *Jing*, restrains excess sweating

Table 3: *Tui-na* techniques used to treat a 13 year old cat with renal failure

Tui-na Technique	Actions[1]
Yi-zhi-chan (single thumb)	Promotes *Qi* flow and regulates *Zang-Fu* organs
Nie-fa (pinching)	Invigorates *Qi* and Blood and regulates Spleen and Stomach

FIGURE LEGENDS

- All figure legends should be typed in *New Times Roman 10 point font* and placed as a numbered list at the end of the paper *after* the **Tables** section.
- The format should be written with the first letter of the word "Figure" capitalized followed by the number, a colon and the legend in sentence structure (only first letter capitalized) (Example: **Figure 1:** A 10 year old male dachshund, with back pain, receiving acupuncture at *Hua-tuo-jia-ji*, from vertebrae T10-L2).
- Legends should be succinct and *provide essential information only*, but adequately explain findings in the figure.
- List all acupuncture points visible in photographs of animals receiving acupuncture.

FIGURE FORMATS

- Figures may be inserted at the end of the manuscript with *the appropriate legend inserted below* each one and placed in the order they are discussed in the text.
- Figures may also be sent as separate files, but should be clearly labeled with the figure number.
- Simple figures such as line drawings, bar graphs and line graphs prepared in *Excel* should be saved *as Excel files (.xls)* or *jpeg files*, titled with the corresponding figure number.
- Line drawings and graphs that were not prepared in Excel are also acceptable, but *should be submitted as jpeg files*.
- Figures created with software programs that use proprietary graphic formats (Example: SigmaPlot, Statistix cannot be used; most such software

programs have the capability to save figures in a *jpeg* format).

- Minimum resolution for line drawings and charts is 1,000 dots per inch.
- All photographs except radiographs should be of high quality clarity and color, saved in a *jpeg* format and be at least 1MB.
- The Editor-in-Chief reserves the right to crop photographs or return photographs to the author for cropping or reformatting when needed.
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AAVA Updates

Maurice Casey DVM

Dr. Maurice Casey

As the newly elected President of the AAVA I would like to thank all of you that have entrusted me with the care and guidance of the AAVA for the next year. We had a very well received Annual meeting in Savanah, Ga. March 24-26. The city of Savannah was most accommodating and friendly. Michelle Tilghman DVM, was our newly elected member to the Board of Directors (BOD). Michelle lectures extensively throughout the Country on Acupuncture and rehabilitation for companion animals. She brings a wealth of knowledge and energy to our BOD. She complements the rest of the Board Members that include; Lynelle Graham, Vice President, Keum Hwa Choi, immediate past President, Mili Bass, Secretary/Treasurer, Robert Schwyzer, Carol Grifford, and Kevin May. This is a wonderful group of people to work with and I look forward to our Board having a successful year. We have already accomplished a few important things since our annual meeting. First and foremost, we have satisfied our requirements to maintain our seat on the AVMA. A large thanks goes to the Chi Institute, as their support in this very important endeavor was a major factor in us being able to accomplish this. Having a seat on the AVMA gives Acupuncture credibility and a chance to make friends and alliances for our future goal of acceptance of Acupuncture as a Specialty Board.

We also approved a hands on "Wet Lab" for equine practitioners on June 24, 2017 at the Aiken Horse Rescue in Aiken, S.C. We are very fortunate to have Dr. Chris Cahill, a renowned Veterinary Acupuncturist and a Past President of the AAVA as our Presenter at this Educational Opportunity. Dr. Cahill has an Acupuncture Practice in Lexington, KY where he has worked on many of the top TB horses in the world, both on the race track and in the breeding shed. He brings over 35 years of practical experience with acupuncture to share with us. Don't miss this one.

One of the main goals that I would like to see the AAVA accomplish during my tenure as President, is that we are the "umbrella organization" for all US Veterinary Acupuncturists, regardless of their certification training. We open our arms to IVAS, Chi Institute, and MAV graduates. We welcome you all to our Organization, to our Annual Meetings, and we invite you to submit papers to the AAVA Annual Meeting, which will be held on April 13-15, at the Inn and Spa at Loretto, 211 Old Santa Fe Trail, Santa Fe, NM.

I look forward to a productive and energetic year.

Thank you,
Maurice

AATCVM Updates

Bruce Ferguson DVM, MS President AATCVM

Bruce Ferguson, DVM, MS with my wife Suzanne Hughes, enjoying the lovely "Spring" weather (in December) in Florida. Get out and breathe the fresh aroma!

Hello Everyone! I hope that this letter finds you all appreciating your lives. I am not going to say "thoroughly enjoying your lives". Not every day is a "great" day in the sense of "perfect, pain free, no stress". Nonetheless, every day that we are sentient and alive is still a miracle, IF you are open to what is actually unfolding before your eyes, rather than what you "want" to be happening. It is said that the secret to a happy life is not necessarily getting what you want, but rather wanting what you get. Please be certain to grasp that last concept, for when you are truly grateful for the chances that life brings you, then happiness is automatically a consequence.

Today I am smiling because of the following situation which has come into my awareness. I am once again reminded of the miracle of the "vertebrate" nervous system. My wife is currently studying Neuroscience and what a long way the science has come since most of us studied the fundamentals in our veterinary training. Now neuroscientists have mapped a huge number of brain cells, areas, activities, and possible functions. Even though our understanding of some very fundamental events of the nervous system are still unknown to us (e.g. how is a "rose" image stored in the brain and how might it be recalled at a later time?), we can still almost visualize the cascade of events which occurs as we simply walk. The pressure receptors, the golgi tendon organs, the muscle spindle organs, the motor tracts to the effector muscles, and the sensory tracts returning and passing through the cerebellum, brainstem, and into the cortex.

And we know that all of this is happening at an amazing speed, with depolarization and polarization of neurons occurring dozens to hundreds of time per second. We can measure many small details and attempt to build upon them. But, if you can for just a few seconds use your visual imagination (and just what is that?), and attempt to see what the brain, spinal cord, and peripheral nerves are doing as we simply take a few steps in one direction, you may see a flashing, dancing "light of depolarization" and similar light of membrane potential changes as the neurons "do their stuff", pumping ions madly and sending waves of electrical activity down the axons. I recently had such a vision while practicing Qigong and it was truly a vision of the miracle of our bodily CNS function.

So why am I rambling on about this today? It is my feeling that our daily lives are full, much too full of information, and this information overload grows daily. At some point we simply "shut down" and stop processing. I understand as much as any of you why we feel compelled to accept no more information and take a "brain break". This is why I urge you to relax occasionally and see the Big (Miraculous) Picture. It is not an attempt to put any more "stuff" into your brain, but rather to give your brain a chance to smile and reflect upon its own wonderment. Because this package of cell bodies, axons, dendrites, synapses, and "scaffolding" cells of which we now believe the astrocytes, oligodendrocytes, and others, are

actual participants rather than just mute structures, has the ability to reflect on its own construction. So, to say it simply, we have this fatty-rich tissue which is capable of contemplating its own existence in this universe. How amazing is that?

Well, knowing this to be true, contemplating it, visualizing the possible dance of membrane potentials and neurotransmitter's tickling the post-synaptic membranes and continuing the "dance".....simply reminds me of the miracle of life and the Nervous System. And, of course, we are not 100% certain, but it is highly likely that this lovely nervous system is the material basis for the related miracle of acupuncture.

Please remind yourself occasionally of this daily miracle, perhaps it will help you to appreciate your life. I know that it certainly has increased my daily appreciation of this beautiful life.

AATCVM Website Updates

Visit the AATCVM online at www.aatcvm.org to see our new search bar! You'll find the search bar in the top right corner of the website once you have logged in with your AATCVM/WATCVM user name and password. Use the search bar to find specific article topics or authors quick and easily.

IVAS Updates

A Letter from the IVAS President 2016-2018: Uwe Petermann DVM, CVA

Dr. Uwe Petermann

Dear members of the IVAS family and all veterinary acupuncturists worldwide,

When you hold this journal in your hands, many of us will already be at the IVAS "Family Meeting" in Cairns, Australia. This is the 43rd Annual IVAS Congress on Veterinary Acupuncture being held jointly with the Australian Veterinary Acupuncture Group (AVAG) at the Hilton Cairns, August 23 - 26, 2017.

It is an unbelievable success story, to look back on a history of 43 years when the IVAS Family was founded by a handful of enthusiastic veterinary acupuncturists at a time when only a rare veterinarian had heard about this treatment modality. In addition, if she/he had heard about it, likely it might not be something positive! These days we are celebrating, yes really celebrating our 43th International Annual Congress!!

In my eyes the "Annual Family Meeting", our Congress, is one of the many important things we do in our society. The Board of Directors has planned for the future with the Congress venues planned through 2020. In 2018, the Congress meets in Tel Aviv, Israel; in 2019 it will be Wroclaw, Poland and back in the USA for 2020.

The 2018 IVAS Congress in Tel Aviv will be a combined joint Congress with the Asian Society for Traditional Veterinary Medicine (ASTVM). Our House of Delegates and Board member Dr. Sagiv Ben-Yakir is the current President of this society and can bring us together with all the colleagues from the Near and Far East including China and Japan. This is a unique opportunity for IVAS to expand to this region of the world and come into contact with different approaches to acupuncture and broaden one's horizon.

I hope to see many of you from all over the word at the 44th Annual IVAS and ASTVM Congress in Tel Aviv, Israel in 2018!! "The Holy Land Congress." How many chances do you have in your life to go to the Holy Land which is the Holy Land for many religions? Take this opportunity and join the "Family Congress"!

No one should miss the unique experience of attending a Congress and feel the friendship, hear the laughter, and listen to the talks. You will be surrounded by so many wonderful people who are your friends or will become your friend as you enjoy the music and the party at the Banquet Dinner. I can't imagine missing the IVAS Annual Congress even one single year. This will be my 19th IVAS Congress in a row! Each of these Congresses was special, and during each of these, there was the spirit of internationality, friendship, wisdom, knowledge and human kindness which is so typical for the IVAS family!

Best wishes,
Uwe

IVAS CONTINUING EDUCATION AND UPCOMING EVENTS

PRINCIPLES OF CHINESE FOOD THERAPY

February 11-12, 2017 IVAS held a Continuing Education Event in Fort Collins, Colorado on "The Principles of Chinese Food Therapy" with Drs. Connie DiNatale and Linda Boggie. We had 22 participants attend coming from across the United States, Canada and the furthest came from Trinidad and Tobago. The weekend was filled with great information, lots of cooking and some downright funny comedy between these two!

Maybe tasting some heart and liver would not have been high on the list of favorite foods to try; but the participants tasted all of the recipes and felt responses along with different sensations in their bodies when trying them out. By tasting and experiencing the food first hand this reinforced the topics and connected all the dots.

If you were just starting out with food therapy or an old pro, this weekend had something for everyone that attended. We hope to see many of you at the next IVAS Continuing Education Event!

IVAS HERBAL CERTIFICATION

The International Veterinary Acupuncture Society (IVAS) offers Certification in Veterinary Chinese Herbal Medicine through a flexible format via online education. The course consists of 8 Modules that are self-paced to fit your busy schedule and can be taken wherever your laptop takes you. This course offers licensed veterinarians clinically oriented education and skills through foundational training in the field of Chinese Herbal Medicine.

If you want to extend your knowledge and add Chinese Herbal Medicine to your armory, then the IVAS Certification in Veterinary Chinese Herbal Medicine offers you the chance to expand your treatment options in a very rational, effective and evidence-based way. This course is RACE approved and delivered by the government accredited College of Integrative Veterinary Therapies (CIVT).

For more information visit the IVAS website at www.ivas.org.

Veterinarians attending one of the lectures in the IVAS continuing education class “The Principles of Chinese Food Therapy” in Fort Collins, Colorado. Participants are shown here working hard to absorb all the information shared with them over this 2 day course!

Dr Linda Boggie demonstrating the ease of preparing crockpot recipes for animals.

This is a tonifying recipe prepared during class and ready to taste!

Dr Connie DiNatale sharing her Food Therapy knowledge and demonstrating her Iron Chef abilities.

A completed diet preparation demonstrating a good texture which is moist but not soupy.

WATCVM Updates

Mushtaq A Memon, BVSc, PhD, CVA, Executive Director of the WATCVM

Dr. Mushtaq Memon

Annual Meeting: The WATCVM Board of Directors (BOD) annual meeting will be held on August 17, 2017 at Westin Prince Toronto, Canada. The meeting agenda includes the new BOD nominations, WATCVM accreditation of TCVM Institutes, WATCVM's assistance in development and support for TCVM student clubs at veterinary colleges, and BODs reports on TCVM activities in various countries and regions of the world.

Ms. Dakota Sproule: I am very pleased to welcome Ms. Dakota Sproule as a part of TCVM family. Dakota is going to play an important role to stream line the WATCVM online membership process, assisting in fundraising, updating the website, and assist in WATCVM day-to-day activities.

WATCVM Newsletter: The 1st WATCVM Newsletter, replacing the TCVM newsletter was distributed to all American Association of TCVM (AATCVM) and WATCVM Members. If you have not received your copy, please contact Ms. Dakota Sproule at dakota@watcvm.org The newsletter will be published and distributed electronically during fall, winter and spring.

I would like to hear from you what you liked and didn't like about the newsletter, and share your ideas for improving the newsletter. Our goal is to make the newsletter the voice of all TCVM practitioners and students worldwide. Please send me your ideas at watcvm@gmail.com

WATCVM/AATCVM membership: Now with one membership fee you become member of both organizations - WATCVM/AATCVM. With $85 membership fee, the veterinarian will become member of both – National (e.g. AATCVM, German Association of TCVM) as well as WATCVM. The WATCVM will work with the national organization to share membership dues.

Benefits of the WATCVM membership:

1. Free access to the online American Journal of TCVM
2. Free access to the TCVM Forum to receive free TCVM advice for clinical cases
3. Free access to the online WATCVM Newsletter
4. Free access to the online TCVM News
5. 5% discount of International Annual TCVM Conference registration
6. To be included on a TCVM referral list in the WATCVM official Website

In addition to the above mentioned benefits, your membership will help to accomplish the goals of WATCVM, which are to develop standards for TCVM education and practice, to raise funds to support research and provide scholarships for veterinary students and faculty members to learn TCVM. The WATCVM would supports the national and regional organizations to unite the global TCVM community through promotion, teaching and publication of research on all aspects of TCVM.

Please join or renew your membership on line at http://www.aatcvm.org/ If you have any questions or need help in completing the membership online, feel free to contact Ms. Dakota Sproule at dakota@watcvm.org

Submitted by,
Mushtaq A Memon, BVSc, PhD, CVA
Executive Director, WATCVM
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Basic Science Studies

A Controlled *In-Vitro* Study Comparing Efficacy of Two Commercial Antibiotic Topical Compounds and Two Chinese Herbal Medicine Topicals Against Bacteria Cultured from 31 Canine Pyoderma Cases

Michael D Bartholomew DVM, MS

ABSTRACT

Canine pyoderma is a common and frustrating disease to treat in veterinary practice. Antibiotic resistance continues to be problematic forcing a search for effective alternative therapies. The objective of this controlled *in-vitro* experimental study was to document the antibacterial activity of two Chinese herbal medicines [Golden Yellow Powder (modified *Ru Yi Jin Huang*) and Coptis (*Huang Lian*)] and compare them to two conventional antibiotic topical medications (Zymox and Animax) against bacteria cultured from 31 clinical cases of canine pyoderma. Test material efficacy was determined by measured zones of inhibition (ZOI) against bacteria on sheep blood agar plates. ZOI results demonstrated that both herbal medicines at concentrations of 5g/20ml and 10g/20ml had statistically significant values for antibacterial activity when compared to placebo control ($p < 0.001$) and when compared to the antibacterial topical Zymox ($p < 0.001$). The single herb Coptis (*Huang Lian*) had greater ZOI activity when compared to both antibiotic topicals, Zymox and Animax, with a 95% confidence level. Finally, Coptis had statistical significance when compared to Golden Yellow Powder ($p<0.001$). The results from this study suggest that both Chinese herbal medicines tested have effective antibacterial activity against bacteria cultured from canine patients with pyoderma and show promise either as an adjunct to conventional antibacterial therapy or as primary treatment.

Key words: Chinese herbal medicine, pyoderma, traditional Chinese veterinary medicine, Golden Yellow Powder, bacterial culture, Coptis, methicillin-resistant Staphylococcus aureus, MRSA

ABBREVIATIONS

TCVM	Traditional Chinese veterinary medicine
TCM	Traditional Chinese medicine
ZOI	Zone of inhibition
GY5	Golden Yellow Paste 5g/20ml water
GY10	Golden Yellow Paste 10g/20ml water
Cop5	Coptis 5g/20ml water
Cop10	Coptis 10g/20ml water
ZFS	Zymox topical full strength
AFS	Animax ointment full strength
Cm	Centimeters
ML	Milliliter
G	Grams
MRSA	Methicillin-resistant Staphylococcus aureus
Spp	Species

From: The Chi Institute of Chinese Medicine, Reddick, FL and The Animal Hospital of Dunedin, Dunedin, FL

Canine pyoderma is one of the most commonly seen diseases in veterinary practice.[1,2] It is characterized by localized or generalized bacterial skin infection accompanied by a variety of lesions such as erythema, papules, pustules, crusts, alopecia and ulceration of the skin. While simple pyoderma can easily be treated, complex pyoderma resulting from recurrent infections is difficult to resolve and can cause great discomfort and suffering in both canine and feline patients.[3] Diagnosis is accomplished through a combination of physical examination, cytology of skin preparations, along with culture and sensitivity testing.

Conventional therapies for bacterial pyoderma include oral antibiotics and topical antibacterials including ointments, sprays, and shampoos.[3] Recommended antibiotic therapy for superficial pyoderma can range from 30 days to as long as 3 months for chronic, recurrent or deep pyoderma.[3] This recurrence and chronicity combined with limited options for approved antibacterial products

leaves the veterinary practitioner reaching for the same drugs repeatedly, leading to multi-drug resistant strains of bacteria. One solution to this growing problem is to create newer generations of antibacterials, but their use will inevitably lead to resistant strains of bacteria. A different and more creative solution to the dilemma this creates is to explore therapies that have been used for thousands of years such as Chinese herbal medicine which is an integral part of Traditional Chinese Medicine (TCM) and Traditional Chinese Veterinary Medicine (TCVM).

While TCVM herbal therapy is still considered new to the western world, it has been practiced in China for centuries and continues to be a large part of Chinese medical practice today. Thousands of years of documented information on the properties, processing techniques, safety, toxicity, cautions, contraindications, and combinations of herbal medicines have led to the compilation of the Chinese Herbal Materia Medica seen today.[4] According to a recent publication, herbal therapy accounts for an estimated 40% of all health care in China. A search of medical literature yields a plethora of human studies using Chinese herbal medicine to treat skin disorders.[5, 6] A number of herbal extracts have been identified that show promising antibacterial activity or shared synergism with antibiotics. Extracted published data indicates an effect against a number of microorganisms including MRSA and drug resistant Pseudomonas bacteria with the antibacterial properties of herbal medicines not affected by antibiotic resistance of organisms.[7, 8] A review on the antimicrobial effects of Heat clearing herbs concluded that they have shown an antibacterial effect by decreasing bacterial adherence to epithelial surfaces and exert selective inhibition of microbial growth.[9]

According to TCVM, disease occurs when there is an imbalance within the body and the smooth flow of energy through the Channels or Meridians is interrupted.[10] TCVM pathogens, such as Heat, Cold, Damp and Wind can disrupt energy flow and are associated with disease production.[10] Wind-Heat and Damp-Heat are the TCVM diagnostic patterns most commonly identified with canine pyoderma.[11] Wind-Heat presents a superficial pattern where the pathogen invades at the level of the Wei Qi (Defensive Qi). Presenting TCVM clinical signs are commonly characterized by a red dry tongue, rapid floating pulses, pruritis and skin inflammation.[12] Damp-Heat, is a deeper invasion of the skin and is typically a more chronic condition. TCVM clinical signs may include a red swollen tongue, pruritus, rapid forceful or slippery pulses along with inflamed skin with weeping eruptions.[12] Shen Disturbance may accompany both patterns due to the severe pruritus and pain associated with Stagnation.[10, 12]

The Chinese herbal medicines, Golden Yellow Powder[a] (modified *Ru Yi Jin Huang San)* and the single herb Coptis[b] (*Huang Lian*) are both used topically for skin conditions presenting with the conventional Western diagnosis of pyoderma or TCVM pattern of Damp-Heat and have been used successfully in clinical settings by the author (Tables 1 and 2).[4, 13] . The primary TCVM principles of treatment achieved with topical application of these Chinese herbal medicines include clearing Heat, moving Blood, drying up the Damp, detoxifying, and relieving pain.[11] In addition, scientific literature demonstrates antibacterial activity of Golden Yellow Powder and Coptis (*Huang Lian*) when used topically and suggests potential for treating acute and chronic wounds. [4, 13]

The objective of this controlled *in-vitro* experimental

Table 1: Ingredients of the Chinese herbal medicine Coptis (*Huang Lian*)[a] and their actions.

***Pin Yin* Name**	**English Name**	**Actions[11]**
Huang Lian	Coptis	Clear Heat/Damp Heat/Detoxify

Table 2: Ingredients of the Chinese herbal medicine Golden Yellow Powder[b] and their actions.

***Pin Yin* Name**	**English Name**	**Actions[11]**
Tian Hua Fen	Tricosanthes	Clear Heat/Promote Body Fluid
Da Huang	Rheum	Clear Heat/Dissipate Swelling
Huang Bai	Phellodendron	Clear Heat/Detoxify
Jiang Huang	Curcuma	Activate Blood/Relieve Pain
Bai Zhi	Angelica	Relieve Pain/Clear Wind Cold
Cang Zhu	Atractylodes	Dry Damp
Chen Pi	Citrus	Move *Qi*/Relieve Pain
Gan Cao	Glycyrrhiza	Harmonize
Hou Po	Magnolia	Dry Damp/Move *Qi*
Huang Lian	Coptis	Clear Heat/Detoxify

study was to document the antibacterial activity of two Chinese herbal medicines (Golden Yellow Powder, Coptis [*Huang Lian*]) and compare them to two commercial antibiotic topical medications (Zymox[c], Animax[d]) against bacteria cultured from 31 clinical cases of canine pyoderma (Tables 1-5). The primary hypothesis was that both Chinese herbal medicines would demonstrate antibacterial activity when compared to placebo control. In addition, it was hypothesized that the Chinese herbal medicines would be as effective as the conventional topical antibiotics and that the Chinese herbal formula (Golden Yellow Powder) would have greater antibacterial activity than the single Chinese herb, Coptis (*Huang Lian*).

METHODS AND MATERIALS

The cases of canine pyoderma used for this study were recruited from the patient population presented for bacterial pyoderma at the Animal Hospital of Dunedin, Dunedin, Florida, USA. Patients with any weight, age, breed, sex, or duration of disease were included in the study. The only exclusion criteria were animals presenting with otitis. A diagnosis of pyoderma, made by the author, was sufficient to enroll the patient in the study.

Patients who demonstrated signs of bacterial

Table 3: Ingredients of Zymox (Topical Cream)[c]

ZYMOX Ingredients	
Aloe Vera	Lactoferrin
Benzyl Alcohol	Lactoperoxidase
Dextrose	Lysozyme
Glucose Oxidase	Potassium Iodide
Glycerin	Propylene Glycol
Glyceryl Polymethacrylate	Purified Water
Isopropyl Myristate	Titanium Dioxide
	Zinc Gluconate

Table 4: Ingredients of Animax[d]

ANIMAX Ingredients	
Nystatin	100,000 units
Neomycin Sulfate	2.5 mg
Thiostrpton	2,500 units
Triamcinolone acetonide	1 mg
In a polyethylene and mineral oil base	

Table 5: Bacteria cultured from each of the 31 clinical cases enrolled in the study

Patient Number	**Bacterial Culture Identification**
1	*Staphylococcus pseudointermedius*
2	*Staphylococcus pseudointermedius*
3	*Comomonas testeroni*, methicillin-resistant *Staphylococcus pseudointermedius*
4	*Staphylococcus pseudointermedius*
5	*Staphylococcus pseudointermedius*
6	Bacillus spp, coagulase-negative *Staphylococcus* spp
7	*Staphylococcus pseudointermedius*
8	*Staphylococcus schleiferi* subsp *coagulans*
9	*Staphylococcus pseudointermedius*
10	Methicillin-resistant *Staphylococcus pseudointermedius*
11	Methicillin-resistant *Staphylococcus schleiferi coagulans*

Table 5 continued

12	*Escherichia coli, Proteus mirabilis*
13	*Staphylococcus schleiferi* subsp *coagulans*, Bacillus App
14	*Staphylococcus schleiferi* subsp *coagulans*
15	*Staphylococcus pseudointermedius*, *Acinetobacter lwoffii*
16	Methicillin-resistant *Staphylococcus pseudointermedius*
17	*Citrobacter koseri*, *Staphylococcus pseudointermedius*
18	Coagulase-negative Staphylococcus spp
19	Methicillin-resistant *Staphylococcus schleiferi coagulans*
20	Methicillin-resistant *Staphylococcus pseudointermedius*
21	*Staphylococcus pseudointermedius*
22	*Staphylococcus pseudointermedius*
23	*Staphylococcus pseudointermedius*
24	*Staphylococcus pseudointermedius*
25	*Staphylococcus pseudointermedius*
26	*Staphylococcus pseudointermedius*
27	Micrococcus luteus, coagulase-negative Staphylococcus spp
28	*A) Methicillin-resistant coagulase-negative Staphylococcus spp, B)Bacillus spp
29	*^A) *Staphylococcus schleiferi* subsp. *coagulans*, *Moraxella osloensis*; B) methicillin-resistant *Staphylococcus schleiferi coagulans, Moraxella osloensis*; C) Bacillus spp
30	*Staphylococcus pseudointermedius*
31	A) Bacillus spp, alpha-hemolytic Streptococci, B) *Staphylococcus aureus*

*Indicates where bacterial isolates were obtained from each patient
^Indicates *Moraxella osloensis* was grown twice from the same patient in different isolates

pyoderma and met inclusion criteria were admitted to the study after the owner's consent was obtained using the client consent form. Each animal was then assigned a case number. An initial sterile swab was taken from the location of the skin lesion associated with the bacterial pyoderma. To obtain the sample, a sterile swab was removed from its wrapper and was rolled across the affected area. The sample was immediately transferred to the surface of a new sheep's blood agar plate[e]. The plate was labeled with the date and assigned a case number and referred to as Plate 1. The sample plate was then transferred to an incubator[f] set at 75.9°F where the plate would be monitored for bacterial growth.

After growth was observed (Figure 1), a sample of Plate 1 growth was obtained using a sterile Curity swab[g]. This swab was sent to Antech Laboratories[h] with paperwork stating the date and case number for further growth and bacterial identification by the commercial laboratory. Efforts were made to isolate as many different bacterial colonies as possible for growth. In addition to the sample sent to the commercial laboratory, another sterile swab was used to collect a sample of growth from Plate 1 and transfer it to a new Sheep's Blood Agar Plate, called Plate 2.

After transferring the bacterial colonies to Plate 2, a sterile new 6 mm biopsy punch was used to create 7 wells in the agar plate. Each of the 6 test substances assessed in the study (Tables 1-4) was placed in these wells (Figure 2) along with the control mixture. Test substances were prepared as follows: Golden Yellow Powder[a] (GY5) was mixed at a concentration of 5g of Golden Yellow Powder[a] to 20ml of boiling water, Golden Yellow Powder[a] (GY10) was mixed at a concentration of 10g of Golden Yellow Powder[a] to 20ml of boiling water, Coptis[b] (Cop5) was mixed at a concentration of 5g Coptis[b] (*Huang Lian*) powder to 20ml of boiling water, Coptis[b] (Cop10) was mixed at a concentration of 10g Coptis[b] (*Huang Lian*) powder to 20ml of boiling water, the Control was mixed at a concentration of 10g white baking flour to 20 ml of boiling water. The 2 commercial products, Zymox[c] (ZFS) and Animax[d] (AFS) had a consistency that worked well for this experiment and both were used at full strength in the agar wells. After thorough mixing, each test substance was stored individually in a syringe in a cool, dry area to be used throughout the study.

Each test substance was introduced into a well via syringe. The exact amount placed in each well was determined by the mixture having adequate contact with the agar surface (Figure 2). Each well was given a number that was recorded on the plate along with other pertinent

Table 6: Agar Wells containing test substance mixtures with well number and abbreviation used for each mixture throughout the study.

Well #	Treatment	Abbreviation Used
Well 1	Zymox	ZFS
Well 2	Animax	AFS
Well 3A	Golden Yellow 5g/20ml water	GY5
Well 3B	Golden Yellow 10g/20ml water	GY10
Well 4A	Coptis 5g/20ml water	Cop5
Well 4B	Coptis 10g/20ml water	Cop10
Control	Flour 10g/20ml water	

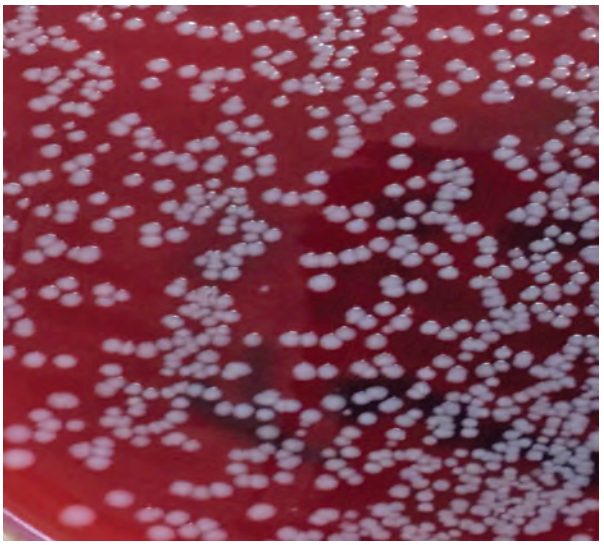

Figure 1: Example of bacterial growth from a canine patient with pyoderma on one of the sheep's blood agar Plates in the study (Plate 1).

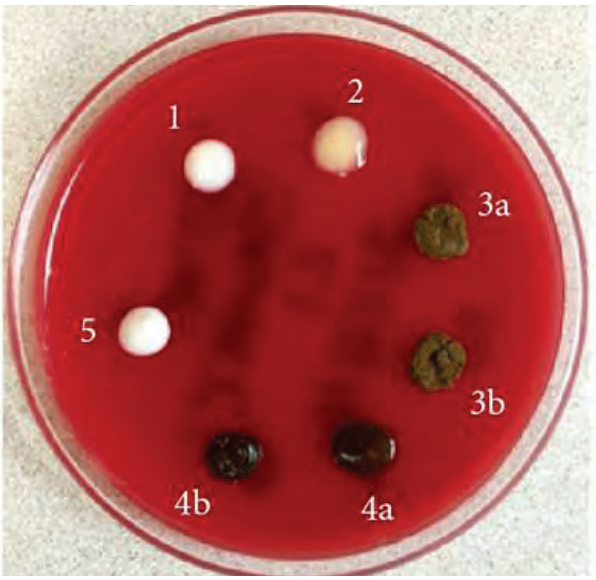

Figure 2: Plate 2 demonstrated the wells created for each test solution by a 6mm biopsy punch into a sheep's blood agar plate. The approximate locations of the test substances as demonstrated in the picture include: ZFS located at no. 1, AFS at no. 2, GY5 at no. 3a, GY10 at no. 3b, Cop5 at no. 4a, Cop10 at no. 4b and placebo control at no. 5.

Figure 3: After plating the bacteria and introducing the treatments into individual wells, the plate has been labelled and is ready for the incubator.

Figure 4: A plate just removed from incubator demonstrating antibacterial activity of a test substance represented by a Zone of Inhibition (ZOI) around one of the Coptis preparations after 24 hours of bacterial growth.

information such as date and case number. Well numbers were consistent throughout the study and were assigned to a specific test substance to avoid confusion (Table 6, Figure 2). The plate was then placed in an incubator (Figure 3) and the bacteria were allowed to grow for 24 hours.

When the plates were removed from the incubator, they were individually assessed by the author for bacterial growth (Figure 4). Response to the anti-bacterial action of the test substance was assessed by 2 criteria: presence of a visible Zone of Inhibition (ZOI) eliminating bacterial growth around the test mixture and measurement of the ZOI with a tape measure in centimeters (cm). Results were recorded for each well on an agar plate throughout the study on the data collection template. After all the samples were evaluated and the template completed for all test substances, the data was collected for analysis.

A paired t-test was used to statistically evaluate two superiority hypotheses (herbal test substance compared to control, single herb compared to herbal formula) based on measurement of ZOIs representing anti-bacterial activity of a test substance. Since the hypothesis was a non-inferiority hypothesis (claiming at least as effective), the determination of rejection of the null hypothesis was based on the 95% confidence interval of the pair-t statistic, with the non-inferiority threshold being set to -0.05cm. In addition, using McNemar's Chi-Squared Test as a secondary statistical analysis allowed the study to also evaluate statistical significance with respect to the rate of visible ZOI results (defined as: ZOI > 0.1 cm). All tests with p-values < 0.05 were considered statistically significant The number of pyoderma cases (sample size) in the study totaled 31 cases, which ensured that the employed pair-t test had more than a 95% test power to detect (reject null hypothesis) a mean difference between two treatments being equal to the sample standard deviation (of differences) with 95% confidence.

RESULTS

There were 31 cases enrolled in the study with the average age of patients in the study 7.89 years with a range of 8 months to 15 years old. Spayed and neutered dogs represented the largest population in the study at 18 and 10 respectively followed by intact animals with 2 females and 1 male. There was a wide variety of breeds represented in the study.

Bacterial identification of case samples submitted to Antech Laboratory revealed that a varied number of bacteria were cultured from the skin of patients with canine pyoderma (Table 6). *Staphylococcus pseudointermedius* (17 isolates) was the most commonly isolated bacteria in the study (Figure 5). Fifteen other bacterial isolates included: methicillin-resistant *Staphylococcus pseudointermedius*, *Comomonas testeroni*, Bacillus species (spp), coagulase-negative Staphylococcus spp, *Staphylococcus schleiferi* subspecies (subsp) *coagulans*, methicillin-resistant *Staphylococcus schleiferi* subsp *coagulans*, *Escherichia coli*, *Proteus mirablilis*, *Acinetobacter lwoffi*, *Citrobacter koseri*, *Micrococcus luteus*, *Moraxella osloensis*, alpha-hemolytic Streptococcus, and *Staphylococcus aureus*.

There were 39 pathogenic bacteria and 6 non-pathogenic bacteria identified. Methicillin resistant organisms were cultured in 8 of the 31 cases (26%). Nine clinical cases had multiple bacteria isolated from a single submitted sample while 27 pyoderma cases had 1 bacterial isolate each. The submission of multiple colonies of bacteria to the commercial laboratory for culture from patients that had infections with multiple organisms were not uniformly successful in getting bacterial identification on all isolates submitted. The total number of bacteria cultured was greater than the total patient number due to multiple bacteria cultured from some canine cases.

Visual assessment was made of the ZOI of each test substance on each plate. To balance the weight of analysis between clinical cases with more than 1plate of bacterial growth with cases containing only 1 bacterial isolate (1 plate), the observed ZOIs within the same patient were averaged. After this pre-processing, the mean circumference of ZOIs from Cop10 was 0.56 cm with a 94% visible ZOIs (29/31 circumference of ZOIs > 0.1cm), Cop5 was 0.51 cm with a 97% visible ZOIs

Table 7: Comparison of ZOI mean circumference and visible ZOI's >0.1cm for test solutions

Test Solution	ZOI Mean circumference (cm)	% Visible ZOI >0.1cm
COP 10	0.56	94% (29/31)
COP 5	0.51	97% (30/31)
AFS	0.26	100% (31/31)
GY 10	0.13	90% (28/31)
GY 5	0.12	90% (28/31)
ZFS	0.07	77.4% (24/31)
Control	0.01	10% (3/31)

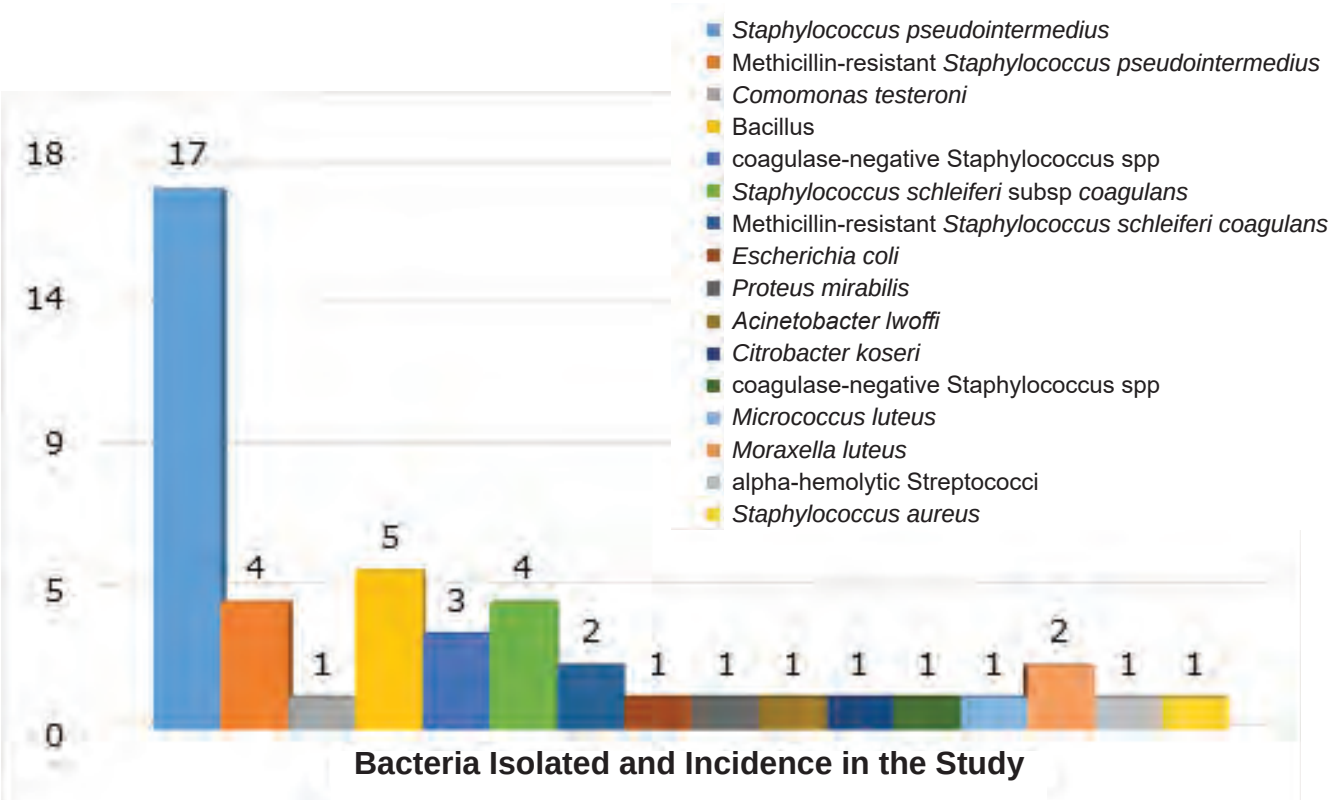

Figure 5: Bacteria cultured from 31 clinical canine pyoderma cases in the study.

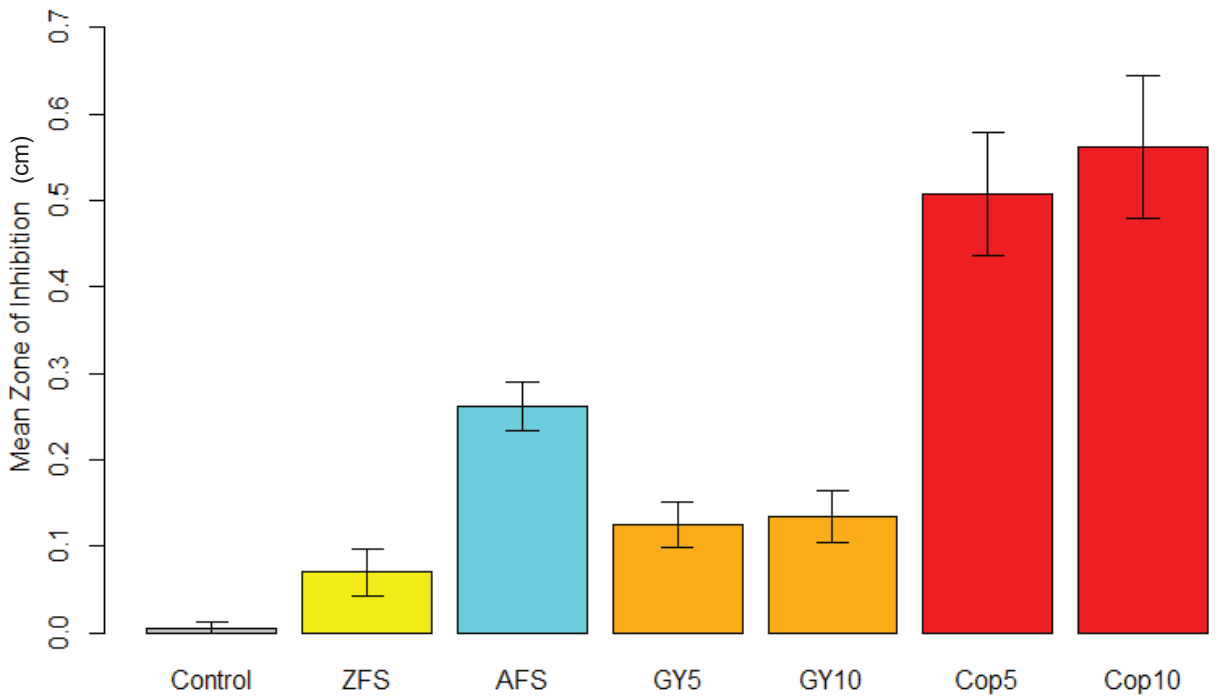

Figure 6: Mean ZOI in centimeters (cm) for each treatment substance tested for antibacterial activity against 31 canine pyoderma cases enrolled in the study. Larger mean ZOI indicates greater level of antibacterial activity against susceptible bacteria. The control contained 10g of flour with 20ml of water. AFS and ZFS were used full strength. Golden Yellow Powder (GY5 and GY10) contained 5g or 10g of Golden Yellow Powder with 20 ml of water, respectively. Coptis (Cop 5 and Cop 10) contained 5g or 10g of Coptis with 20 ml of water, respectively.

(30/31 circumference of ZOIs >0.1cm), AFS was 0.26 cm with a 100% visible ZOIs (31/31 circumference of ZOIs > 0.1cm), GY10 was 0.13 cm with a 90% visible ZOIs (28/31 circumference of ZOIs > 0.1cm), GY5 was 0.12 cm with a 90% visible ZOIs (28/31 circumference of ZOIs > 0.1cm), ZFS was 0.07 cm with a 77.4% visible ZOIs (24/31 circumference of ZOIs > 0.1cm) and the placebo control was 0.01 cm with a 10% visible ZOIs (3/31 circumference of ZOIs > 0), (Table 7, Figures 6 and 7).

When assessing antibacterial activity against specific bacteria of interest, visible ZOI were observed in 8/8 plates against methicillin-resistant Staphlococcus spp for both concentrations of Coptis (Cop5, Cop10). For Golden Yellow Powder, a visible ZOI was noted on 7/8 plates and 6/8 plates for GY5 and GY10, respectively. When unable to separate out bacterial colonies in this study, GY5 and GY10 yielded visible ZOI in 6/6 plates while CY5 and CY10 demonstrated ZOI in 4/6 plates. Separate statistical evaluation was not performed on test substance activity against individual bacterial species.

Table 8: Summary of statistical evaluation of the 3 hypotheses using mean ZOI representing anti-bacterial activity of a test substance using the paired t-test or the confidence interval of paired t-statistic.

	GY5	**GY10**	**Cop5**	**Cop10**
ZOI (mean ± SD)	0.124 ± 0.074	0.135 ± 0.085	0.507 ± 0.199	0.561 ± 0.229
p-value (ZOI better than **Control** = 0.006 ± 0.018)	2.66×10^{-10} (**)	1.18×10^{-9} (**)	1.48×10^{-14} (**)	3.98×10^{-14} (**)
p-value (ZOI at least as good as **ZFS** = 0.070 ± 0.076)	2.00×10^{-6} (**)	1.10×10^{-6} (**)	8.00×10^{-14} (**)	7.01×10^{-14} (**)
p-value (ZOI at least as good as **AFS** = 0.261 ± 0.079)	1.00	1.00	3.50×10^{-9} (**)	8.00×10^{-10} (**)
p-value (Gy5 vs. Cop5)	8.02×10^{-11} (**)			
p-value (Gy10 vs. Cop10)	1.07×10^{-11} (**)			

$p < 0.05$ = Significant (*); $p < 0.01$ = Very significant (**)

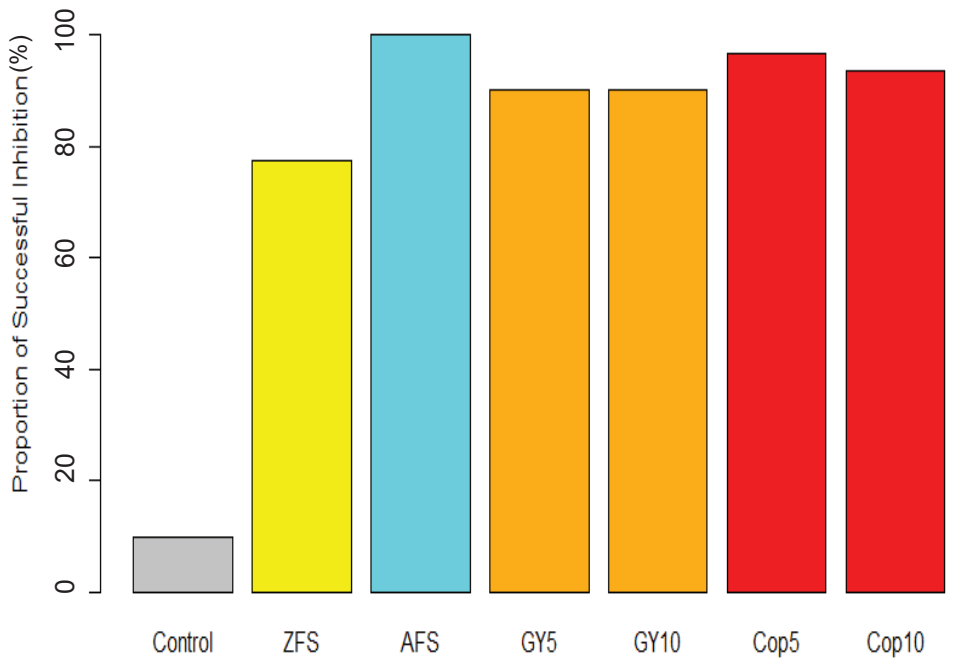

Figure 7: Percentage of visible (zone size > 0.1 cm) ZOIs for each treatment solution tested for antibacterial activity against 31 cases of canine pyoderma in the study.

Three hypotheses using the measured ZOIs representing anti-bacterial activity of a test substance were statistically tested using the paired t-test or the confidence interval of paired t-statistic (herbal test substance compared to control, herbal test substance compared to antibiotic test substance, single herb compared to herbal test substances). All 4 herbal medicine test substances (GY5, GY10, Cop5, Cop 10), demonstrated antibacterial activity when compared to placebo control with a statistically significant p-value < 0.001 (p-value = 2.66×10^{-10}, 1.18×10^{-9}, 1.48×10^{-14}, and 3.98×10^{-14}, respectively). When compared to the antibacterial topical product (ZFS), all 4 herbal test substances demonstrated efficacy statistically not less than ZFS (p-value = 2.00×10^{-6}, 1.10×10^{-6}, 8.00×10^{-14}, and 7.01×10^{-14}, respectively). Only Cop5 and Cop10 demonstrated efficacy statistically not less than AFS (p-value = 3.50×10^{-9}, 8.00×10^{-10}, respectively). Finally, the single Chinese herb (Cop10, Cop5) when compared to the Chinese herbal formula (GY10, GY5) demonstrated improved ZOI with statistically significant p-value < 0.001(p-value =1.07×10^{-11} and 8.02×10^{-11} for Cop 10 vs. GYP10 and Cop 5 vs. GYP5 respectively), Table 8.

The three hypotheses were again tested using percent visible ZOIs (visible zone equals a measurement >0.1cm), instead of measured ZOI, with a McNemar's Chi-squared test. Comparing the herbal test substances and control yielded similar results to the first analysis with GY5, GY10, Cop5, and Cop10 all having statistically significant antibacterial activity when compared to placebo control (p-value = 2.67×10^{-6}, 2.67×10^{-6}, 1.19×10^{-7}, and 2.0×10^{-7}, respectively). Secondly, results from the four herbal test articles compared to the 2 antibiotic topical therapies revealed the following: the ZOI presence rates of all 4 were as high as that of the ZFS (p-value = 1.25×10^{-5}, 2.20×10^{-6}, 3.40×10^{-16}, and 1.70×10^{-14}, respectively); when compared to the AFS, only Cop 5 had a ZOI presence rate statistically not lower than AFS (p-value = 0.035). Finally, comparison of the single herb and the herbal formula was not statistically significant for GY5 vs Cop5 and GY10 vs Cop10 (p-values = 0.077 and 0.182, respectively), although GY5 vs Cop5 could be considered moderately significant (Table 9).

DISCUSSION

This is the first controlled *in-vitro* experimental study written in English evaluating the antibacterial efficacy of the topical use of Chinese herbal medicines such as Golden Yellow Powder and Coptis (*Huang Lian*) against bacteria cultured from clinical cases of canine pyoderma. The results of this study demonstrated that both Golden Yellow Powder and Coptis (*Huang Lian*) when compared to the control have antibacterial activity ($p<0.001$). In addition to satisfying the first study hypothesis (herbal test substances would have antibacterial effects), a secondary hypothesis that there would be comparable efficacy between the commercial topical antibiotic products commonly used for canine pyoderma and the Chinese herbal medicines was confirmed. All 4 herbal test substance concentrations (GY5, GY10, Cop5, Cop10) had greater antibacterial efficacy than the topical antibiotic, ZFS ($p<0.001$), however, only Coptis at both concentrations (Cop5, Cop10) had a statistically significant antibacterial activity when compared to AFS ($p<0.001$).

In addition to the primary objectives of the study (antibacterial activity of the test solutions), it was hypothesized that the Chinese herbal formula (Golden Yellow Powder) and single herb (Coptis) would have a significant difference in antibacterial efficacy. It was proposed that the herbal formula would have greater antibacterial activity since it was a compilation of several herbal ingredients that would provide local effects that

Table 9: Summary of statistical evaluation of the 3 hypotheses using percent visible ZOIs (visible zone equals a measurement >0.1cm) with a McNemar's Chi-squared test

	GY5	GY10	Cop5	Cop10
Percentage ZOI	77.4%	77.4%	96.8%	93.5%
p-value (Percentage ZOI higher than **Control** = 0%)	2.67×10^{-6} (**)	2.67×10^{-6} (**)	1.19×10^{-7} (**)	2.00×10^{-7} (**)
p-value (Percentage ZOI at least as high as **ZFS** = 35.5%)	1.25×10^{-5} (**)	2.20×10^{-6} (**)	3.40×10^{-16} (**)	1.70×10^{-14} (**)
p-value (Percentage ZOI at least as high as **AFS** = 100%)	1.00	1.00	0.035 (*)	0.425
p-value (Gy5 vs. Cop5)	0.077			
p-value (Gy10 vs. Cop10)	0.182			

$p < 0.05$ = Significant (*); $p < 0.01$ = Very significant (**)

would improve antibacterial efficacy. In addition, the herbal formula contained the single herb (Coptis). It has been documented that the use of Coptis (*Huang Lian*) in a formula will enhance the antibacterial effect over ten-fold.[14] This study demonstrated in an *in-vitro* environment, however, the single herb Coptis (Huang Lian) statistically outperformed the herbal formula (Golden Yellow Powder; p value<0.001). Explanation of this may be that performance of the herbal test substances were influenced by several factors. First the efficacy of the Chinese herbal medicines may have been affected by their dilution with water versus use of full strength uniformly blended commercial products. Second, mixture of a single herb with water may have yielded a more consistent mixture than an herbal formula containing 10 different herbs thus resulting in a more potent antibacterial activity for the single herb. Thirdly, the herbal formula may be more effective than the single herb in an *in-vivo* environment versus the present *in-vitro* test.

In all, 16 different isolates were cultured from the 31 canine pyoderma cases (Table 6). *Staphlococcus pseudointermedius* was the most common bacterial isolate from the clinical canine pyoderma cases used in the study (Figure 5). This is consistent with The Merck Veterinary Manual which reports this organism as the primary pathogen of dogs with pyoderma.[4] Of interest is the frequency of isolation of methicillin-resistant staphlococcus spp which occurred in 26% of the cases (8/31). The bacterial isolates in this study support the growing concern that antibiotic resistance is a problem in canine pyoderma cases presenting for treatment in veterinary clinics.

Coptis[a] (*Huang Lian*) has been widely known for its broad-spectrum antibacterial properties.[15] The pharmaceutical actions of this herbal are thought to be derived from the alkaloids berberine, coptisine, worenine, palmatine, jatrorrhizine, epiberberinem, columbamine, and magnoflorine it contains. Other active compounds include ferulic acid and chlorogenic acid.[15] The palmatine and berbine in this herb demonstrated an inhibitory effect on *Staphlococcus aureus* when treating impetigo in a human clinical study.[16] In addition, berberine is bactericidal on *Vibrio cholera* by preventing bacteria from attaching to mucosal and epithelial surfaces and was effective against *Streptococcus agalactiae* with effects on the cell membrane and synthesis of DNA.[9, 17] It is effective against multiple pathogens including but not limited to *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Beta-hemolytic Streptococcus, Leptospira*, *Bacillus proteus, Mycobacterium tuberculosis, Bacillus anthracis, Bacillus cereus* and *Vibrio parahaemolyiticus* with the greatest efficacy against *Escherichia coli*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis* with scholarly research dating back to the 1940's.[14, 18, 19, 20] Four of those pathogens, *Escherichia coli*, *Staphylococcus aureus*, Beta-hemolytic Streptococcus, and *Pseudomonas aeruginosa* were commonly cultured from the skin of patients with pyoderma in the author's clinic. In 9 dermatological cases of exudative erythema multiforme, local and oral administration of Coptis was shown to have significant effect.[14]

Golden Yellow Powder, unlike Coptis (*Huang Lian*), is a Chinese herbal formula (classical antecedent *Ru Yi Jin Huang)* and is not comprised of a single herb, but is a combination of 10 different herbs that work together to perform a primary action and to balance the formula. Rhuem (*Da Huang*), serves as the main herb in the formula that clears and drains Heat downward and cools Blood. The Minister herbs, Phellodendron (*Huang Bai*) and Coptis (*Huang Lian*) are used to clear Heat. Magnolia (*Hou Po)* and Atractyoles (*Cang Zhu)* dry up Damp while Tricosanthes (*Tian Hua Fen)* promotes Body Fluid. The Adjuvant herbs, Tangerine (*Chen Pi*) and Curcuma (*Jiang Huang)* by smoothing the flow of *Qi* and Blood relieve pain. Angelica (*Bai Zhu*) clears Wind-Cold and dissipates swelling while Glycyrrhiza (*Gan Cao*) harmonizes the herbal formula.[11]

Clinical studies evaluating the Chinese herbal formula, *Ru Yi Jin Huang* in humans demonstrated effective treatment of amiodarone induced phlebitis in 36 patients while in another study it demonstrated superior performance when compared to the antibiotic cefuroxime sodium for acute mastitis.[11] When evaluating *Ru Yi Jin Huang's* treatment effect on skin ulcers with swelling in 32 human patients, a 90.62% efficacy was demonstrated.[11] In experimental studies with mice and rabbits, antibacterial effects with improved pain thresholds and 1 week resolution of superficial staphylococcus skin infection was demonstrated, respectively.[11] The rabbit study also demonstrated decreases in IL-1β and TNF-α.. Similar activity was observed with the Adjuvant herb in the formula, Curcuma (*Jiang Huang)*, where extracted curcumin demonstrated antibacterial activity through inhibiting the expression of IL-1β mRNA in rat enteritis models.[11] Chen studied the antibacterial effects of 10 herbs, one of them a Minister herb in this formula, Phellodendron (*Huang Bai)* with the Harmonizer herb Glycyrrhiza (*Gan Cao*) and found these 2 had synergistic activity on inhibiting Methicillin-resistant Staphylococcus aureus (MRSA) bacteria.[11] Additional pharmaceutical studies have shown anti-inflammatory effects of other Minister herbs in the formula such as Atractyoles (*Cang Zhu)* and Tangerine (*Chen Pi).*[11]

One of the limitations of this study was that it took place in a private veterinary practice clinical setting so the population was limited to patients who were seen at the practice location. In addition to the restricted population, the number of cultures was limited during this study by cost and time. The conventional treatments were used at full strength due to their suitable consistency, however, the Chinese herbal medicines which were powders had to be diluted with enough water to make a paste. This could have

affected the outcome of the study, especially the efficacy of GY5 and GY10 which as an herbal formula would then contain lower concentrations of individual ingredient herbs such as Coptis (*Huang Lian*). In addition, preparing a consistent mixture containing multiple ingredients is more difficult. Still, even at diluted concentrations, GY5, GY10, Cop5, and Cop10 outperformed ZFS and Cop5 and Cop10 outperformed AFS. It is interesting to note that visible ZOIs (> 0.1cm) were present on culture plates in 33/36 plates for GY5, 32/36 plates for GY10, 34/36 plates for Cop5 and 34/36 plates for Cop10 indicating consistant antibacterial theraputic effect for all.

There are still a number of areas left to explore. Expansion of the breadth of this study to include a larger population, different species and participants from different geographical areas would give a broader look at the utility of these Chinese herbal medicines. Studies that isolate individual bacterial organisms, particularly those of special interest such as MRSA could test antibacterial activity of various Chinese herbal medicines against each specific organism allowing more specific recommendations for single herbs or herbal formulas. Conducting research studies on animal models or at clinical settings would allow more complete efficacy assessment of herbal formulas which contain ingredients that may permit better dermal penetration versus single herb activity

As practitioners face more challenges with multi-drug resistant bacteria, it becomes imperative to seek alternatives to conventional antibiotics. While the number was not large, when addressing Methicillin-resistant Staphlococcus spp in this study, visible zones of inhibition were observed in 8/8 plates for both concentrations of Coptis. For GY5, a visible ZOI was noted on 7/8 plates and for GY10, 6/8 plates. These results are particularly interesting and deserve further investigation with a larger study as they have the potential to be an effective alternative or adjunct to conventional therapies when drug resistant bacteria are present. In addition, these Chinese herbal medicines should be assessed as to their effectiveness against multi-organism pyodermas. During this study on some cases where it was not possible to separate out individual bacterial colonies in a multi-organism infection, it is of interest to note that GY5 and GY10 yielded visible ZOI in 6/6 plates and COP5 and COP10 in 4/6 plates.

In conclusion, ZOI results demonstrated that both Chinese herbal medicines at concentrations of 5g/20ml and 10g/20ml had statistically significant values for antibacterial activity when compared to placebo control ($p < 0.001$) and when compared to the antibacterial topical Zymox ($p < 0.001$). The single herb Coptis had greater ZOI activity when compared to both antibiotic topicals, Zymox and Animax, with a 95% confidence level. Both tested Chinese herbal medicines have effective antibacterial activity against bacteria cultured from canine pyoderma cases and show promise, in particular, for antibiotic resistant chronic cases of pyoderma either as an adjunct to conventional therapy or as the primary treatment.

Acknowledgements

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FOOTNOTES

a. Golden Yellow Powder, Jing Tang Herbal, Reddick Florida, USA
b. Coptis Powder, Jing Tang Herbal, Reddick Florida, USA
c. Zymox (Topical Cream), PKB Animal Health, Westmont, IL USA
d. Animax, Dechra Veterinary Products, Overland Park, KS. USA
e. Trypic Soy Agar (TSB II) with 5% Sheep's Blood; Becton, Dickinson and Company, Sparks, MD 21152, USA
f. Incubator, Brand Unknown
g. Curity Cotton Tipped Applicator, Covidien llc, Mansfield, MA. USA
h. Antech Laboratories, Irvine, CA. USA www.antechdiagnostics.com

REFERENCES

1. Sischo W. Regional Distribution of Ten Common Skin Diseases in Dog. J Am Vet Med Assoc 1989; 195 (6):752-6.
2. Greene CE. Infectious Diseases of the Dog and Cat. St Louis, Missouri: Elsevier Health Sciences 2013: 878.
3. The Merck Veterinary Manual, 9th ed. Whitehouse Station, NJ: Merck & Co Inc. 2005: 694-697.
4. Xie H. Chinese Veterinary Herbal Handbook, 3rd ed. Reddick, FL: Jing Tang Publishing 2011: 366, 287, 290.
5. Dorai A. Wound Care with Traditional, Complementary, and Alternative Medicine. Indian Journal of Plastic Surg 2012; 45(2): 418-424.

6. Rister R. Japanese Herbal Medicine The Healing Art of KamPo. Garden City Park, NY: Avery Publishing Group 1999: 5.
7. Chan B, Lau C, Jolivalt C et al. Chinese Medicinal Herbs Against Antibiotic Resistant Bacterial Pathogens. Science against microbial pathogens: communicating current research and technological advances Formatex 2011: 773-781.
8. Liu C, Cham T, Yang C et al. Antibacterial Properties of Chinese Herbal Medicines Against Nosocomial Antibiotic Resistant Strains of Pseudomonas aeruginosa in Taiwan. Am J Chin Med 2007; 35(6): 1047-1060.
9. Muluye R, Bian Y, Alemu P. Anti-inflammatory and Antimicrobial Effects of Heat-Clearing Chinese Herbs: A Current Review. J Tradit Complement Med 2014; 4(2): 93-98.
10. Xie H, Preast V. Traditional Chinese Veterinary Medicine, Fundamental Principals, Vol 1. Reddick, FL; Jing Tang Publishing 2002: 10-16, 209-240, 80.
11. Ma A. Clinical Manual of Chinese Veterinary Herbal Medicine. Ma A (ed). Gainesville, FL: Ancient Art Press 2016: 238, 300, 301.
12. Xie H, Wedemeyer L, Chrisman C, Trevisanello, L. Practical Guide to Traditional Chinese Veterinary Medicine. Reddick, FL: Chi Institute Press 2014: 562, 563, 483-490.
13. Xie H, Preast V. Xie's Chinese Veterinary Herbology. Ames, Iowa: Wiley-Blackwell 2010: 133, 539, 540.
14. Chen J, Chen T. Chinese Medical Herbology and Pharmacology. City of Industry, CA: Art of Medicine Press 2004: 143.
15. Xie H, Preast V. Xie's Chinese Veterinary Herbology. Ames, Iowa: Wiley-Blackwell 2010: 133, 134.
16. Dharmananda, Subhuti, et al. Impetigo and Its Treatment: Inhibitors of Staphylococcus. *http://www.itmonline.org/arts/impetigo.htn*
17. Peng L, Kang S, Yin Z, Jia R et al. Antibacterial activity and mechanism of berberine against Streptococcus agalactiae. Int J Clin Exp Pathol 2015; 8(5): 5217-5223.
18. Lee G, Doh E, Chang J, Kil G. Antibacterial Activity of Caesalpinia sappan and Coptis chinensis Extracts against Bacillus cereus and Vibrio parahaemolyticus. Kor. Herbology 2010: 25 (3): 111-116.
19. Chang N. Exp Biol Med (Maywood) 1948; 69(1): 141-143.
20. Wang V. Chinese Medical Journal 1950; 68 (5/6): 169-172.

Clinical Studies

The Effect of Oral *Yunnan Baiyao* on Periprocedural Hemorrhage and Coagulation Parameters in Dogs Undergoing Nasal Biopsy: A Randomized, Controlled, Blinded Study

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ABSTRACT

The purpose of this randomized, controlled, blinded study was to evaluate the effect of orally administered *Yunnan Baiyao* on measures of blood loss and coagulation in dogs undergoing nasal biopsy. Nineteen client-owned dogs presenting for nasal biopsy were randomized to receive *Yunnan Baiyao* or lactose placebo. All dogs had baseline blood pressure, platelet count, prothrombin time and activated partial thromboplastin time performed at presentation and a buccal mucosal bleeding time performed before and after capsule administration. Some dogs also had thromboelastography and blood loss measurements (9/19), just thromboelastography (6/19), and only blood loss (4/19) measurements performed. Measurements of blood loss included total blood loss, blood loss as a percentage of body weight and time to stop bleeding. Results of the study demonstrated time to stop bleeding was significantly shorter ($p<0.05$) in the YB group (300 ± 12 sec) compared to the control group (367 ± 9 sec) and was not influenced by underlying disease process. Although there was decreased blood loss as a percentage of body weight in the *Yunnan Baiyao* treated group (14%) when compared to the control group (25%), the difference did not reach statistical significance. There was no effect of *Yunnan baiyao* on measures of coagulation including buccal mucosal bleeding time or thromboelastography. The results of this study support the use of *Yunnan baiyao* prior to nasal biopsy in dogs. Further studies are needed to determine the clinical use of *Yunnan baiyao* for other procedures and diseases associated with increased risk of hemorrhage.

Key words: activated partial thromboplastin time, blood loss, clotting, coagulation, dog, ginseng, hemostasis, hemorrhage, nasal, biopsy, thromboelastography, thromboplastin time, *Yunnan Baiyao*

ABBREVIATIONS

α angle	Alpha angle
ANCOVA	Analysis of covariance
aPTT	Activated partial thromboplastin time
BMBT	Buccal mucosal bleeding time
K	Clot formation time
MA	Maximum amplitude
PT	Prothrombin time
R	Reaction time
TEG	Thromboelastography
TCM	Traditional Chinese medicine
UT-VMC	University of Tennessee Veterinary Medical Center
YB	*Yunnan Baiyao*

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Yunnan Baiyao (YB) is a Chinese herbal medicine that has been used for centuries for its hemostatic effect.[1,2] It was first developed in the Yunnan province of China in 1902 and is now a nationally protected Chinese herbal medicine approved by the Chinese state food and drug administration.[3,4] The hemostatic efficacy and safety of *Yunnan Baiyao* (YB) has been demonstrated across multiple species.[4,5-13] Both topical and oral application of YB significantly shortened bleeding time in rats and time to blood clot formation in both rabbits and humans.[5-8] In humans, pre-operative oral YB administration significantly reduced intra-operative blood loss associated with various types of surgery.[4,9,10,14] The underlying mechanism of action of YB has not yet been fully elucidated but it is believed to exert its hemostatic effect via effects on both primary and secondary hemostasis.[15-19]

Despite the widespread anecdotal use of YB in veterinary medicine for dogs with bleeding tendencies, few studies have evaluated its efficacy in dogs. Dogs appear

to tolerate YB and there are no reported adverse effects after topical or oral administration.[3,13,14] Nasal biopsy is a common diagnostic procedure performed for the workup of nasal disease in dogs, and iatrogenic hemorrhage is an anticipated complication.[20-22] Life-threatening hemorrhage is a rare but potential complication of nasal biopsy, and in a large retrospective study of 119 dogs protracted hemorrhage occurred in 2 cases, one of which was fatal.[20,22,23] Mild bleeding occurs more commonly and is managed with digital pressure and packing of the nasal cavity with sponges until bleeding subsides.[21,24] Currently, there are no other methods to control bleeding and better strategies to prevent or reduce post-procedural hemorrhage are needed.

The primary objective of this study was to evaluate the effect of YB on clinical hemorrhage following nasal biopsy in dogs as measured by blood loss and time to stop bleeding. A secondary objective was to evaluate the effect of YB on measures of coagulation including buccal mucosal bleeding time (BMBT) and thromboelastography (TEG). It was hypothesized that oral administration of YB to dogs prior to nasal biopsy would significantly decrease post-procedural hemorrhage and improve coagulation parameters when compared to a placebo control.

MATERIALS AND METHODS

Dogs who were presented to the University of Tennessee Veterinary Medical Center (UT-VMC) internal medicine service for nasal biopsy between March 2015 and August 2016 were enrolled prospectively. The study was approved by the Institutional Animal Care and Use Committee of the University of Tennessee, and informed owner consent was obtained for all dogs before study enrollment. Dogs were excluded from enrollment if they had a history of any coagulation disorder or allergy to YB, hypertension, or if they were treated with any type of anticoagulant in the previous week. Presence or absence of epistaxis at presentation was recorded.

This study was a controlled, blinded and randomized clinical trial. Dogs were randomized via a random number generator to treatment (YB) or placebo control group. Owners, statisticians and primary investigators were blinded to which treatment the dog received. As one of two class-1 protected traditional Chinese medicines, the exact formula of YB remains classified. Due to demand for quality assurance, the product is now labeled to identify its major components per 0.5g serving (Table 1). The contents of the YB[a] capsules were emptied into larger capsules (maintaining 0.5g per capsule) so that they were identical to lactose placebo capsules made by the UT-VMC Pharmacy. Due to the absence of published dosages or pharmacokinetic studies on YB in dogs, dogs were dosed based on expert recommendation[b]. Each dog received 2 doses of drug, one dose at 6 pm the evening before and one dose at 6 am the morning of the procedure as follows: <11 lb.: 1 capsule; 12-20 lb.: 2 capsules; 21-35 lb.: 3 capsules; 36-50 lb.: 4 capsules; >50 lb.: 5 capsules. Any side effects were noted.

At the time of enrollment, an unsedated Doppler systolic blood pressure was obtained from all dogs on the left forelimb in right lateral recumbency by a single investigator (LA). Dogs were excluded from the study if systolic blood pressure was >160 mmHg. Blood was collected for platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT) and TEG. Platelet number was determined on whole blood in EDTA with an automated hematology analyzer[c], using the methods recommended by the manufacturer. PT and aPTT were measured on plasma within 30 minutes of sample collection with an automated coagulometric analyzer[d], using the methods recommended by the manufacturer. A BMBT was performed on the left maxillary lip with the dog in right lateral recumbency using a disposable, single-use, spring-loaded lancet[e]. Dogs were excluded from further analysis if laboratory work revealed platelet count <147,000, PT > 13 sec, PTT > 90.6 sec or BMBT > 4 minutes. When platelet values were not available due to clumping, dogs were included if a clinical pathology technologist estimated the value to be adequate. A second blood sample was collected for post-treatment TEG 2 to 3 hours after the second YB dose was administered prior to premedication for anesthesia. A post-treatment BMBT

Table 1: Ingredients of the Chinese herbal medicine *Yunnan Baiyao*[a] and their pertinent actions.

***Pin Yin* Name**	**English Name**	**Amount**	**Actions (if known)[25]**
San Qi	Notoginseng	40%	Activates blood circulation, stops bleeding
Li Zhi Hao	Diels plant	17%	
Shan Yao	Yam rhizome	13%	Tonifies *Qi*, nourishes Spleen, Stomach, Lung and Kidney *Yin*, treats wasting and thirsting syndrome
Chuan Shan Long	Makino root	12%	Dispels wind and dampness, invigorates blood, opens channels and collaterals, relieves cough, dissolves phlegm
Lao Guan Cao	Erodium/geranium	7%	Dispels wind and dampness, stops diarrhea
Ku Liang Jiang	Dioscorea parviflora	6%	
Bai Niu Dan	Sheep's ear	5%	

was performed at that time on the right maxillary lip with the dog in left lateral recumbency.

For TEG analysis, citrated whole blood samples were run within 60 minutes of collection using manufacturer recommended methods[f]. Two samples were collected from each patient, the first at time of enrollment and the second within 2 to 3 hours of administering the second medication dose. Briefly, whole blood was drawn from the subject into a 0.109 M sodium citrate vacutainer collection tube. Samples were driven a short distance (1.7 kilometers) to the University of Tennessee Medical Center and processed by their research staff within 60 minutes of collection. The citrated whole blood and all reagents were allowed to equilibrate to room temperature for 10 minutes. The blood was then gently inverted 3 times before adding 1 mL of the citrated whole blood to 40 µL of kaolin reagent. The sample was capped and gently inverted 5 times to mix. Disposable cups and pins were loaded into the TEG analyzer. Next, 20 µL of 0.2 M calcium chloride solution was added to the disposable cup along with 340 µL of the kaolin treated-citrated whole blood. The mixture was pipetted 3 times to ensure homogenous mixing. The test was initiated, and the tracings were monitored a short time to ensure the software generated no error messages. The same analyzer was utilized for all samples tested. All tests were conducted at 37˚C. The accompanying TEG analytical software was utilized. Assayed variables included alpha angle (rate of clot formation), K (clot formation time), R (reaction time) and MA (maximum amplitude).

A standardized anesthetic protocol was followed. Premedication with hydromorphone[g] (0.05-0.2 mg/kg IM or IV) and midazolam[h] (0.2-0.5 mg/kg IM or IV once) was followed by propofol[i] induction (4-6 mg/kg IV) and isoflurane maintenance at 0.5-4%. Fluids (Normosol-R) were maintained at a constant rate of 5 mL/kg/hr. Mean arterial pressure was maintained between 70 and 100 mmHg. No crystalloid fluid boluses or colloids were used due to possible effects on hemostasis.[26-28] Dopamine[j], dobutamine[k] and ephedrine[l] were used as needed to maintain mean arterial pressure within this target range. All anesthetic complications, including hypo- or hypertension were documented. Post-operative pain control was provided with hydromorphone[g] (0.05-0.2 mg/kg IV q 4-6 h) or tramadol[m] (2-5 mg/kg PO q 8-12 h) as needed. Post-procedural anti-anxiety was managed with acepromazine[n] (0.0025-0.02 mg/kg IV q 4-6 h) or trazadone[o] as needed.

Nasal biopsy was performed under the direct supervision of a board-certified specialist (SO, JS), by a board-certified internal medicine specialist (SO) or an internal medicine resident (LA). For patients that underwent rhinoscopy prior to biopsy, a target irrigation fluid volume of 2.5 L (1.25 L per side) 0.9% saline was used. For cases that did not have rhinoscopy performed 1.25 L of room temperature saline was flushed through each nasal passage using a red rubber catheter prior to nasal biopsy. Biopsies were collected from both nasal passages by means of blind biopsy technique using uterine cup biopsy forceps. The number of biopsies performed to achieve a goal of 4 good quality biopsy specimens per side was at the discretion of a single investigator (LA). The number of biopsies and their site was recorded and biopsy specimens were weighed. The total time of biopsy collection along with the time to stop bleeding were recorded for each side. Cessation of bleeding was determined by consensus by two investigators (SO, LA) when no additional blood was dripping from the nares and a stable clot had formed. Time to stop bleeding was recorded as the longest time of the two sides measured.

Following rhinoscopy or nasal saline flush, the oropharynx was suctioned and fresh 4x4 gauze squares were placed at the back of the oropharynx prior to biopsy. Gauze 4x4 squares were used to collect any blood from the nares during the biopsy procedure until cessation of bleeding occurred for each nare. Total blood loss was quantified by weighing the blood soaked gauze, including that from the oropharynx, and subtracting the weight of an individual gauze square, multiplied by the number of gauze squares used. Gauze weight was recorded on the same scale with 0.1-gram resolution for improved sensitivity. Blood loss as a percentage of the dog's weight was also calculated. A single investigator (LA) performed all blood loss measurements.

A final diagnosis was determined by histopathologic evaluation of biopsy samples, computerized tomography findings and/or cytological exam on an individual case basis. The final diagnosis was categorized into 1 of 3 categories; neoplasia, inflammatory disease or other, which included fungal disease or foreign body. Histopathology was performed by a board certified anatomic pathologist through the UT-VMC anatomic pathology service.

Statistics were performed using SAS software[p]. A rank transformation was applied to the coagulation measures BMBT and TEG in order to meet the required statistical assumptions. No transformation was required for blood loss measures. A two-way analysis of covariance (ANCOVA) was used to analyze blood loss measures including total blood loss, blood loss as a percentage of body weight and time to stop bleeding. The two between subject factors were treatment and disease. A repeated measures mixed effects split plot ANCOVA was used to analyze BMBT and TEG measures (α angle, K, MA, R). The between subjects factor was treatment and the within subjects factor was time. In all cases a compound symmetry covariance matrix was applied to the data to better model covariates that did not change between pre and post time measurements. A p-value < 0.05 was considered statistically significant. Backwards variable elimination was performed in order to eliminate statistically insignificant covariates from each analysis in order to more accurately assess the impact of significant explanatory variables. Backwards elimination of the covariates age, body weight, blood pressure, platelet count, PT and PTT were performed for coagulation measures. Backwards elimination of the covariates age,

body weight, epistaxis at presentation, blood pressure, biopsy number, weight of biopsies, weight of biopsies as a percentage of body weight were performed for blood loss measures. Normality was assessed via the Shapiro-Wilk test for normality. Equality of variances was accessed with the Levene Test for Equality of Variances. All statistical assumptions regarding analysis of variance were met.

RESULTS

Nineteen client-owned dogs presenting to the UT-VMC for nasal biopsy were prospectively enrolled between March 2015 and August 2016. All dogs had blood pressure, platelet count, PT and aPTT performed at presentation. No dogs were excluded based on these results. Ten dogs were randomized to receive YB and 9 dogs a placebo control. Multiple breeds of dogs were enrolled and included mixed-breeds (7), Border Collies (3), Dachshunds (2), Beagles (2), a Weimaraner (1), Norwegian Elkhound (1), Pharaoh Hound (1), Boston Terrier (1), and Maltese (1). Mean age of the YB group was 9.4 years (range 5-12) and mean age of the control group was 9.9 years (range 3-14). The YB group consisted of 4 castrated male dogs and 6 spayed females and the control group consisted of 4 castrated male dogs and 5 spayed females.

Diagnostic tests performed in control and YB treated dogs are shown in Table 2. All 19 dogs had baseline coagulation values and BMBT measurements performed. Nasal biopsies were performed in 13/19 dogs. In the other 6 dogs, biopsies were not pursued due to a lack of indication (i.e. foreign body, fungal disease), delay in biopsy due to CT machine malfunction, or following imaging, biopsy was declined by owners. Blood loss parameters associated with nasal biopsy was therefore limited to these 13 dogs. TEG was performed in 15/19 dogs. TEG was not yet available in the 4 dogs where only blood loss measurements were performed.

The results of blood loss parameters including total blood loss, blood loss as a percentage of body weight and time to stop bleeding are shown in Table 3. Total blood loss and blood loss as a percentage of body weight were not significantly different between YB and control groups. A noticeable biological trend was present for blood loss as a percentage of body weight with a relative hemorrhage risk reduction calculation of 56.4% for the YB treated group. Underlying disease category had no significant effect on total blood loss or blood loss as a percentage of body weight for either group. There was no significant effect of age, body weight, blood pressure, presence of epistaxis at presentation, biopsy number or weight of biopsies on total blood loss or blood loss as a percentage of body weight. There was a significant positive association between biopsy weight as a percentage of body weight and blood loss as a percentage of body weight (F16.48, dif=1,7, p= 0.004).

Time to stop bleeding was significantly shorter in the YB group (300 ± 12 sec) compared to the control group (367 ± 9 sec) (F19.46, dif=1,4, p= 0.01) and was not influenced by underlying disease process (neoplastic vs. inflammatory) (Figure 1). There was no significant effect of body weight, weight of biopsies or weight of

Table 2: Number of control and YB treated dogs that underwent each diagnostic test

	Baseline Coagulation Values[a]	BMBT[b]	TEG[c]	Nasal Biopsy	Total Loss	Loss % BW	Bleeding Time
Control Dogs	9	9	6	7	7	7	7
YB Treated Dogs	10	10	9	6	6	6	6

[a]Baseline values include: blood pressure, platelet count, PT, aPTT; [b] BMBT= Buccal mucosal bleeding time; measured prior to and after YB administration; [c]TEG (thromboelastography): alpha angle, K, R, MA; Total Loss = Total blood loss; Loss % BW = Blood loss as a percentage of body weight; Bleeding Time = Time to stop bleeding

Table 3: Total blood loss, blood loss as a percentage of body weight and time to stop bleeding in dogs receiving YB versus placebo control

Parameter	YB N=6	Control N=7	*p*-value
Bleeding Time (sec)	300 ± 12	367 ± 9	0.01*
Loss % BW (%)	14 ± 9	25 ± 23	0.69
Total Loss (grams)	38 ± 27	27 ± 15	0.86

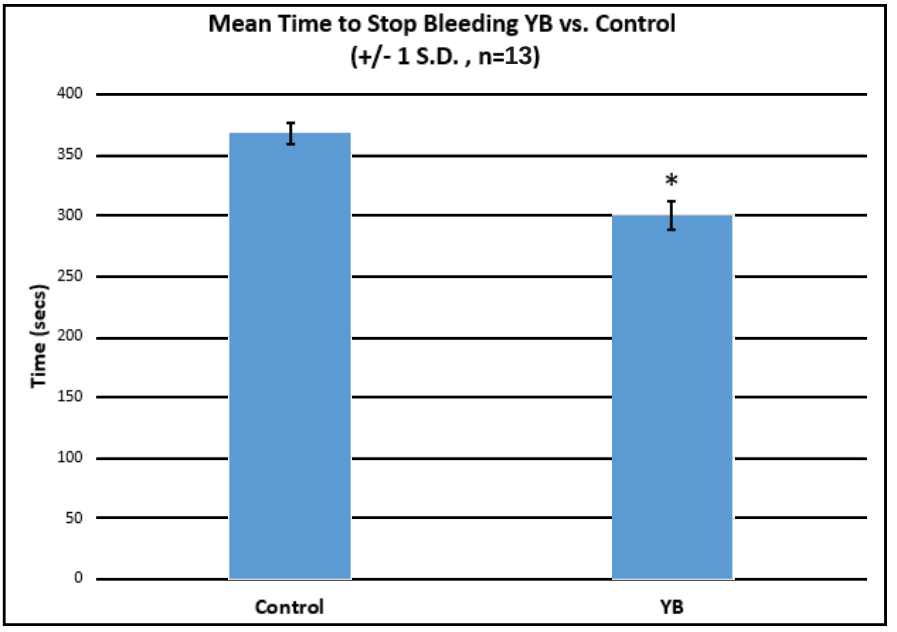
*p<0.05 = statistically significant; All values expressed as mean +/- standard deviation; Total Loss = Total blood loss; Loss % BW = Blood loss as a percentage of body weight; Bleeding Time = Time to stop bleeding; YB = *Yunnan Biayao*

Table 4: Comparison of BMBT and TEG parameters in dogs receiving YB versus placebo control

Parameter	YB N=10		Control N=9		*p*-value	
	Pre	Post	Pre	Post	Time	Treatment
BMBT (sec)	**N=10** 113 ± 41 (70-178)	**N=10** 96 ± 35 (43-154)	**N=9** 115 ± 21 (90-150)	**N=9** 93 ± 36 (30-135)	0.09	1.00
TEG – α angle	**N=9** 75 ± 6 (64-85)	**N=9** 75 ± 7 (63-85)	**N=6** 72 ± 7 (61-78)	**N=6** 73 ± 5 (67-77)	0.37	0.44
TEG – K	**N=9** 68 ± 25 (50-130)	**N=9** 69 ± 26 (50-130)	**N=6** 78 ± 46 (50-160)	**N=6** 81 ± 39 (50-145)	0.96	0.85
TEG – MA	**N=9** 69 ± 8 (56-81)	**N=9** 69 ± 7 (56-81)	**N=6** 56 ± 23 (11-73)	**N=6** 64 ± 7 (55-73)	0.35	0.26
TEG – R	**N=9** 104 ± 45 (45-175)	**N=9** 127 ± 64 (50-280)	**N=6** 103 ± 38 (65-165)	**N=6** 104 ± 45 (45-175)	0.49	0.13

$p<0.05$=statistically significant

All values expressed as mean +/- standard deviation followed by range in parentheses; *p*-value category "time" represents the difference between parameters at two time points within an individual treatment group (YB or control); *p*- value category "treatment" represents the difference between parameters between YB and control groups; BMBT = Buccal mucosal bleeding time; TEG = Thromboelastography; YB = *Yunnan Baiyao*; K = Clot formation time; MS = Maximum amplitude; R = Reaction time



*$p = 0.01$; $p< 0.05$ = statistically significant; YB = *Yunnan Baiyao*

Figure 1: Time to stop bleeding was significantly shorter ($p = 0.01$) in the YB treated group compared to control group and was not influenced by underlying disease process.

biopsies as a percentage of body weight on time to stop bleeding. Age (F80.58, dif=1,4, p= 0.0009) and presence of epistaxis at presentation (F337.54, dif= 1,4, p< 0.001) were significantly associated with a longer time to stop bleeding. Conversely, blood pressure (F288.01, dif=1,4, p< 0.001) and biopsy number (F155, dif=1,4, p= 0.0002) were significantly associated with a shorter time to stop bleeding. Treatment effect remained significant even when the effect of these covariates was considered.

Results of coagulation testing (BMBT and TEG) are shown in Table 4. BMBT was not significantly different between YB and control groups nor within individual treatment groups over time. There was no significant effect of age, weight, blood pressure, platelet count, PT, or PTT on BMBT.

There was no significant effect of treatment on TEG variables including rate of clot formation (α angle), time to achieve clot strength (K) or time to initial clot formation (R). TEG variables at different time points within a treatment group were also not significant for any of these variables. There was no significant effect of age, body weight, blood pressure, platelet count, PT, or PTT on clot strength (K). There was a significant positive association between rate of clot formation (α angle) with increasing body weight (F5.62, dif=1,7, p= 0.049) and platelet count (F10.56, dif=1,7, p= 0.02). All platelet counts, however, were within reference range or deemed adequate by clinical pathology technologist review. A significant positive association was present between time to initial clot formation (R) and PTT (F5.37, dif=1,12, p= 0.038). All PTT values were within reference limits. No clear solution for the reduced model evaluating the effect of covariates on clot strength (MA) was available, as statistical analysis could not be performed due to mathematical convergence errors and biased results.

No adverse effects were noted during or after treatment in either group. In one patient, the anesthetic protocol was abandoned as the patient required a fluid bolus due to refractory hypotension. This patient was included in TEG analysis but blood loss measurements were not performed. The remaining patients were maintained within target parameters with the described anesthetic protocol.

DISCUSSION

In this study, oral administration of YB to dogs prior to nasal biopsy resulted in significantly shorter bleeding times compared to control ($p<0.05$). This result was significant even when considering the effect of covariates. Total blood loss was not significantly different between YB and control groups, but there was a trend toward decreased blood loss as a percentage of body weight in the YB treated group (14%) when compared to the control group (25%). There was no effect of YB on measures of hemostasis, including BMBT or TEG.

The shortened bleeding times in the present study demonstrated by YB oral treatment suggest an enhanced hemostatic effect which has been well established in the literature.[5,6] YB has been shown to increase platelet reactivity and aggregation *in-vitro* and act as a procoagulant at the level of the prothrombinase complex.[15-19] The component of YB responsible for its hemostatic effect has not been definitively determined but its main ingredient, notoginseng, has been known for its hemostatic properties with saponins linked to its efficacy.[3,7,29,30] Recently, nanofibers have also been identified in YB and are proposed to play a role in platelet aggregation leading to clot formation and wound repair.[18,31]

Few studies have evaluated the clinical use of YB in veterinary medicine. Open cervix pyometra was successfully treated in 18 of 19 dogs with oral YB, however no control group was available for comparison in the study.[11] In an abstract of 2 dogs treated with oral YB, no significant differences between pre- and post-administration thromboelastography (TEG) values were found.[12] More recently oral administration of YB demonstrated some interesting trends between pre- and post-administration buccal mucosal bleeding times (BMBT), platelet numbers and some TEG values that suggest improved hemostasis with YB administration.[13]

To the authors' knowledge, the present study is the only study to investigate the effect of oral YB on measures of blood loss in dogs. Bleeding time was significantly shorter in YB treated dogs and numbers indicate the possibility of decreased blood loss as a percentage of body weight. Further research with increased number of cases is warranted to investigate this trend. In an unpublished study by one of the primary investigators (CE) a significant reduction in bleeding time was found after topical application of YB to experimentally induced wounds in 10 healthy beagle dogs when compared to control. There was no significant difference in total blood loss between groups, however blood loss as a percentage of body weight was not examined.

Covariate analysis of bleeding time revealed that both age and epistaxis were significantly associated with a longer time to stop bleeding. Patients with epistaxis at presentation did not have significantly more total blood loss or blood loss as a percentage of body weight. The role of increased age on bleeding tendency is unclear and this finding is of questionable significance. Age does not appear to be a common predictor of blood loss in human patients. Review of the literature revealed a single study in which age (>60 years) was reported to be an independent risk factor for intraoperative blood transfusion in patients undergoing free tissue transfer.[32]

There was a significant positive association between biopsy weight as a percentage of body weight and blood loss as a percentage of body weight ($p<0.05$). This association suggests quantity of tissue removed during the biopsy process has some effect on blood loss, particularly when evaluating blood loss as a percent of body weight. This study result has questionable significance as removal of a greater quantity of tissue would naturally result in a greater quantity of blood removal or loss.

The present study also evaluated the effect of YB on measures of coagulation and no significant effect of treatment was found. Laboratory assessment of hemostasis in canine patients includes measures of both primary and secondary hemostasis. Tests of primary hemostasis include platelet enumeration to rule out thrombocytopenia and BMBT for evaluation of thrombocytopathia.[24,33] Although BMBT testing is most often performed to evaluate for prolonged time to clot formation, a shortened BMBT could potentially be expected with platelet activation.[13] In a previously published abstract, the pre-operative oral administration of YB to 6 healthy ponies undergoing halothane anesthesia significantly shortened the template bleeding time compared to pre-treatment values.[34] Recently, 8 healthy beagle dogs demonstrated decreasing trends in BMBT at certain time points following administration of YB.[13] In the present study, no significant effect of YB on BMBT was evident. It is possible that the effects of YB are only present when active bleeding is occurring and that these results may have been different if BMBT was performed during nasal biopsy. Although BMBT is a useful adjunct to *in-vitro* measures of coagulation, its sensitivity and specificity have been questioned due to high variability in results secondary to iatrogenic factors.[35,36] Other platelet function tests including flow cytometry and platelet aggregometry may be more sensitive means of assessing platelet function than BMBT and would have been useful in the present study.[36,37] Unfortunately these tests were not available to the authors at time of enrollment.

Tests of secondary hemostasis evaluate the extrinsic, intrinsic and common pathways and include PT, aPTT and TEG.[24,33] TEG is a more global test of hemostasis that provides information on both the quality and kinetics of clot formation from initiation through fibrinolysis.[38-40] The four most important measured variables used to interpret TEG tracings are reaction time (R), clot formation time (K), α angle and maximal amplitude (MA) the latter of which is a measurement of clot strength.[38,40] A hypercoagulable state would be indicated by a decrease in R and K values and an increase in α angle and MA values.[38] In the present study, no significant differences were found for TEG variables α angle, K or R between YB treated dogs and control. Unfortunately, MA was unable to be assessed, as there was no appropriate statistical model. Previously, differences in TEG parameters after YB administration were demonstrated in 2 dogs (statistically non-significant).[12] This study was published only as an abstract and a description of methodology was not included limiting comparison. In a recently published study of oral administration of YB in healthy Beagles, no statistically significant differences in TEG variables were present compared to control dogs. It is worth noting, however, that more profound decreases in R and increases in α angle in the YB group were seen, although these differences did not reach statistical significance in this small study.[13]

There was a significant positive association between time to initial clot formation (R) and PTT. Since PTT provides information on the extrinsic and common pathways, it is influenced by coagulation factors that would also affect many of the TEG variables. The association, however, between PTT was only present with R in the current study. The distinction between the extrinsic and intrinsic arms of the coagulation cascade is more of an *in-vitro* rather than *in-vivo* phenomenon and therefore the relationship between these variables might not be so black and white.[41] The significance of this finding is unknown.

There are several limitations to the present study. The study was likely underpowered to detect small differences between groups including the impact of underlying disease on blood loss measures and coagulation making the probability of a type II error significant. Variability in patient population was not controlled including no standardization of prior therapy. Evaluation prior to study inclusion was focused on nasal disease and basic systemic evaluation. It is possible that dogs had concurrent disease affecting hemostatic tendencies. The length of the nasal cavity was not recorded, and it is possible that time to stop bleeding could be influenced by nose length. For example, it is possible that dolicocephalic dogs have more intranasal blood clot formation and, therefore, less external bleeding and reduced time to stop bleeding.

The disease categories utilized in the present study were broad and non-specific. Dogs categorized as inflammatory and neoplastic disease had a variety of underlying histopathologic diagnoses and it is possible that dogs with the same disease were presented at different stages. There was also variability in the diagnostic tests performed in each patient with some dogs having rhinoscopy prior to biopsy while others only had biopsy performed. Equivalent volume saline irrigation was performed in all dogs to minimize the vasoconstrictive and hemostatic effects of saline during rhinoscopy. Additional trauma, however, caused by the rhinoscopy procedure itself might have led to increased hemorrhage which was not accounted for.

Another limitation is the degree of subjectivity with regards to blood loss measures. The time to stop bleeding was subjectively assessed by two of the primary investigators by visual consensus of clot formation. Blood loss was measured by weighing blood soaked gauze. The gauze from the oropharynx was also variably soaked with nasal secretions and fluid, which could have influenced weight. Tests such as BMBT, PT, aPTT and TEG are subject to significant technical difficulties and there is a wide range of normal results.[35,36,38,41-45] Although the same individuals performed these tests, variability in operator technique and time to run samples could have influenced results.

The dose of YB used in this study was based on expert opinion as currently no pharmacokinetic studies have been performed in dogs. It is possible that the oral dose,

frequency or duration of administration were not adequate. Pharmacokinetic studies are needed to determine optimal oral dose and frequency in dogs. Additionally, because the exact mechanism of action of YB has not be elucidated in the dog, it is possible that the effects of YB are only present when active bleeding is occurring, and would affect results of BMBT and TEG that were performed prior to biopsy.

In conclusion, pre-procedural oral administration of YB to dogs undergoing nasal biopsy resulted in significantly shorter time to stop bleeding following biopsy. There was no effect of YB on measures of coagulation. The objectives of the study were satisfied and no adverse effects of oral YB were noted. Although oral administration of YB prior to nasal biopsy did not appear to improve coagulation parameters, the hypothesis that it would significantly decrease post-procedural hemorrhage was supported by the shortened bleeding time and trend toward decreased blood loss as a percentage of body weight. While results of this study support the use of YB prior to nasal biopsy in dogs, additional studies are needed to determine the clinical use of YB for other procedures or medical conditions associated with hemorrhage in dogs along with pharmacokinetic studies to determine optimal oral dosing of YB in dogs.

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FOOTNOTES

a. Yunnan Baiyao Group Co., Ltd., Chenggong New District, Kunming, Yunnan Province, China
b. Dr. Huisheng Xie, DVM, PhD, founder and president of Chi Institute of Traditional Chinese Veterinary Medicine, Reddick, Florida, USA
c. Bayer ADVIA™120, Siemens Diagnostics, Tarrytown, NY, USA
d. ACL 9000, Beckman Coulter, Inc., Bree, CA, USA
e. ITC Surgicutt Adult, 1.0 mm depth, 5.0 mm length, Accriva Diagnostics, Edison, NJ, USA
f. TEG® 5000 Hemostasis Analyzer System, Haemonetics Corporation, Braintree, MA, USA
g. Hydromorphone HCl Injection, West-Ward, Eatontown, NJ, USA
h. Midazolam Injection, West-Ward, Eatontown, NJ, USA
i. Propoflo 28, Zoetis Inc., Kalamazoo, MI, USA
j. Dopamine HCl Injection, Hospira Inc., Lake Forest, IL, USA
k. Dobutamine Injection, Hospira Inc., Lake Forest, IL, USA
l. Ephedrine Sulfate Injection, Akorn Inc., Lake Forest, IL, USA
m. Tramadol Hydrochloride Tablets, Sun Pharmaceutical Industries, Inc., Cranbury, NJ, USA
n Acepromazine Maleate Injection, West-Ward, Eatontown, NJ, USA
o Trazodone Hydrochloride Tablets, Qualitest Pharmaceuticals, Huntsville, AL, USA
p. SAS version 9.4 release TS1M3, SAS Institute, Cary, NC, USA

REFERENCES

1. Wynn SG, Marsden S. Manual of Natural Veterinary Medicine. St. Louis, MO: Mosby Inc 2003: 203.
2. Wynn SG, Foug BJ. Veterinary Herbal Medicine. Philadelphia, PA: Mosby Inc 2007: 99-119, 294-384.
3. Liu XX, Wang L, Chen XQ et al. Simultaneous quantification of both triterpenoid and steroidal saponins in various *Yunnan Baiyao* preparations using HPLC-UV and HPLC-MS. J Sep Sci 2008; 31:3834-3846.
4. Tang ZL, Wang X, Yi B et al. Effects of preoperative administration of *Yunnan Baiyao* capsules on intraoperative blood loss in bimaxillary orthognathic surgery: a prospective randomized, double-blind, placebo-controlled study. Int J Oral Maxillofac Surg 2009; 38:261-266.
5. Ogle CW, Dai S, Ma J. The hemostatic effects of the Chinese herbal drug *Yunnan Bai Yao*: a pilot study. Am J Chinese Med 1976; 4:147-152.
6. Ogle CW, Dai S, Cho CH. The hemostatic effects of orally administered *Yunnan Bai Yao* in rats and rabbits. Comp Med East West 1977; 5:155-160.
7. White CM, Fan C, Chow M. An evaluation of the hemostatic effect of externally applied notoginseng and notoginseng total saponins. J Clin Pharmacol 2000; 40:1150-1153.
8. Chengde F. A comparison of the hemostatic effects of notoginseng and *Yun Nan Bai Yao* to placebo control. J Herbal Pharmacother 2005; 5:1-5.
9. Pan SF, Sun Y, Li F et al. Effects of *Yunnan Baiyao* on peri-operative bleeding of patients undergoing cervical open-door laminoplasty: a multi-center randomized double-blind placebo-control trial. Zhonghua Yi Xue Za Zhi 2006; 86:1888-1890.
10. Li NC, Pan BN, Wang HJ et al. The effect of *Yunnan Baiyao* on reduction of intra-operative bleeding of the patients undergoing transurethral resection of prostate. Zhongua Yi Xue Za Zhi 2007; 87:1017-1020.
11. Salgado B, Paramo R, Sumano H. Successful treatment of canine open cervix pyometra with *Yun-Nan-Pai-Yao*, a Chinese herbal preparation. Vet Res Commun 2007; 31:405-412.
12. Patterson W. Measurement of the effects of *Yunnan Pai Yao*, a Chinese herbal preparation, on hemostasis in rabbits and dogs. FASEB Journal 1998; 12(4):

A191.
13. Egger C, Gibbs D, Wheeler J et al. The effect of *Yunnan Baiyao* on platelet activation, buccal mucosal bleeding time, prothrombin time, activated partial thromboplastin time, and thromboeslastography in healthy dogs: A randomized, controlled, blinded study. Amer J of Trad Chinese Vet Med 2016; 11(2): 27-36.
14. Shmalberg J, Hill RC, Scott JC. Nutrient and metal analysis of Chinese herbal products marketed for veterinary use. J Anim Physiol Anim Nutr 2013; 97:305-314.
15. Chew EC. Effects of *Yunnan Bai Yao* on blood platelets: an ultrastructural study. Comp Med East West 1977; 5:169-175.
16. Chew EC. *Yunnan Bai Yao*-induced platelet release in suspensions of washed platelets. Comp Med East West 1977; 5:271-274.
17. Jianfeng Y, Weimin Y, Zhuohui G et al. Effects of *Yunnan Baiyao* on the expression of platelet membrane glycoproteins. The Chinese Pharmaceutical Association 2004; 21:100-103.
18. Lenaghan SC, Xia L, Zhang M. Identification of nanofibers in the Chinese herbal medicine: *Yunnan Baiyao*. J Biomed Nanotechnol 2009; 5:472-476.
19. Loyd K, Cohn L, Smith SA. In vitro effects of *Yunnan Baiyao* on coagulation. Abstract: Proceedings of the American College of Veterinary Internal Medicine Annual Meeting, 2014.
20. Brown, NL. Sneezing and Nasal Discharge. Textbook of Veterinary Internal Medicine 7th Ed, Ettinger SJ, Feldman EC (eds). St Louis, MO: Saunders 2009: 269-274.
21. Hawkins, EC. Diagnostic tests for the nasal cavity and paranasal sinuses. Small Animal Internal Medicine 5th Ed, Nelson RW, Couto GC (eds). St Louis, MO: Elsevier 2014: 224-233.
22. Forbes Lent SE, Hawkins EC. Evaluation of rhinoscopy and rhinoscopy-assisted mucosal biopsy in diagnosis of nasal disease in dogs: 119 cases (1985-1989). J Am Vet Med Assoc 1992; 9:1425-1429.
23. Rawlings CA. Diagnostic rigid endoscopy: otoscopy, rhinoscopy and cystoscopy 2009; 39(5): 849-868.
24. Geiger T, Northrup N. Clinical approach to patients with epistaxis. Comp Cont Educ Pract 2004; 26(1): 30-44.
25. Chen JK, Chen TT. Chinese medical herbology and pharmacology 2nd Ed. City of Industry, CA: Art of Medicine Press 2012.
26. Chohan AS, Greene SA, Grubb TL et al. Effects of 6% hetastarch (600/0.75) or lactated Ringer's solution on hemostatic variables and clinical bleeding in healthy dogs anesthetized for orthopedic surgery. Vet Anaesth Analg 2011; 38:94-105.
27. Morris BR, Delaforcade A, Lee J et al. Effects of in vitro hemodilution with crystalloids, colloids and plasma on canine whole blood coagulation as determined by kaolin-activated thromboelastography. J Vet Emerg Crit Care 2016; 26(1):58-63.
28. Wurlod VA, Howard J, Francey T et al. Comparison of the in vitro effects of saline, hypertonic hydroxylethyl starch, hypertonic saline, and two forms of hydroxyethyl starch on whole blood coagulation and platelet function in dogs. J Vet Emerg Crit Care 2015; 25(4):474-487.
29. Polesuk J, Amodeo JM, Ma TS. Microchemical Investigation of Medicinal Plants. X. Mikrochimica Acta 1973; 4:507-517.
30. Fan C, Song J, White CM. A comparison of the hemostatic effects of notoginseng and *Yun Nan Bai Yao* to placebo control. J Herb Pharmacother 2005; 5(2):1-5.
31. Santos-Martinez MJ, Inkielewicz-Stepniak I, Medina C et al. The use of quartz crystal microbalance with dissipation (QCM-D) for studying nanoparticle-induced platelet aggregation. Int J Nanomedicine 2012; 7:243-55.
32. Kolbenschlag J, Schneider J, Harati K et al. Predictors of intraoperative blood transfusion in free tissue transfer. J Reconstr Microsurg 2016; Epub ahead of print.
33. Callan, MB. Petechiae and ecchymoses. Textbook of Veterinary Internal Medicine 7th Ed, Ettinger SJ, Feldman EC (eds). St Louis, MO: Saunders 2009: 269-274.
34. Graham L, Farnsworth K, Cary J. The effect of *Yunnan baiyao* on the template bleeding time and activated clotting time in healthy halothane anesthetized ponies. International Veterinary Emergency and Critical Care Symposium, San Antonio, TX, 2002. J Vet Emerg Crit Care 2002; 12(4):279
35. Marks SL. The Buccal mucosal bleeding time. J Am Anim Hosp Assoc. 2000; 36:289-290.
36. Bromel C. Buccal mucosal bleeding time. Textbook of Veterinary Internal Medicine 7th Ed, Ettinger SJ, Feldman EC (eds). St Louis, MO: Saunders 2009: 354-365.
37. Christopherson PW, Spangler EA, Boudreaux MK. Evaluation and clinical application of platelet function testing in small animal practice. Vet Clin North Am Small Anim Pract 2012; 42(1):173-188.
38. Donahue SM, Otto CM. Thromboelastography: A tool for measuring hypercoagulability, hypocoagulability, and fibrinolysis. J Vet Emerg Crit Care 2005; 15(1):9-16.
39. Thawley VJ, Sanchez MD, Drobatz KJ et al. Retrospective comparison of thromboelastography results to postmortem evidence of thrombosis in critically ill dogs: 39 cases (2005-2010). J Vet Emerg Crit Care 2016; 26(3):428-436.
40. Chandler WL. The thromboelastography and the

thromboelastograph technique. Semin Thromb Hemost 1995; 21(4):1-6.
41. Green RA, Thomas JS. Hemostatic disorders: coagulopathies and thrombosis, Textbook of Veterinary Internal Medicine, 4th Ed, Ettinger SJ, Feldman EC (eds). Philadelphia, PA: Saunders 1995: 1946–1963.
42. Geiger T. Bleeding disorders: epistaxis and hemoptysis. Textbook of Veterinary Internal Medicine 7th Ed, Ettinger SJ, Feldman EC (eds). St Louis, MO: Saunders 2009: 354-365.
43. McMichael M, Goggs R, Smith S et al. Systematic evaluation of evidence on veterinary viscoelastic testing part 1: system comparability. J Vet Emerg Crit Care 2014; 24(1):23-29.
44. Flatland B, Koenigshof AM, Rozanski EA, et al. Systematic evaluation of evidence on veterinary viscoelastic testing part 1: Sample acquisition and handling. J Vet Emerg Crit Care 2014; 24(1):30-36.
45. Delaforcade A, Goggs R, Wiinberg B, et al. Systematic evaluation of evidence on veterinary viscoelastic testing part 3: Assay activation and test protocol. J Vet Emerg Crit Care 2014; 24(1):37-46.

A Randomized and Controlled Study of the Efficacy of *Yin Qiao San* Combined with Antibiotics Compared to Antibiotics Alone for the Treatment of Feline Upper Respiratory Disease

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ABSTRACT

A randomized controlled study was designed to investigate the efficacy of a combined treatment of the Chinese herbal medicine *Yin Qiao San* and antibiotic medicine (Group B; N = 32) for treating naturally occurring upper respiratory infections in shelter cats, where conventional antibiotic medicine alone was used as the control treatment (Group A; N = 19). A numerical clinical sign scoring system was devised with points given for conjunctivitis, sneezing, nasal discharge, lower respiratory signs, systemic signs and oral cavity involvement. Clinical sign scores for each cat were obtained before treatment and on days 2, 4, 6, 8 and 10 after initiation of treatment. The mean clinical scores of each group on days 2, 4, 6, 8 and 10 were compared to pre-treatment scores using the Wilcoxon signed rank test to determine the significance of clinical sign score reduction. There was no significant reduction in mean clinical sign scores for Group A on Days 2, 4 and 10, but a significant reduction occurred on Days 6 and 8 (p=0.002 and 0.0006, respectively). On the other hand, by Day 4 in Group B there was a significant improvement (p<0.001), and remained significant throughout Days 6, 8 and 10. No adverse effects occurred. The combination of *Yin Qiao* and Clavamox or doxycycline resulted in faster and more significant clinical improvement of cats with URI than antibiotics alone and was cost effective and had no adverse side effects.

Key words: Feline upper respiratory infection, herpes virus, calicivirus, *Yin Qiao San*, *Yin Chiao San*, Chinese herbal medicine, traditional Chinese veterinary medicine, cats

ABBREVIATIONS

FCV	Feline calicivirus
FHV-1	Feline herpes virus 1
IL-1 β	Interleukin 1 beta
IL-6	Interleukin 6
M±SD	Mean plus or minus the standard deviation
TCVM	Traditional Chinese veterinary medicine
TFF3 mRNA	Trefoil factor 3 messenger ribonucleic acid
TGF-β	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
URI	Upper respiratory infection(s)

Feline upper respiratory infections (URI) are extremely common in cats in animal shelters due to poor ventilation, stress-induced immunosuppression and overcrowded conditions.[1-3] Conjunctivitis, rhinitis and gingivostomatitis are obvious in some cats while others are asymptomatic carriers. Although caused by a variety of organisms, feline herpes virus 1 (FHV-1) and feline calicivirus (FCV) are the most common infectious agents.[1-3] *Mycoplasma felis, Bordetella bronchiseptica,* and *Chlamydophila felis* infections also occur.[4-5] The presence of these organisms can be confirmed by reverse transcription polymerase chain reaction (RT-PCR) assays.[5] Other secondary bacterial infections can further complicate the disease process. Conventional treatment often includes amoxicillin-clavulanic acid, cefovecin, doxycycline and fluroquinolones.[4,6] Antiviral treatments with mefloquine and lithium chloride have been recently evaluated *in-vitro* for FCV infections, but *in-vivo* studies are lacking.[7,8] Vaccination programs may reduce the severity or control the spread of infections, but many strains are vaccine resistant.[3] The result is that many cats are euthanized in order to control outbreaks or are unadoptable because of a severe URI. Although conventional treatments exist, the recovery is slow and other treatment options are needed to hasten recovery.

From a traditional Chinese veterinary medicine (TCVM) perspective upper respiratory infections may be

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associated with Wind-Heat, Wind-Cold and Damp Heat.[9] The External pathogens Wind and Cold invade the body through the nose causing sneezing and a serous nasal discharge. If *Wei Qi* and Lung *Qi* are weak, Wind-Cold transforms into Wind-Heat causing upper respiratory disease with fever and a thick, yellow nasal discharge. The pulses are strong and rapid reflecting Excess Heat and the tongue is red and dry. Feline URI is often associated with a Wind-Heat pattern.[9]

A variety of Chinese herbal medicines have been prescribed for the treatment of human URI.[10] After a 2014 systemic review and meta-analysis of 7 acceptable randomized controlled trials, it was concluded that there was evidence that Chinese herbal medicines had potential positive effects in the treatment of human URI.[10]

Yin Qiao San, a Chinese herbal medicine suggested to treat Wind-Heat URI, was first recorded in *Wen Bing Tiao Bian* (Detailed Analysis of Epidemic Febrile Disease) written by Wu Tang in 1798.[11] The formula is named for its 2 King herbs *Jin Yin Hua* (Lonicera) and *Lian Qiao* (Forsythia) (Table 1). The primary actions are to relieve exogenous pathogenic factors, clear Heat and eliminate toxins (Table 1). The authors (Hirsch and Xie) have successfully used *Yin Qiao San* to prevent and treat URI in horses, dogs and cats.[9,11] *Yin Qiao San* has also been successfully used to treat pneumonia and pleuropnemonia of cattle in China.[11] In a case report, acupuncture and a modified *Yin Qiao San*[a] (with *Ban Lan Gen* [Isatis] and *Lu Gen* [Phragmites] added and *Dan Dou Chi* [Soya] deleted) were used to successfully treat upper respiratory disease in a geriatric Quarter horse.[12] One author (Hirsch) has also used *Yin Qiao San* clinically along with conventional antibiotic therapy to treat feline URI in cats in animal shelters.[9] Although experiential evidence and case reports have been published, no randomized controlled clinical studies are currently published in English on the effects of *Yin Qiao San* for the treatment of URI in cats or other animals.[8,10]

The objective of this randomized controlled clinical study in shelter cats with naturally occurring URI was to demonstrate the effectiveness of *Yin Qiao San* when combining its use with conventional antibiotics. The hypothesis was that a combination of *Yin Qiao San*[b] and Clavamox[c] or doxycycline would result in faster and more significant clinical improvement of cats with URI than either antibiotic alone and have no adverse side effects.

MATERIALS AND METHODS

Cats for the study were recruited from 3 different animal shelters in the United States: Cape May Animal Shelter in Cape May County, New Jersey, the Ulster County Society for the Prevention of Cruelty to Animals, in Kingston, New York and the Atlantic County Animal Shelter, Pleasantville, New Jersey. All animals were handled and housed according to the guidelines for standards of care in animal shelters and participation in the study was on a volunteer basis.[13]

A clinical sign scoring system was devised based on the presence or absence and type of conjunctivitis, sneezing, nasal discharge, lower respiratory signs, systemic signs and oral cavity involvement as outlined in Table 2 and Figure 1. The scoring system was a modification of an earlier published scoring system for URI in cats.[4] The scores were recorded and treatments were administered by 2-4 technicians from each of the 3 participating agencies. All technicians underwent training to enhance the consistency of scoring. Technicians scoring were aware of whether the cat was in Group A or B as they also administered the treatments.

Inclusion criteria were cats showing 1 or more of the signs of URI; that is, a clinical sign score of 1 or above (Table 2). Cats were excluded from the study if they had no evidence of URI or were deemed unable to be safely handled to examine the oral cavity or administer medication. As a cat with URI was identified they were randomly assigned to one of the groups using a computerized random number generator[d]. Cats for which an even number was generated were placed in a control group (Group A) receiving antibiotics alone and cats with odd numbers were placed in a treatment group (Group B) receiving antibiotics and *Yin Qiao San*[b] combined. Antibiotics included amoxicillin-clavulanate (Clavamox[c]) liquid at a dose of 13.75 mg/kg body weight twice daily or doxycycline (compounded) at approximately 4.5 mg/kg

Table 1: Ingredients of the Chinese herbal medicine *Yin Qiao* and their actions[11]

***Pin Yin* Name**	**English Name**	**Amount %**	**Actions**
Jin Yin Hua	Lonicera	17.9	Clears Heat and Toxins
Lian Qiao	Forsythia	17.9	Clears Heat and Toxins, reduces swelling
Niu Bang Zi	Articum	10.7	Disperses Wind-Heat in Lung Channel
Jie Geng	Platycodon	10.7	Opens Lung *Qi*, relieves cough, dissolves Phlegm
Bo He	Mentha	10.7	Disperses Wind and Heat
Dan Dou Chi	Soja	8.95	Relieves the Exterior
Gan Cao	Glycyrrhiza	8.95	Harmonizes the formula
Jing Jie	Schizonepeta	7.1	Opens the surface to release Exterior pathogens
Dan Zhu Ye	Lophatherum	7.1	Clears Heat, relieves restlessness, promotes diuresis

body weight once daily and was continued throughout the study. *Yin Qiao San*[b] tablets were administered twice daily at a dose of ½ tablet for cats under 5 pounds and 1 tablet for cats 5 pounds and over (approximately 0.13-0.25 g) twice daily and also continued throughout the study.

The daily clinical sign score for each cat was the sum

Table 2: Clinical scoring system for the evaluation of cats with upper respiratory infections

Anatomy	Clinical Signs		Points Assigned
Eyes	Conjunctivitis*	Serous conjunctivitis	1
		Mucopurulent conjunctivitis	2
	Keratitis		4
Nose	Sneezing		1
	Nasal discharge*	Mucopurulent only	2
		Mucopurulent with blood	3
Lungs	Lower respiratory*	Coughing	1
		Crackles on auscultation	2
		Dyspnea	3
Oral cavity	Salivation		1
	Oral ulcers*	1 ulcer <4mm	1
		Multiple ulcers <4mm	2
		Multiple ulcers >4mm	3
		Bleeding ulcers	4

If signs are not present a score of 0 is given; *select only one clinical sign (highest score)

STUDY DATA SHEET

Name of Subject_____ Number_____ Shelter Name_____

Group 1- Clavamox Only Group 2- Clavamox and Yin Qiao

Under 5 Pounds Over 5 Pounds

SIGN		Initial Date	2 Days Post	4 Days Post	6 Days Post	8 Days Post	10 Days Post
Conjunctivitis	Serous (1)						
	Mucopurulent (2)						
Keratitis	(4)						
Sneezing & Nasal Discharge	Sneezing (1)						
	Mucopurulent (2)						
	Mucopurulent with blood (3)						
Lower Respiratory Signs	Coughing (1)						
	Crackles on auscultation (2)						
	Dyspnea (3)						
Systemic Signs	Anorexia (1)						
	Dehydration (1)						
	Depression (1)						
Oral Cavity	Salivating (1)						
	1 ulcer <4mm (1)						
	>1 ulcer <4mm (2)						
	>1 ulcer >4mm (3)						
	Bleeding ulcers (4)						
Total							

Figure 1: Data sheet completed on each cat before treatment and on days 2, 4, 6, 8 and 10 after initiating treatment

of all the scores in each category on the scoring sheet for that day (Table 2, Figure 1). Clinical sign scores for each cat were obtained before treatment began and on days 2, 4, 6, 8 and 10 after the initiation of treatment. A mean score was calculated[e] for each evaluation day on all the cats in Group A and also all the cats in Group B. Due to the outcome data not being normally distributed (Shapiro-Wilk normality test; $p < 0.05$), Wilcoxon tests (nonparametric version of t test) were applied for mean score comparisons. The Group A and B pretreatment mean clinical scores were statistically compared using the Wilcoxon Rank Sum test[e] (nonparametric version of two-sample t test) to determine if there was a significant difference between the 2 groups prior to treatment. The mean clinical scores of Group A on days 2, 4, 6, 8 and 10 were compared to the pre-treatment (day 0) score for the group using the Wilcoxon Signed Rank test[e] (nonparametric version of paired t test) to determine significance of improvement within the group. The same was done for Group B. A *p* value less than (<) 0.05 was considered statistically significant.

RESULTS

Fifty cats met the inclusion criteria and became part of the study. There were fewer even numbers than odd numbers so there were 19 in the Group A (antibiotics alone) and 31 in Group B (antibiotics and *Yin Qiao San*). In Group A, 14 cats weighed less than or equal to (< or =) 5 pounds and 5 cats that weighed over 5 pounds. In Group B 18 cats weighed < or = 5 pounds and 13 cats weighed over 5 pounds. Being a multicenter study, 17 cats were located at Cape May Animal Shelter, 11 cats at the Ulster County Society for the Prevention of Cruelty to Animals and 22 cats at the Atlantic County Animal Shelter (Table 3). Gender, breed, neutered status and other physical traits (such as color) were not used as characteristic parameters. Also, duration in the shelter environment was not considered. In Group A 17/19 (89%) cats were administered Clavamox and 2/19 (10.5%) doxycycline. In Group B 21/31 (68%) cats were administered Clavamox and 10/31 (32%) doxycycline.

The individual and group mean clinical sign scores are listed in Tables 4 and 5 from Day 0 (prior to treatment)

Table 3: Number of cats in each treatment group enrolled at each shelter

	Cape May Animal Shelter	Ulster County Society for the Prevention of Cruelty to Animals	Atlantic County Animal Shelter	
Group A	9	4	6	**19**
Group B	8	7	16	**31**
	17	**11**	**22**	**50**

Table 4: Individual and group clinical scores at different time points in cats receiving antibiotics only (Group A)

Cat Number	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Shelter*
1	1	1	1	1	1	0	CM
2	2	1	1	1	0	0	CM
3	1	2	1	0	0	0	CM
4	2	3	0	0	1	4	UC
5	2	2	2	0	1	4	UC
6	2	1	1	0	0	0	UC
7	3	3	1	1	1	0	CM
8	7	8	7	6	1	1	CM
9	1	2	3	1	0	0	UC
10	3	1	1	1	0	1	CM
11	5	3	5	5	2	3	CM
12	5	5	5	4	4	7	AC
13	4	5	3	1	1	6	AC
14	2	3	4	2	2	2	AC
15	1	1	1	1	1	0	AC
16	1	1	1	1	0	0	AC
17	1	1	1	1	1	0	AC
18	2	1	2	1	1	0	CM
19	2	1	3	1	1	0	CM
M±SD	2.47±1.68	2.37±1.89	2.26±1.85	1.47±1.68	0.95±0.97	1.47±2.25	

M±SD = mean plus or minus the standard deviation; *CM = Cape May Animal Shelter in Cape May County, NJ; UC= Ulster County SPCA, in Kingston, NY; AC= Atlantic County Animal Shelter, in Pleasantville, NJ

and on Days 2, 4, 6, 8 and 10 after initiation of treatment. The clinical sign scores on initial inclusion prior to treatment ranged from 1-7 (M±SD = 2.47±1.68) for Group A, and 1-6 (M±SD = 2.77±1.31) for Group B. Although the mean score prior to treatment for Group A appeared to be less than Group B, when compared statistically, there was no significant difference (p= 0.1598). Side-by-side comparisons of the mean clinical sign scores between Group A and Group B at different time points are shown in Figure 2.

The results of the comparisons of mean clinical sign scores of Group A (antibiotics alone) on Day 0 and subsequent examination days are outlined in Table 6. There was no significant reduction in clinical sign scores for Group A on Days 2 and 4 (p=0.644 and 0.503, respectively), but significant reduction occurred on Days 6 and 8 (p=0.002 and 0.0006, respectively). However by day 10 the mean clinical sign score had increased again and was not significantly different than prior to treatment (p=0.082).

The results of the comparisons of mean clinical sign scores of Group B (antibiotics and *Yin Qiao San*) on Day 0 and subsequent examination days are outlined in Table 7. Similarly, on Day 2 the improvement was not significant (p = 0.609). But by Day 4 there was a significant improvement (p = 0.0008), and remained significant on Days 6, 8 and 10 (p = $7.9\times10^{-6,}$ 9.1×10^{-6}, and 0.0002, respectively). No adverse side effects were noted in cats receiving *Yin Qiao San* combined with Clavamox or doxycycline.

In both groups the mean clinical sign score on Day 10 increased when compared to Day 8 (Tables 4 and 5). This was because in Group A 6/19 (32%) cats (2 from each shelter) had increased clinical sign scores and Group B had 4/31 (13%) cats (1 or 2 from each shelter) had increased clinical scores (Tables 3 and 4). Although the mean clinical sign score of Group B was increased on Day 10, there was still a very significant improvement compared to the Day 0 scores. The worsening of 6 cats in Group A resulted in a loss of significant improvement when Day 10 was compared to Day 0 for the group.

Table 5: Individual and group clinical scores at different time points in cats receiving antibiotics and *Yin Qiao* san (Group B)

Cat Number	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Shelter
20	3	0	3	3	1	1	UC
21	5	5	4	1	1	0	UC
22	3	3	3	1	0	0	CM
23	2	2	0	2	1	2	UC
24	1	1	1	0	0	0	CM
25	6	5	4	4	1	1	CM
26	2	1	2	0	0	0	UC
27	2	2	1	0	0	1	UC
28	5	4	3	3	3	4	CM
29	5	5	4	4	4	4	CM
30	6	1	1	0	0	0	UC
31	3	5	4	2	2	2	UC
32	3	1	1	1	1	0	CM
33	3	3	3	3	3	6	AC
34	2	2	0	0	0	0	AC
35	2	3	4	2	2	1	AC
36	4	4	4	4	4	6	AC
37	2	2	2	2	2	1	AC
38	2	4	1	0	0	0	AC
39	2	4	1	0	0	0	AC
40	2	4	1	0	0	0	AC
41	2	2	1	1	1	1	AC
42	2	1	1	1	1	1	AC
43	2	2	2	1	2	2	AC
44	3	3	3	2	1	0	AC
45	2	2	1	1	1	1	AC
46	2	2	1	1	1	1	AC
47	2	2	1	1	1	1	AC
48	2	2	1	1	1	1	AC
49	2	1	1	1	2	2	CM
50	2	1	1	1	1	0	CM
M±SD	2.77±1.31	2.59±1.43	1.94±1.31	1.39±1.26	1.19±1.14	1.26±1.65	

M±SD = mean plus or minus the standard deviation; *CM = Cape May Animal Shelter in Cape May County, NJ; UC= Ulster County SPCA, in Kingston, NY; AC= Atlantic County Animal Shelter, in Pleasantville, NJ

In addition to the within-group (self-control) analysis for observing and comparing how quickly the improvement took place in each group, the study further conducted the between-group comparisons in mean improvement score on each of the 5 post-treatment examining days (Days 2, 4, 6, 8, and 10). For each of the 5 examining days, Group B had better mean improvement score than Group A. However, the results from the statistical tests (Wilcoxon Rank Sum test) did not support the statistical significance (with 0.05 significance level) of the greater improvements in Group B over Group A on any of the post-treatment examining days (p = 0.741, 0.058, 0.302, 0.819, and 0.540, respectively).

DISCUSSION

This is the first randomized controlled investigation of the effectiveness of using a Chinese herbal medicine combined with conventional antibiotics for the treatment

Table 6: Group A comparison of mean clinical scores on pretreatment Day 0 to Days 2, 4, 6, 8 and 10 after initiation of treatment to determine significant improvement

Days Compared	Pretreatment Score	Day Score	*p*-value	Significance
Day 0 to Day 2	2.47±1.68	2.37±1.89	0.644	Not significant
Day 0 to Day 4	2.47±1.68	2.26±1.85	0.503	Not significant
Day 0 to Day 6	2.47±1.68	1.47±1.68	0.002	Very significant
Day 0 to Day 8	2.47±1.68	0.95±0.97	0.0006	Very significant
Day 0 to Day 10	2.47±1.68	1.47±2.25	0.082	Not significant

$p<0.05$= Significant; $p<0.01$= Very significant

Table 7: Group B comparison of mean clinical scores on pretreatment Day 0 to Days 2, 4, 6, 8 and 10 after initiation of treatment to determine significant improvement

Days Compared	Pretreatment Score	Day Score	*p*-value	Significance
Day 0 to Day 2	2.77±1.31	2.59±1.43	0.609	Not significant
Day 0 to Day 4	2.77±1.31	1.94±1.31	0.0008	Very Significant
Day 0 to Day 6	2.77±1.31	1.39±1.26	7.9×10^{-6}	Very significant
Day 0 to Day8	2.77±1.31	1.19±1.14	9.1×10^{-6}	Very significant
Day 0 to Day 10	2.77±1.31	1.26±1.65	0.0002	Very significant

$p<0.05$= significant; $p<0.01$= very significant

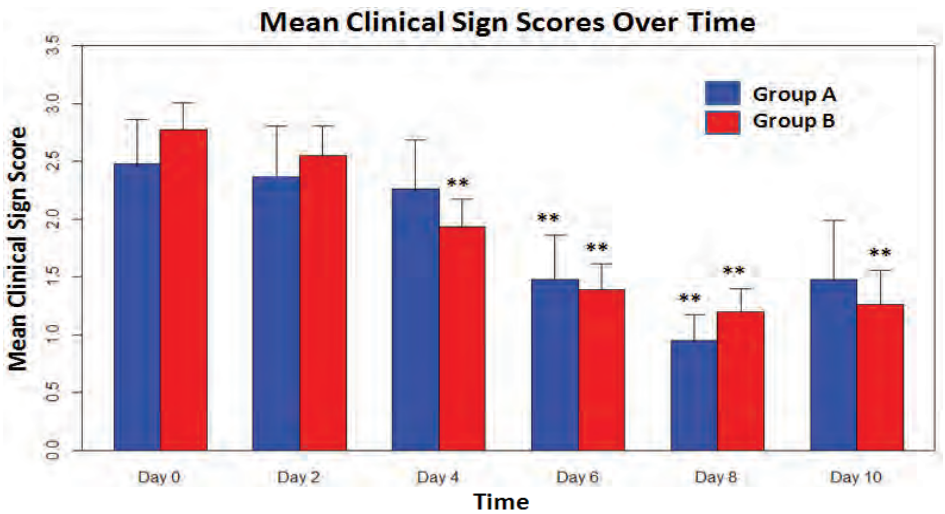

Figure 2: Changes in mean clinical sign scores from Day 0 prior to treatment and on Days 2, 4, 6, 8 and 10 after initiation of treatment; * significant reduction of clinical sign score $p<0.05$ as compared to pretreatment score for the group; ** very significant reduction of clinical sign score $p<0.01$ as compared to pretreatment score for the group; Group A (antibiotics alone) had a very significant reduction of clinical signs on Days 6 and 8, but not on any other days: Group B (antibiotics plus *Yin Qiao San*) started having a very significant reduction of clinical signs by Day 4 and remained very significant on Days 6, 8 and 10 of treatment.

of feline URI. The results of the study supported the hypothesis that a combination of *Yin Qiao San* and Clavamox or doxycycline would result in faster clinical improvement of cats with URI than either Clavamox or doxycycline alone and have no adverse side effects. By Day 4 cats treated with *Yin Qiao San* and antibiotics had significantly reduced clinical sign scores, and the improvement remained significant through Days 6, 8 and 10. On the other hand, the group treated with antibiotics alone showed significant improvement only after Day 6. This is especially important in a shelter situation, as affected cats are less likely to be euthanized because of their disease and become available for adoption faster.

On Day 10 of treatment, 6 cats in Group A and 4 cats in Group B had higher clinical sign scores than on Day 8 indicating worsening of clinical signs. Unable to control exposure to URI organisms from incoming cats, it was suspected that these cats contacted FCV in the shelter, which resulted in worsening of the URI. *Yin Qiao San* seems to have had a protective effect in Group B as only 13% of cats had increased clinical scores on Day 10 compared to 32% of cats in Group A. Since the cats in Group B on *Yin Qiao San* were doing so much better than Group A, the technicians later added *Yin Qiao San* to the treatment of several cats in Group A. Treatment on all cats was continued until the clinical signs resolved.

Experimental and clinical research provides some insights into the possible conventional mechanisms of action of *Yin Qiao San* on the respiratory system.[14-19] It is suspected that *Yin Qiao San* has protective effects on the respiratory system by improving the function of the respiratory mucosal immune system.[14] In an experimental murine study, a combined formula of *Xi Jiao Di Huang Tang* and *Yin Qiao San* inhibited the production of inflammatory cytokines and free radicals in mice with viral pneumonia caused by the influenza virus.[15] In another murine model of influenza viral pneumonia, *Yin Qiao San* reduced histological evidence of damage to lung tissue and reduced influenza virus hemagglutination titers compared to controls.[16]

In a human study of patients with dementia, the administration of either *Ma Xing Gan Shi Tang, Yin Qiao San* or *Xiao Qing Long Tang* reduced the number of patients developing pneumonia, a common complication for dementia patients, compared to those patients not receiving Chinese herbal medicine.[17] In another randomized study of humans with H1N1 influenza virus infections, treatment outcomes of 4 groups were compared: 1) combination of *Ma Xing Shi Gan* and *Yin Qiao San* alone, 2) the antiviral medication oseltamivir (Tamiflu®) alone, 3) a combination of *Ma Xing Shi Gan, Yin Qiao San* and oseltamivir and 4) a control group with no treatment.[18] Time to fever resolution was significantly less for the combination of *Ma Xing Shi Gan, Yin Qiao San* and oseltamivir compared to oseltamivir alone. In another study *Yin Qiao San* significantly reduced fever in children demonstrating its anti-pyretic effects.[19]

The anti-inflammatory and anti-viral effects of *Yin Qiao San* come primarily from the King herbs *Jin Yin Hua* (Lonicera) and *Lian Qiao* (Forsythia) along with the Minister herbs *Jie Geng* (Platycodon), *Bo He* (Mentha) and *Niu Bang Zi* (Articum).[11] In an *in-vivo* murine model of acute lung inflammation induced by lipopolysaccharide, *Jin Yin Hua* (Lonicera) increased the expression of interleukin 10 (IL-10), an important anti-inflammatory cytokine, which resulted in reduction of the proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1 β) and interleukin 6 (IL-6).[20] A reduction of proinflammatory cytokines was also found in an *in-vitro* study of the anti-inflammatory effects of flavonoids extracted from *Jin Yin Hua* (Lonicera) on microglia stimulated with lippolysaccharide.[21]

Anti-inflammatory mechanisms of Forsynthin, a major lignin found in *Lian Qiao* (Forsythia) has been studied in *in-vitro* and *in-vivo* murine models of viral and bacterial pneumonia, sepsis and cytokine-driven inflammation.[22] It was found that Forsynthin acts as an immunosuppressant by inhibiting phosphodiesterase 4 (PDE4) in inflammatory and immune cells. Further Forsythia has been shown to have direct effects on the influenza A viruses.[23] *Jin Yin Hua* (Lonicera), *Lian Qiao* (Forsythia) and *Jie Geng* (Platycodon) alone and combined were studied in another murine model of lipopolysaccharide induced lung inflammation.[24] Animals in the group that were administered a combination of all 3 herbs had reduced lung inflammation on histopathological examination compared to the untreated control group. Significantly or very significantly reduced ($p<0.05$ or $p<0.01$) transforming growth factor beta (TGF-β), released from macrophages following inflammatory stimuli, as well as reduced IL-1β and WBC counts were found in bronchial alveolar lavage fluid in the herbal treated group. Further, trefoil factor 3 messenger ribonucleic acid (TFF3 mRNA), which protects respiratory mucosa, was very significantly increased ($p<0.01$) in lung tissue from the group administered all 3 herbs. The conclusion was *Jie Geng* (Platycodon) acts synergistically with the King herbs found in *Yin Qiao San* to further reduce inflammatory cytokines and protect lung tissue.[24] The anti-viral activity of aqueous extracts of *Bo He* (Mentha) against Influenza A, Newcastle disease virus, herpes simplex virus and vaccinia virus have been shown since the 1960s and the anti-inflammatory, antibacterial and anti-viral effects has been extensively studied *in-vitro* and *in-vivo* since that time.[25] *Niu Bang Zi* (Articum) another major component of *Yin Qiao San* has been shown to have anti-inflammatory, anti-oxidant, anti-bacterial and antifungal effects in many *in-vitro* and *in-vivo* studies.[26,27]

Limitations to the study included housing cats in a shelter facility and statistical inter- versues intra-group comparison. The ideal clinical study situation would have been 50 cats housed in 1 location, isolated from other

incoming cats, living under the same conditions, treated with the same antibiotic and evaluated by one veterinarian or well trained technician that was blinded to the treatment group. In reality the variables in clinical studies cannot always be that well controlled. The uncontrollable variables in the current multicenter animal shelter clinical study included: 1) varying administrative organizations, policies and commitment to the study, 2) use of different antibiotics by different shelters, 3) differences in interpretation of clinical signs by multiple technicians, 4) technician bias because they were unblinded to the group assignment and 5) exposure of cats to viruses and secondary bacterial infections associated with the large turnover of cats in a shelter environment.

During the study design, it was determined that a sample size of 25 subjects in each group (50 total) was sufficient to ensure at least 90% test power (for both paired-t tests and nonparametric Wilcoxon Sign Rank tests for each within-group analysis) to detect a difference of one sample standard deviation with a 0.05 significance level. Such a sample size would also give 89% power (for both two sample t tests and nonparametric Wilcoxon Rank Sum tests for between-group comparison) to detect a difference of one (pooled) sample standard deviation with a 0.05 significance level. In order to get 50 cats for the study within the time frame, involvement of 3 different animal shelters was necessary. Two shelters exclusively used Clavamox for feline URI, but would switch to doxycycline if suspected FCV outbreaks occurred. The other shelter used either Clavamox or doxycycline. Due to work schedules and number of available technicians at any given time, more than 1 technician at each of the 3 locations completed the clinical sign scoring sheet on the predetermined days. Although training sessions for technicians were provided, interpretation of clinical signs most likely varied slightly among individuals. In addition, due to limited technician availability, the study was conducted unblinded, with a technician scoring and treating the same cat. Without being blinded there could have been positive or negative bias that affected the interpretation by the technician based on a preconceived notion of whether Chinese herbal medicine would be helpful or not. Even with all these study challenges, the differences in clinical scores before treatment compared to days 6, 8 and 10 in the 31 cats treated with antibiotics and *Yin Qiao San* were very significant ($p<0.01$). The difference in the groups was so clear to those working with them that eventually many cats were given *Yin Qiao San* along with antibiotics until the URI had resolved.

With all these challenges, statistically valid inter-group comparison was difficult to demonstrate versus intra-group comparison of animals with their own pretreatment baseline. Based on the results from the first (within-group) analysis, it would be anticipated that only on Day 4 the mean inter-group improvement score in Group B was significantly greater than that in Group A. This hypothesis was supported marginally ($p = 0.058$) by this between-group comparison. A study with a larger number of animals (and more balanced groups) in facilities with better quarantine procedures could provide more statistical power to claim this significance.

In summary it was found that for naturally occurring URI of cats, a combination of *Yin Qiao San* and either Clavamox or doxycycline resulted in faster resolution of clinical signs than with antibiotics alone. There were no adverse side effects and the *Yin Qiao San* tablets were relatively easy to administer. *Yin Qiao San* costs 6-8 cents/tablet and with the usual dose of 0.5-1 tablet twice daily, based on their weight, *Yin Qiao San* was also cost effective. Discounted Clavamox in the shelter situation is usually about $3/day/cat and compounded doxycycline liquid required for feline administration can cost even more depending on the source of the drug. Clinicians should be aware that some human over-the counter *Yin Qiao San* preparations may also contain acetaminophen, which causes feline liver and red blood cell damage resulting in mortality in some animals. Also TCVM clinicians should be alert to modifications in the original *Yin Qiao San* formulations with the addition and deletion of other herbal ingredients. Based on the results of this study *Yin Qiao San*, along with Clavamox or doxycycline is recommended for the treatment of feline URI. In the future, blinded, prospective studies are needed to compare antibiotics alone versus *Yin Qiao San* administered either alone or combined with antibiotics to determine the optimum treatment of feline URI.

FOOTNOTES

a. Jing Tang Herbal, Reddick FL; www.tcvmherbal.com
b. *Yin Chiao Chieh Tu Pien* (extra concentrated tablets) by Plum Flower Brand, Mayway, San Francisco CA
c. Clavamox, Zoeitis Corporation, Kalamazoo, MI
d. https://www.easycalculation.com/statistics/standard-deviation.php
e. The R Foundation for Statistical Computing; R version 3.2.2
f. Roche Pharmaceuticals, Basel, Switzerland;, http://www.roche.com/

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REFERENCES

1. Bannasch MJ, Foley JE. Epidemiologic evaluation of multiple respiratory pathogens in cats in animal

shelters. J Feline Med Surg 2005; 7(2):109-119.
2. Dinnage JD, Scarlett JM, Richards JR. Descriptive epidemiology of feline upper respiratory tract disease in an animal shelter. J Feline Med Surg 2009; 11(10):816-825.
3. Gourkow N, Lawson JH, Hamon SC, Phillips CJC. Descriptive epidemiology of upper respiratory disease and associated risk factors in cats in an animal shelter in coastal western Canada. The Canadian Vet J 2013; 54(2):132-138.
4. Hartmann AD, Helps CR, Lappin MR et al. Efficacy of pradofloxacin in cats with feline upper respiratory tract disease due to Chlamydophila felis or Mycoplasma infections. J Vet Intern Med 2008; 22(1):44-52.
5. Fernandez M, Manzanilla EG, Lloret A et al. Prevalence of feline herpesvirus-1, feline calicivirus, Chlamydophila felis and Mycoplasma felis DNA and associated risk factors in cats in Spain with upper respiratory tract disease, conjunctivitis and/or gingivostomatitis. J Feline Med Surg 2016 [Epub ahead of print].
6. Litster A, Wu C, Constabale P. Comparison of the efficacy of amoxicillin-clavulanic acid, cefovecin, and doxycycline in the treatment of upper respiratory tract disease in cats housed in an animal shelter. J Am Vet Med Assoc 2012; 241(2):218-226.
7. McDonagh P, Sheehy PA, Fawcett A, Norris JM. Antiviral effect of mefloquine on feline calicivirus in vitro. Vet Microbiol 2015; 176(3-4):370-377.
8. Wu H, Zhang X, Liu C et al. Antiviral effect of lithium chloride on feline calicivirus in vitro. Arch Virol 2015; 160(12):2935-2943.
9. Hirsch D. Respiratory disorders. Practical Guide to Traditional Chinese Veterinary Medicine. Xie H, Wedemeyer L, Chrisman C (eds). Reddick, FL: Chi Institute Press 2014:259-263.
10. Wang C, Wang H, Liu X et al. Traditional Chinese medicine for the treatment of influenza: a systematic review and meta-analysis of randomized controlled trials. J Trad Chin Med 2014; 34(5):527-31.
11. Song D, Schmalberg J, Xie H. Herbal formulas to release the Exterior. Xie's Chinese Veterinary Herbology. Xie H, Priest V (eds) Ames, IA: Wiley Blackwell 2010:352-353.
12. Baley P. Acupuncture and Chinese herbal therapy for acute upper respiratory disease in a geriatric Quarter Horse. Amer J of Trad Chinese Vet Med 2009; 4(1):52-57.
13. Newbury S, Blinn MK, Bushby PA et al. Guidelines for standards of care in animal shelters 2010: http://www.sheltervet.org/assets/docs/shelter-standards-oct2011-wforward.pdf.
14. Liu LS, Lei N, Lin Q et al. The effects and mechanism of *Yin Qiao* Powder on upper respiratory tract infection. Int J Biotech Well Indus 2015; 4(2):57-60.
15. Wu Y, Li JQ, Jian M et al. Effect of *Xi Jiao di Huang Tang* and *Yin Qiao San* on inflammatory cytokines and free radicals in mice with pneumonia caused by influenza virus. Chinese J of Info on Trad Chinese Med 2012; 19(7):28-30.
16. Hao OM, Wang XF. Experimental research of *Yin Qiao San* extract on treatment of influenza viral pneumonia in mice. World J of Integrated Trad and West Med 2009; 4(9):630-632.
17. Lin SK, Tsai YT, Lo PC et al. Traditional Chinese medicine therapy decreases the pneumonia risk in patients with dementia. Medicine 2016; 95(37):e4917.
18. Chen W, Bin C, Liu QQ et al. Oseltamivir compared with Chinese traditional therapy *Ma Xing Shi Gan* and *Yin Qiao San* in the treatment of H1N1 influenza: a randomized trial. Annals of Internal Med 2011; 155(4):217-225.
19. Liew WK, Loh WY, Chiang WC et al. Pilot study of the use of *Yin Qiao San* in children with conventional antipyretic hypersensitivity. Asia Pacific Allergy 2015; 5(4):222-229.
20. Kao ST, Liu CJ, Yeh CC. Protective and immunomodulatory effect of flos Lonicerae japonicae by augmenting IL-10 expression in a murine model of acute lung inflammation. J Ethnopharmacol 2015; 168:108-15.
21. Han MH, Lee WS, Nagappan A et al. Flavonoids isolated from flowers of Lonicera japonica Thunb. inhibit inflammatory responses in BV2 microglial cells by suppressing TNF-α and IL-β Through PI3K/Akt/NF-kb signaling pathways. Phytother Res 2016; 30(11):1824-1832.
22. Coon TA, McKelvey AC, Weathington NM et al. Novel PDE4 inhibitors derived from Chinese medicine forsythia. PLoS One 2014; 9(12):e115937.
23. Zhou Z, Li X, Liu J et al. Honeysuckle-encoded atypical microRNA2911 directly targets influenza A viruses. Cell Res 2015; 25(1):39-49.
24. Li YH, Zheng FJ, Huang Y et al. Synergistic anti-inflammatory effect of Radix Platycodon in combination with herbs for clearing-heat and detoxification and its mechanism. Chin J Integr Med 2013; 19(1):29-35.
25. McKay DL, Blumberg B. A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperta L.). Phytotherapy Res 2006; 20(8):619-633.
26. Rajasekharan SK, Ramesh S, Satish AS, Lee J. Anti-biofilm and Anti-β-lactamase activities of burdock root extract and chlorogeneic acid against Klebsiella pneumoniae. J Microbiol Biotechnol 2016; Epub ahead of print.
27. Chan YS, Cheng LN, Wu JH. A review of the pharmacological effects of Arctium lappa (burdock). Inflammopharmacol 2011; 19(5):245-254.

Epidemiology

Case-Control Study of Exposure Factors Associated with Gastrointestinal Side Effects in Dogs after Treatment with Chinese Herbal Medicine

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ABSTRACT

A case-control study was conducted to identify exposure factors associated with gastrointestinal (GI) side effects (i.e., vomiting, diarrhea, or anorexia) in dogs after treatment with Chinese herbal medicine. Investigated exposure factors included age, gender, breed, spay/neuter status, body weight, presenting complaint(s) at admission, herbal treatment(s) used and duration of treatment, as well as other medication(s) and diet(s) used during treatment. A total of 34 case dogs and 34 control dogs were enrolled in the study. Using logistic regression analysis, short duration of treatment (≤ 5 days) was associated with a diagnosis of GI side effects in study dogs ($p < 0.001$). Although the frequency of dogs affected with side effects after treatment with Chinese herbal medicine is very low, traditional Chinese veterinary practitioners and dog owners are advised to monitor dogs for GI side effects during the first five days after treatment with Chinese herbal medicine.

Key words: Traditional Chinese veterinary medicine, exposure factors, side effects, dogs, Chinese herbal medicine, epidemiology

ABBREVIATIONS

CI	Confidence intervals
GI	Gastrointestinal
OR	Odds ratio
SD	Standard deviation
TCVM	Traditional Chinese Veterinary Medicine

Chinese herbal medicine is widely and safely used for treatment and prevention of diseases in both humans and companion animals in the United States and other Western countries.[1-4] In companion animals, the risk of side effects after treatment with Chinese herbal medicine is considered low as evidenced by a recent survey conducted by the Chi Institute, Florida USA where the prevalence of adverse side-effects was 0.4% (63/15,000).[5] For comparison, review of clinical trials and post marketing surveillance of the common and safely used pharmaceutical (tramadol) in 21,000 humans had an overall higher incidence of adverse effects between 1 and 6%.[6] An even larger oral safety data evaluation of a fluoroquinolone antibiotic, based on >46,000 and >7,000 patients in post marketing surveillance and clinical trials, respectively had 7.1% nausea and 5.2% diarrhea rates and was judged as well tolerated and a good option for diabetic or elderly patients and those with renal impairment.[7]

Similar to drugs, surveillance of therapeutic related adverse effects in patients receiving Chinese herbal medicine is important. A review of those herbs that might be used in veterinary medicine suggested that small numbers have demonstrated potential to cause renal, hepatic, cardiac and nervous system toxicity along with gastrointestinal (GI) side effects.[8] Their toxicity could be related to several factors including: toxic properties of the herb, improper substitution or combination of herbs in herbal formulas, improper herb extraction and processing techniques, overdose, inappropriate long-term usage, interaction with concurrent conventional medications, individual animal sensitivity to a particular herbal medicine, and incorrect traditional Chinese veterinary medicine (TCVM) pattern diagnosis and prescription.[8]

Based on clinical experience of the authors, although the frequency of GI side effects in companion animals after treatment with Chinese herbal medicine is low, there is interest among TCVM practitioners to elucidate exposure factors that may predispose companion animals to development of this side effect. To our knowledge, there are no published, epidemiologic research studies that have

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examined the relationship between exposure factors and the development of side effects after treatment with Chinese herbal medicine in companion animals. The objective of this epidemiologic study was to identify exposure factors associated with GI side effects (i.e., vomiting, diarrhea, anorexia) in dogs following administration of a Chinese herbal medicine in a clinical setting using a case-control study design.

MATERIALS AND METHODS

A structured online questionnaire with exposure data of interest was prepared by the first author and tested by attending veterinarians at three selected veterinary clinics in Florida. After preliminary use by these clinics, a revised questionnaire was prepared using a Survey Monkey instrument[b] and sent via e-mail to 8,000 veterinary clinics in the United States, Australia and European countries. The list of veterinary clinics was provided by the Chi Institute in Reddick, Florida, USA. This study was approved by the University of Florida's Institute of Animal Care and Use Committee[a].

The questionnaire contained three parts (A, B, C) with a total of 35 questions. Part A included three questions about the veterinary clinic (i.e., number of dogs admitted for veterinary care in the last 6 or 24 months, number of dogs treated with Chinese herbal medicine and number of dogs identified with side effects after treatment with Chinese herbal medicine). Part B included 17 questions about the last case dog (i.e., a dog with side effects after treatment with Chinese herbal medicine) admitted to the clinic. The questions covered signalment of the dog (age, sex, breed, body weight, spay/neuter status), the presenting complaint, date of diagnosis, treatment (including treatment duration), side effect(s) observed and past history (diet, GI sensitivity, food allergy). Finally, Part C included 15 questions about the last control dog given a Chinese herbal medicine without side effects admitted to the clinic before or after the case dog (the same questions included in Part B, with the exception of the two questions about side effects).

The questionnaire-survey was first posted on July 23, 2014 requesting veterinary practitioners to identify dogs diagnosed with side effects in the last 6 months. A total of 130 surveys were received over a 1-week period (July 23 – July 31, 2014). The questionnaire was modified on August 7, 2014 requesting veterinary practitioners include dogs diagnosed with side effects in the previous 24 months in an attempt to increase the study sample size. An additional 90 surveys were received bringing the total number of surveys submitted for this study to 220 from 205 clinics (15 surveys submitted by veterinarians in same clinic). The survey was closed on October 9, 2014.

Questionnaire-surveys returned during the 12-week period were reviewed by the authors. Exclusion criteria for study enrollment included submission of an incomplete survey and clinics without documented cases of Chinese herbal medicine side effects. The investigation was designed as a case-control study. Inclusion criteria for a case dog was the most recent dog treated with Chinese herbal medicine which experienced gastrointestinal side effects (vomiting, diarrhea or anorexia) during the 6-24 months after treatment. Control dogs had identical selection parameters but without side effects subsequent to receiving Chinese herbal medicine. The duration of risk exposure (6 to 24 months) was justified based on clinical observations by one of the authors (HX) and to increase the number of enrolled dogs.

The frequency of investigated exposure factors was compared between case and control dogs by using logistic regression analysis. Control and case dogs (ratio 1:1) were matched by clinic and admission date. The sample size of 34 control and 34 case dogs provided 95% confidence and 80% power to declare associations between the outcome of interest (GI side effects) and one or more investigated exposure factors (40% exposure) with an odds ratio (OR) = 5.3 as statistically significant. Sample size calculations were performed using online free-software[c].

Statistical analysis used unconditional logistic regression to model the odds of side effects in study dogs. In the univariable analysis, variables with a p value $\leq$ 0.20 were considered eligible for multivariable analysis. Associations between exposure variables ($p \leq 0.20$) were examined and when a pair of variables was associated by use of a chi-square test (two tailed), the exposure variable judged as most biologically plausible was used as a candidate in the multivariable analysis. To determine the best fitting model, the variable with the smallest p value in the univariable analysis was entered into the model first. Each of the remaining variables, thereafter, was added to the model containing the first variable to test whether its addition significantly improved the fit of the model. The variable with the highest likelihood ratio statistic (chi-square test with one degree of freedom) was selected for addition to the model and the process was then repeated. Variables with a p value ≤ 0.10 were retained in the model. The variable for age was forced into the model due to possible longer exposure to Chinese herbal medicines. Explanatory variables retained in the model were examined for confounding by adding each variable to the model and assessing the changes in the odds ratios (i.e., > 20%) of the remaining variables in the model.

In the final model, the adjusted OR and 95% CI were reported. The OR was used as an epidemiologic measure of association between a factor and a diagnosis of GI side effect. When a particular factor was not associated with GI side effects, the OR was 1. The greater the departure of the OR from 1 (i.e., values larger or smaller than 1), the stronger the association was between a factor and development of GI side effects after treatment. In addition, the null hypothesis that treatment with Chinese herbal medicine was not different between case and control dogs

was tested by using the Wilcoxon rank sum test. In all analyses, values of $p < 0.05$ were considered significant. A commercial software[d] was used for data analysis.

RESULTS

Two-hundred twenty of 8,000 (2.75%) practitioners returned the survey representing 205 individual veterinary clinics with a total caseload of 307,287 dogs. Out of the total caseload number, 54,564 dogs had been treated with Chinese herbal medicines (17.75%). These animals were initially considered for inclusion in the study. A total of 103 clinics of the initial 220 surveys reported treatment side effects (i.e., GI disorders, skin reactions, elevated liver enzymes, hyperactivity, hair loss, increase urination, overheated and ataxia) in dogs (643/54,564 or 1.17%). Surveys from 51 of the 103 clinics reporting side effects had incomplete data (i.e., survey data in sections B and C were incomplete) and were excluded.

The remaining 52 clinics returned questionnaire-surveys which included all information (sections A, B, C) requested. These clinics reported a total canine caseload of 17,256 with 408 dogs diagnosed with Chinese herbal medicine side effects. A gastrointestinal disorder was the most common side effect reported (n = 34 dogs), followed by skin disorders (n = 6 dogs), elevated liver enzymes (n = 4 dogs) and other (n = 8 dogs). In addition, 34 of these 52 clinics reported one or more treated dogs which were diagnosed exclusively with GI side effects.

The case-control study included 34 case dogs affected with GI side effects and 34 control dogs matched by clinic and admission date. The number of case dogs was limited to the number of dogs diagnosed with GI-side effects at the 52 clinics that returned complete surveys. A case dog with GI side effects was the most recent dog treated with Chinese herbal medicine and that was diagnosed by the attending veterinarian with any of the following symptoms: vomiting, diarrhea or anorexia during the following 6-24 months after treatment. In this study, veterinary practitioners were asked to select the dog most recently diagnosed with GI side effects (n = 34 case dogs). Control dogs were treated with Chinese herbal medicine without side effects by the attending veterinarian or dog owner during the following 6-24 months after treatment. On average, control dogs were admitted to the same clinic 119 days before or after case dogs. A control:case ratio of 1:1 was used in this study (n = 34 controls).

Epidemiologic analysis of investigated exposure factors associated with GI side effects in the univariable analysis with values of $p \leq 0.10$ included gender, history of GI tract sensitivity, behavior abnormalities, intervertebral disc disease, seizures, and duration of treatment (Table 1). These were considered eligible for multivariate analysis. The variable for age was not significantly associated with GI side effects (unadjusted OR = 0.48; 95% CI = 0.18, 1.28; $p = 0.14$). The odds of GI side effects in dogs that were treated with Chinese herbal medicines for ≤ 5 days or 6-37 days were almost 100 or 11 times higher respectively, compared to dogs that were treated for a longer time (38-1095 days) (unadjusted OR = 99.90; 95% CI = 13.17, 757.88; $p = 0.001$) (unadjusted OR = 10.90; 95% CI = 2.09, 56.77; $p = 0.004$).

In the multivariable analysis, the variables for duration of treatment ($p < 0.001$), sex ($p = 0.09$), and age ($p = 0.64$) were retained in a full model (Table 2). The odds of GI side effects were 10 times higher in dogs that were treated during 6-37 days, and 105 times higher in dogs treated during 1-5 days, compared to dogs treated during 38-1095 days ($p < 0.001$), after controlling for

Table 1: Univariable logistic regression analysis of investigated exposure factors associated with GI side effects only

Variable	Category	Cases N=34 (100%)	Controls N=34 (100%)	OR	95% CI	*p**
Sex	Female	15 (44)	24 (71)	1.00	Reference	NA
	Male	19 (56)	10 (29)	3.04	1.12, 8.27	0.02
History of GI tract sensitivity	No	22 (69)	28 (87)	1.00	Reference	NA
	Yes	10 (31)	4 (13)	3.18	0.88, 11.51	0.07
Behavior complaints	No	27 (79)	32 (94)	1.00	Reference	NA
	Yes	7 (21)	2 (6)	4.15	0.80, 21.64	0.09
Intervertebral disc disease	No	27 (79)	32 (94)	1.00	Reference	NA
	Yes	7 (21)	2 (6)	4.15	0.80, 21.64	0.09
Seizures	No	33 (97)	28 (82)	1.00	Reference	NA
	Yes	1 (3)	6 (18)	0.14	0.02, 1.25	0.07
Duration of treatment (days)	38 to 1095	2 (6)	20 (61)	1.00	Reference	NA
	6 to 37	12 (35)	11 (33)	10.90	2.09, 56.77	<0.01
	1 to 5	20 (59)	2 (6)	99.90	13.17, 757.88	<0.01

* Only variables with values of $p \leq 0.10$ are shown

NA = Not Applicable; p values reported for each investigated exposure factor; OR = odds ratio; 95% CI = 95% confidence interval

sex and age. The Hosmer–Lemeshow goodness of fit test (1.25; df = 6; $p = 0.97$) indicated there was no evidence of a poor fit for the data. Median duration of treatment with Chinese herbal medicine was shorter in case dogs (5 days) compared to control dogs (60 days) ($p < 0.001$).

The use of a total of 35 Chinese herbal medicines were reported in the 220 surveys (Table 3). There was no statistically significant association found between any single herbal formula and GI side effects. In case dogs, 17 Chinese herbal medicines were used for treatment for ≤ 5 days (Table 4). Modified *Tian Wang Bu Xin Dan* (*Shen* Calmer[e]) was the most common Chinese herbal medicine used in case dogs at 12% (n=4) without use in control dogs ≤ 5 days. An herbal formula of particular interest, *Shen Tong Zhu Yu Tang* (Body Sore[f]), which anecdotally has been associated with GI side effects was given to 7 case dogs and 11 control dogs. Study results revealed no evidence that treatment with this formula was a risk factor for development of GI side effects (unadjusted OR = 0.54; 95% CI = 0.18, 1.63; $p = 0.27$). All other Chinese herbal

Table 2: Final multivariable logistic regression analysis of investigated exposure factors associated with GI side effects only

Variable	Category	Cases N=34 (100%)	Controls N=34 (100%)	OR	95% CI	*p*[#]
Age (years)	1 to 10 11 to 16	18 (53) 16 (47)	12 (35) 22 (65)	1.00 1.39	- 0.34, 5.68	- 0.64
Sex	Female Male	15 (44) 19 (56)	24 (71) 10 (29)	1.00 3.14	- 0.81, 12.17	- 0.09
Duration of treatment (days)	38 to 1095 6 to 37 1 to 5	2 (6) 12 (35) 20 (59)	20 (59) 11 (33) 2 (6)	1.00 10.38 105.37	- 1.88, 57.24 12.73, 872.07	- <0.01** <0.01**

*****($p<0.05$) significant; **($p<0.01$)very significant

[#] Only variables with *p* value ≤ 0.20 from univariate analysis are evaluated; Hosmer–Lemeshow goodness of fit test (1.25; df = 6; $p = 0.97$) indicated there was no evidence of a poor fit for the data

Table 3: Chinese Herbal Medicine Treatment[&]

Variable	Category	Cases N=34 (100%)	Controls N=34 (100%)	OR	95% CI	*p*
Shen Calmer (Classical antecedent *Tian Wang Bu Xin Dan*)	No Yes	29 (85) 5 (15)	32 (94) 2 (6)	1.00 2.76	Reference 0.50, 15.31	NA 0.24
Di Tan Tang	No Yes	32 (94) 2 (6)	29 (85) 5 (15)	1.00 0.36	Reference 0.07, 2.01	NA 0.24
Body Sore (Classical antecedent *Shen Tong Zhu Yu Tang*)	No Yes	27 (79) 7 (21)	23 (68) 11 (32)	1.00 0.54	Reference 0.18, 1.63	NA 0.27
Tendon Ligament (Classical antecedent *Bu Gan Qiang Jing San*)	No Yes	32 (94) 2 (6)	30 (88) 4 (12)	1.00 0.47	Reference 0.08, 2.75	NA 0.40
Wei Qi Booster (Classical antecedent *Si Jun Zi Tang*)	No Yes	28 (82) 6 (18)	30 (88) 4 (12)	1.00 1.61	Reference 0.41, 6.30	NA 0.49
Hindquarter Weakness Formula (Classical antecedent *Bu Qi Zi Yin Tang*)	No Yes	32 (94) 2 (6)	31 (91) 3 (9)	1.00 0.65	Reference 0.10, 4.13	NA 0.64
External Wind (Classical antecedent *Xiao Feng San*)	No Yes	32 (94) 2 (6)	31 (91) 3 (9)	1.00 0.65	Reference 0.10, 4.13	NA 0.64
Di Gu Pi	No Yes	31 (91) 3 (9)	30 (88) 4 (12)	1.00 0.73	Reference 0.15, 3.52	NA 0.69
Stasis Breaker (Classical antecedent *Nei Xiao Wan*)	No Yes	30 (88) 4 (12)	30 (88) 4 (12)	1.00 1.00	Reference 0.23, 4.37	NA 1.0
Double P II *(*Classical antecedent *Da Huo Luo Dan)*	No Yes	33 (97) 1 (3)	33 (97) 1 (3)	1.00 1.00	Reference 0.06, 16.67	NA 1.0

Liu Wei Di Huang or *Rehmannia* 6	No Yes	33 (97) 1 (3)	33 (97) 1 (3)	1.00 1.00	Reference 0.06, 16.67	NA 1.0
Dok's formula *(*Classical antecedent *Du Huo Ji Sheng Tang)*	No Yes	33 (97) 1 (3)	33 (97) 1 (3)	1.00 1.00	Reference 0.06, 16.67	NA 1.0
Stasis in the mansion of the mind (Classical antecedent: *Nao Yu Fang)*	No Yes	33 (97) 1 (3)	33 (97) 1 (3)	1.00 1.00	Reference 0.06, 16.67	NA 1.0
Long Dan Xie Gan Wan	No Yes	33 (97) 1 (3)	33 (97) 1 (3)	1.00 1.00	Reference 0.06, 16.67	NA 1.0
Max formula (Classical antecedent *Nei Xiao Luo Li San*)	No Yes	33 (97) 1 (3)	33 (97) 1 (3)	1.00 1.00	Reference 0.06, 16.67	NA 1.0
Mai Men Dong San or *Ophiopogon Formula*	No Yes	31 (91) 3 (9)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Liver Happy (Classical antecedent *Chai Hu Shu Gan Wan*)	No Yes	32 (94) 2 (6)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Bu Yang Huan Wu	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Release Restraint (Classical antecedent *Yue Ju Wan*)	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Loranthus formula (Classical antecedent *Sang Ji Sheng San*)	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Qi Performance (Classical antecedent *Ba Zhen Tang*)	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Yunnan Bai Yao	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Bu Zhong Yi Qi Tang	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Si Miao San or KE Four Marvels	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Si Wu Tang	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Shu Jin Huo Luo San	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Lily Combination (Classical antecedent *Bai He Gu Jing Tang*)	No Yes	34 (100) 0 (0)	32 (94) 2 (6)	1.00 ND	Reference ND	NA ND
Ding Xian San	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND
Epimedium Formula (Classical antecedent *Sheng Jing San*)	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND
Tian Ma Plus II (Classical antecedent *Tian Ma Gou Teng Yin*)	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND
Wu Bi Shan Yao San	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND
Damp Heat Skin (Classical antecedent *Qing Shi Re Tang*)	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND
Benefit Hips and Knees (Classical antecedent *Xiao Chai Hu Tang Jia Qin Jiao*)	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND
You Gui Wan	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND
Zhi Bai Di Huang Wan	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND

[&] Results of univariable logistic regression analysis

OR = odds ratio; 95% CI = 95% confidence interval; NA = Not applicable; ND = Not determined

medicines used ≤ 5 days had only 1 or 2 dogs with GI side effects and 2 of the herbal formulas (Body Sore[f], External Wind[g]) had 1 control dog that also received the herb with no side effects.

All other results of univariable logistic regression analysis of exposure factors associated with GI side effects in study dogs were not statistically significant. These exposure factors included: signalment and GI history (Table 5), disease syndromes (Table 6), musculoskeletal/neurological disease (Table 7) and TCVM patterns (Table 8).

Table 4: Frequency distribution of Chinese herbal medicines used for ≤ 5 days in case and control dogs

Herbal Formula	Patent Name, Manufacturer	No. Case Dogs	No. Control Dogs
Modified *Tian Wang Bu Xin Dan*	Shen Calmer, Jing Tang Herbal Inc.	4	
Modified *Shen Tong Zhu Yu Tang*	Body Sore, Jing Tang Herbal Inc.	2	1
Modified *Si Jun Zi Tang*	Wei Qi Booster, Jing Tang Herbal Inc.	2	
Di Gu Pi San	*Di Gu Pi*, Jing Tang Herbal Inc.	2	
Modified *Mai Men Dong San*	Ophiopogon Formula, Jing Tang Herbal Inc.	2	
Modified *Xiao Feng San*	External Wind, Jing Tang Herbal Inc.	2	1
Bu Gan Qiang Jin San	Tendon/Ligament Formula, Jing Tang Herbal Inc.	2	
Nei Xiao Luo Li San	Max's Formula, Jing Tang Herbal Inc.	1	
Bu Yang Huan Wu Tang	*Bu Yang Huan Wu*, Jing Tang Herbal Inc.	1	
Yue Ju Wan	Release Restraint, Kan Herb, Inc.	1	
Modified *Sang Ji Sheng San*	Loranthus Formula, Jing Tang Herbal Inc.	1	
Modified *Chai Hu Shu Gan Wan*	Liver happy, Jing Tang Herbal Inc.	1	
Yunnan Bai Yao	*Yunnan Bai Yao, Yunan Bai Yao* Group Co. LTD	1	
Modified *Bu Qi Zi Yin Tang*	Hindquarter Weakness Formula, Jing Tang Herbal Inc.	1	
Modified *Du Huo Ji Sheng Tang*	Dok's formula, Jing Tang Herbal Inc.	1	
Di Tan Tang	*Di Tan Tang*, Jing Tang Herbal Inc.	1	
Liu Wei Di Huang	Rehmannia 6, Jing Tang Herbal Inc.	1	

Table 5: Investigated exposure factors to determine association with GI side effects in study dogs (signalment and GI history)[&]

Variable	Category	Cases N = 34	Controls N = 34	OR	95% CI	*p*-value
Gender	Female	15 (44)	24 (71)	1.00	Reference	NA
	Male	19 (56)	10 (29)	3.04	1.12, 8.27	0.02
Dog has GI Symptom History	No	22 (69)	28 (87)	1.00	Reference	NA
	Yes	10 (31)	4 (13)	3.18	0.88, 11.51	0.07
Age (years)	1 to 10	18 (53)	12 (35)	1.00	Reference	NA
	11 to 16	16 (47)	22 (65)	0.48	0.18, 1.28	0.14
Dog changed the diet during the treatment	No	21 (68)	25 (78)	1.00	Reference	NA
	Yes	10 (32)	7 (22)	1.70	0.55, 5.25	0.35
Breed	Small	13 (38)	11 (32)	1.00	Reference	NA
	Large	21 (62)	23 (68)	1.01	0.98, 1.04	0.43
Dog receiving Multiple Medications	No	16 (47)	14 (41)	1.00	Reference	NA
	Yes	18 (53)	20 (59)	0.79	0.30, 2.05	0.62
Body Weight	Small	16 (47)	17 (50)	1.00	Reference	NA
	Large	18 (53)	17 (50)	1.12	0.43, 2.91	0.80
Neuter/Spay	No	4 (12)	4 (12)	1.00	Reference	NA
	Yes	30 (88)	30 (88)	1.00	0.23, 4.37	1.00
Dog with Food Allergy History	No	31 (97)	31 (97)	1.00	Reference	NA
	Yes	1 (3)	1 (3)	1.00	0.06, 16.71	1.00

[&] Results of univariable logistic regression analysis

OR = odds ratio; 95% CI = 95% confidence interval; NA = Not applicable; ND = Not determined

Table 6: Investigated exposure factors to determine association with GI side effects in study dogs (Disease Syndromes)[&]

Variable	Category	Cases N = 34	Controls N = 34	OR	95% CI	*p*-value
Behavior Abnormalities	No Yes	27 (79) 7 (21)	32 (94) 2 (6)	1.00 4.15	Reference 0.80, 21.64	NA 0.09
Pain	No Yes	19 (56) 15 (44)	15 (44) 19 (56)	1.00 0.85	Reference 0.24, 1.62	NA 0.33
Dog with Multiple Complaints	No Yes	15 (44) 19 (56)	18 (53) 16 (47)	1.00 1.42	Reference 0.55, 3.70	NA 0.46
Elevated Liver Enzymes	No Yes	32 (94) 2 (6)	33 (97) 1 (3)	1.00 2.06	Reference 0.18, 23.86	NA 0.56
Gum Disease	No Yes	33 (97) 1 (3)	32 (94) 2 (6)	1.00 0.48	Reference 0.04, 5.61	NA 0.56
Cancer	No Yes	28 (82) 6 (18)	28 (82) 6 (18)	1.00 1.00	Reference 0.29, 3.48	NA 1.00
Skin Abnormalities	No Yes	29 (85) 5 (15)	29 (85) 5 (15)	1.00 1.00	Reference 0.26, 3.83	NA 1.00
Urinary Incontinence	No Yes	31 (91) 3 (9)	31 (91) 3 (9)	1.00 1.00	Reference 0.19, 5.34	NA 1.00
Chronic Sensitive GI Masses	No Yes	33 (97) 1 (3)	33 (97) 1 (3)	1.00 1.00	Reference 0.06, 16.67	NA 1.00
Renal Abnormalities	No Yes	31 (91) 3 (9)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Ocular Symptoms	No Yes	32 (94) 2 (6)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Bladder or Renal Calculi	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Otitis	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Anemia on Wellness Check	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Fecal Incontinence	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Hookworms	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Cushing's Disease	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Gastrointestinal Disorders	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Agalactia/ Hypogalactia	No Yes	33 (97) 1 (3)	34 (100) 0 (0)	1.00 ND	Reference ND	NA ND
Addison's Disease	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND
Increased ALP only	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND
Night Restlessness	No Yes	34 (100) 0 (0)	33 (97) 1 (3)	1.00 ND	Reference ND	NA ND

[&] Results of univariable logistic regression analysis
OR = odds ratio; 95% CI = 95% confidence interval; NA = Not applicable; ND = Not determined

Table 7: Investigated exposure factors to determine association with side effects in study dogs (Musculoskeletal/ Neurological)[&]

Variable	Category	Cases N = 34	Controls N = 34	OR	95% CI	*p*-value
Intervertebral disc disease	No	27 (79)	32 (94)	1.00	Reference	NA
	Yes	7 (21)	2 (6)	4.15	0.80, 21.64	0.09
Ligament injury or patellar injury	No	26 (76)	27 (79)	1.00	Reference	NA
	Yes	8 (24)	7 (21)	1.19	0.38, 3.74	0.77
Rear or Hindlimb Weakness	No	32 (94)	33 (97)	1.00	Reference	NA
	Yes	2 (6)	1 (3)	1.00	0.18, 23.86	0.56
Seizures	No	33 (97)	28 (82)	1.00	Reference	NA
	Yes	1 (3)	6 (18)	0.14	0.02, 1.25	0.07
Paralysis	No	33 (97)	34 (100)	1.00	Reference	NA
	Yes	1 (3)	0 (0)	ND	ND	ND
Fibrocartilaginous Emboli	No	33 (97)	34 (100)	1.00	Reference	NA
	Yes	1 (3)	0 (0)	ND	ND	ND
Coxofemoral Joint (Hip) Abnormality	No	34 (100)	33 (97)	1.00	Reference	NA
	Yes	0 (0)	1 (3)	ND	ND	ND

[&] Results of univariable logistic regression analysis
OR = odds ratio; 95% CI = 95% confidence interval; NA = Not applicable; ND = Not determined

Table 8: TCVM Pattern[&]

Variable	Category	Cases N = 34	Controls N = 34	OR	95% CI	*p*-value
Bony *Bi* Syndrome	No	33 (97)	33 (97)	1.00	Reference	NA
	Yes	1 (3)	1 (3)	1.00	0.06, 16.67	1.00
Wei Syndrome	No	33 (97)	34 (100)	1.00	Reference	NA
	Yes	1 (3)	0 (0)	ND	ND	ND
Kidney *Jing* Deficiency	No	34 (100)	33 (97)	1.00	Reference	NA
	Yes	0 (0)	1 (3)	ND	ND	ND

[&] Results of univariable logistic regression analysis
OR = odds ratio; 95% CI = 95% confidence interval; NA = Not applicable; ND = Not determined

DISCUSSION

The results identified a statistically significant difference between shorter median duration of treatment with Chinese herbal medicine in case dogs (≤ 5 days) when compared to control dogs (60 days). This finding suggests that treatment with Chinese herbal medicine was generally discontinued after GI side effects were observed in study dogs. In addition, this study finding contradicts the anecdotal belief among some traditional Chinese veterinary medical practitioners that GI and other side effects can occur after a long duration of exposure to Chinese herbal medicines. While there are no published research reports on Chinese herbal medicine in the veterinary literature on duration of exposure and GI side effects in dogs, this syndrome occurs in humans and is commonly acute, dose related, or due to individual sensitivity.[8,9]

The presence of GI side effects did not have a statistically significant association with specific Chinese herbal formulas. Modified *Tian Wang Bu Xin Dan* (*Shen* Calmer) was not used in control dogs and was the most commonly used Chinese herbal medicine among case dogs (n = 4), however, GI side effect incidence did not attain statistical significance. This formula, in traditional Chinese medical principles, calms *Shen*, nourishes Heart *Yin* and Blood, and soothes Liver *Qi*, thereby treating both the outward manifestation of *Shen* disturbance and also the underlying cause.[10] This formula has been shown to be useful in human medicine for the treatment of coronary heart disease, angina, hypotension and arrythmias.[11] Like acupuncture, the selection of the appropriate Chinese herbal medicine is based on the underlying TCVM pattern and perhaps misdiagnosis of the TCVM pattern(s) led to some of the GI side effects. Further studies are needed to confirm if this formula can cause GI side effects in dogs.

This study had several limitations. First, the study sample size used in this case-control study was limited to 34 case dogs and 34 control dogs due to low response to the questionnaire-survey (2.75%). This small sample size precluded a more robust epidemiologic analysis of

exposure factors associated with a diagnosis of GI side effects after treatment with Chinese herbal medicine. Second, dogs with mild clinical signs of GI side effects could have been misclassified as control dogs, with the effect of preventing the identification of exposure factors associated with GI side effects.

In conclusion, short duration of treatment (i.e., ≤ 5 days) was the main exposure factor associated with GI side effects in dogs treated with Chinese herbal medicine. Traditional Chinese veterinary practitioners are advised to monitor dogs for GI side effects during the first five days of administration of Chinese herbs.

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FOOTNOTES

a. IACUC protocol number 201408493
b. Survey Monkey Company https://wwwsurveymonkey.com
c. OpenEpihttp://www.openepi.com/SampleSize/SSCC.htm
d. Statistix 10. Analytical Software. Tallahassee, FL, USA
e. *Shen* Calmer , Jing Tang Herbal Inc, Reddick, FL, USA
f. Body Sore , Jing Tang Herbal Inc, Reddick, FL, USA
g. External Wind, Jing Tang Herbal Inc, Reddick, FL, USA

REFERENCES

1. Cheung F. TCM: made in China. Nature 2011; 480(7378): S82-S83.
2. Xu H, Chen KJ. Complementary and alternative medicine: is it possible to be mainstream? Chinese Journal of Integrative Medicine 2012; 18(6): 403-404.
3. Poppenga RH. Herbal Medicine: Potential for intoxication and interactions with conventional drugs. Clinical Techniques in Small Animal Practice 2002; 17 (1): 6-18.
4. Shmalberg J, Hill RC, Scott KC. Nutrient and metal analyses of Chinese herbal products marketed for veterinary use. Journal of Animal Physiology and Animal Nutrition 2013; 97 (2): 305-314.
5. Trevisanello L. Preliminary Assessment of Adverse Effects of Chinese Herbal Medicine. Proceedings of the 3rd Chinese Veterinary Conference (CVC)/14th International TCVM Annual Conference, Suzhou, Jiangsu Province, China 10/28/2012 - 10/30/2012; 322-334.
6. Cossmann M, Kohnen C, Langford R et al. Tolerance and safety of tramadol use. Results of international studies and data from drug surveillance. Drugs 1997; 53(2):50-62.
7. Ball P, Stahlmann R, Kubin R et al. Safety profile of oral and intravenous moxifloxacin: cumulative data from clinical trials and post marketing studies. Clin Ther 2004; 26(7):940-50.
8. Xie HS. Toxicity of Chinese Veterinary Herbal Medicines. American Journal of Traditional Chinese Veterinary Medicine 2011; 6(2): 45-53.
9. Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosis and management. Lancet 2000; 356: 1255–1259.
10. Xie H. Chinese Veterinary Herbal Handbook. Lauren, Vanessa, Lisa (Ed.). Reddick, Florida: Jing Tang 2011: page 136.
11. Chen J. *Tian Wang Bu Xin Dan* (Emperor of Heaven's Special Pill to Tonify the Heart): Traditional Use, Pharmacological Effects and Clinical Research. Lotus Institute of Integrative Medicine – eLotus Update. Oct 2008.

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Review

Historical Review of the Origins of the Nine Different Schools of Traditional Chinese Medicine

Huisheng Xie DVM, MS, PhD

ABSTRACT

Traditional Chinese Medicine (TCM) has had a broad range of teachings which have been predominately taught in 9 schools since its origins nearly 2000 years ago. The first school which was founded by Zhang Zhongjing 150-219 CE was the earliest to establish and use a Pattern-identification system for disease treatment in TCM which was particularly important when treating exogenous or febrile diseases after invasion of a pathogen. The teachings of this school predominated until the Jin-Yuan Period (1115-1368 CE) when 4 new schools, the Cooling School, the Pathogen-attacking School, The Spleen-Stomach School and the Yin-tonic School were established. The first 2 schools enhanced treatment of Excess Pathogens while the second 2 schools extended treatment for Deficiencies. The final 4 schools were established during the Ming-Qing Dynasties (1368-1912 CE) and included the Warming Tonic School, the School of Warm Diseases, the Fire-Spirit/Yang Warming School and the Integrative School of Chinese and Western Medicine. The Warming Tonic School extended treatment for Deficiency while the School of Warm Diseases improved treatment of epidemic diseases with which the initial signs are fever (Excess Heat). The Fire-Spirit School aimed for the early treatment of Yang Collapse. The Integrative School was founded by a group of well-known physicians in the Qing Dynasty who combined Western Medicine which was brought to China in the 16th century with Chinese Medicine which became known as Traditional Chinese Medicine.

Key words: Traditional Chinese Medicine, Chinese medical history, Traditional Chinese Veterinary Medicine, herbs, herbal formula, Chinese herbal medicine

ABBREVIATIONS

TCM	Traditional Chinese Medicine
TCVM	Traditional Chinese Veterinary Medicine
BCE	Before Common Era (or BC-Before Christ)
CE	Common Era (or AD- Anno Domini)

Like other disciplines of medicine, Traditional Chinese Medicine (TCM) has had a broad range of teachings and schools since its origins nearly 2000 years ago. The school of *Shang Han* (Cold Damage) was initially recorded in the book *Shang Han Za Bing Lun* (Treatise of Cold Damage and Miscellaneous Diseases) which was written by Zhang Zhongjing and published around 200-210 CE (at the end of Han Dynasty).[1] After the school of *Shang Han*, four new schools were established during the Jin-Yuan period (1115-1368 CE) and played an important role in TCM advancement and diversity.[2] The final 4 schools were founded during the Ming-Qing Dynasties (1368 – 1912 CE) (Figure 1). An overview of these 9 different TCM schools and their veterinary clinical applications will be reviewed in this paper.

From: Chi Institute, Reddick, Florida, USA

COLD DAMAGE SCHOOL, *SHANG HAN PAI*[1]

Historical Background

There are 2 books that form the foundation of TCM and have become key basic texts. The first is *Huang Di Nei Jing* (Yellow Emperor's Classic of Medicine) which was originally produced in 475-221 BCE. The second was Zhang Zhongjing's *Shang Han Za Bing Lun* (Treatise of Cold Damage and Miscellaneous Diseases) which founded a unique TCM diagnostic system along with the Cold Damage School. This school was the earliest to establish and use a Pattern-identification system for disease treatment in TCM. Zhang Zhongjing developed this approach around the third century (150-219 CE) and published a detailed description of his diagnostic system, *Shang Han Za Bing Lun*, in 200-210 CE. This school played an important role in the development and advancement of both TCM and Traditional Chinese

Veterinary Medicine (TCVM). It provided a diagnostic method that was particularly important when treating exogenous diseases or febrile diseases after invasion of a pathogen.[1] Historically, almost all TCM and TCVM practitioners had to study Cold Damage.

Following the foundation of the Cold Damage School, there were a number of well-known physicians including Hua Tuo who used this system during the Three Kingdoms (220-280 CE) and the Jin Southern-Northern Dynasties (420-589 CE) with physicians like Ge Hong writing numerous textbooks. A well governed society emerged during the Tang (618-907 CE) and the Song Dynasties (960-1279 CE) with the Tang court government organized authors writing *Xin Xiu Ben Cao* (Newly Revised Materia Medica). During these 2 dynasties, the Office of Imperial Physicians (OIP) was in charge of TCM administration and practice and the Song OIP published the official herbal formulary text book called *Tai Yi Ju Fang* in 1078. When the OIP was changed to *He Ji Ju* (Bureau of Safe Formulary), this book was renamed *He Ji Ju Fang* or *Tai Ping Hui Min He Ji Ju Fang* in 1148. A total of 788 formulas listed in the book were collected from the Han to the Song Dynasty. This book deeply impacted the physicians during that time and many of formulas are still used, such as *Si Jun Zi Tang* (Four Gentlemen) for global *Qi* Deficiency, *Shen Ling Bai Zhu San* (Ginseng, Poria and Atractylodes) for watery diarrhea due to Spleen *Qi* Deficiency, *Si Wu Tang* (Four Substances) for Blood Deficiency, and *Shi Quan Da Bu Tang* (Ten Large Tonification) for *Qi*, Blood and *Yang* Deficiency. Due to the popularity of this book and the governmental suppression of new ideas, significant TCM innovation did not occur until the Jin-Yuan Period (1115-1368 CE) when dramatic political and social changes occurred.

Disease Pathology Philosophy

A Cold Pathogen initially invades the body and induces disease through a pathogenic process called *Shang Han* or Cold Damage (Cold Attack). The essence of the Cold Damage School is *Liu Jing Bian Zheng*, known as the Patterns of Six Channels, Six Meridians, or Six Phases. The Six Channels Pattern is a way of organizing

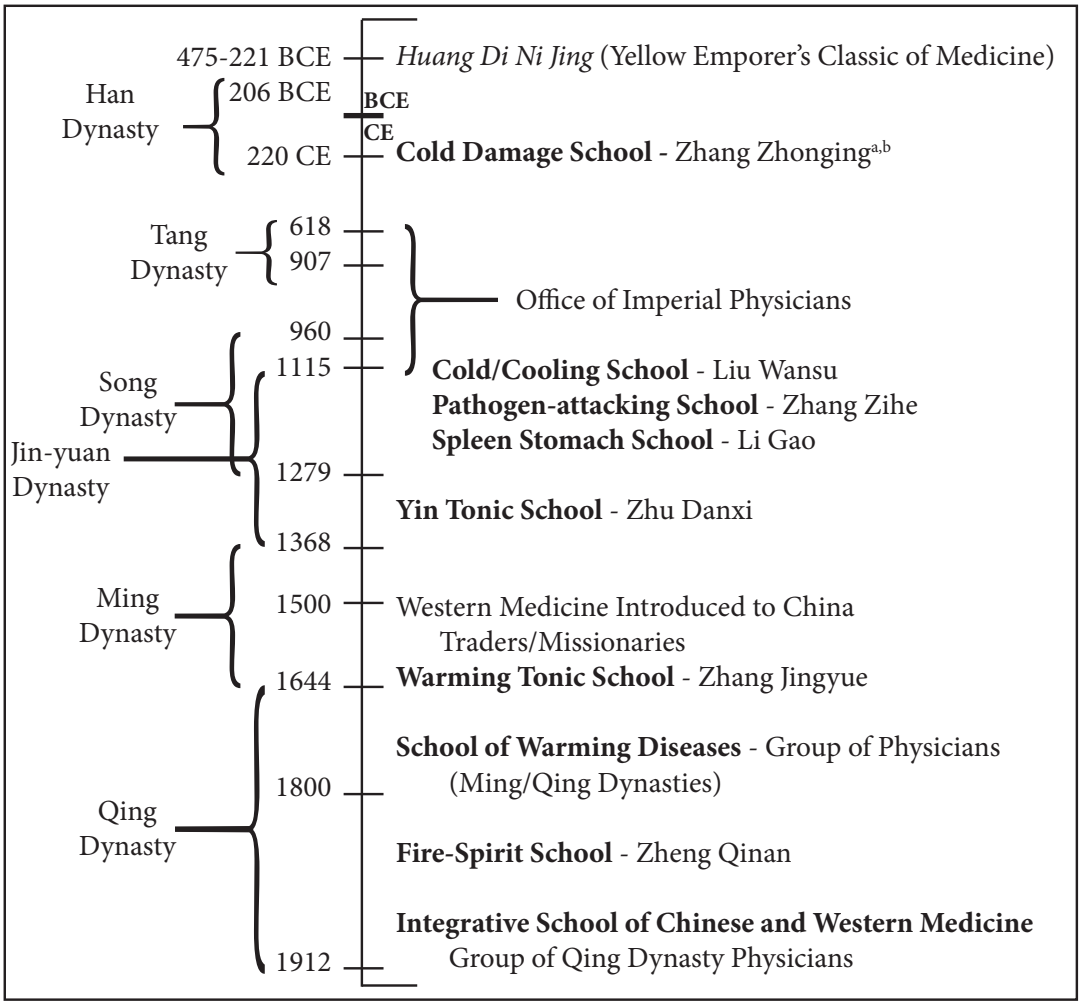


Figure 1: Historical timeline of the Nine Different Schools of TCM

a. *Shang Han Lun* (Treatise of Cold Damage)

b. *Jin Gui Yao Lue* (Synopsis of Prescriptions from the Golden Cabinet)

the six *Yang* Meridians into three pairs and the six *Yin* Meridians into three pairs so that each of the Six Channels is associated with a Meridian pair (Table 1). *Yang-Ming*, for example, includes the Large Intestine and the Stomach Meridians, and *Shao-Yin* refers to both the Kidney and Heart Meridians. Each of these pairs has associations with Exterior or Interior states of disease, particular types of Pathogens (Heat or Cold), and Excess or Deficient states.

The three *Yang* Channels, *Tai-Yang*, *Shao-Yang* and *Yang-Ming*, correlate with a range of Exterior to Interior depth, respectively. In other words, one can catch a "common cold" (*Tai-Yang* Pattern), yet this condition can progress into a deeper infection (*Shao-Yang*), as well as expressing itself into a full Heat condition as the body mounts all its resources against the invading Cold. In general, the *Yang* Patterns tend to be more Exterior conditions relative to the *Yin* Patterns.

Particular Pathogens are prone to enter through the Exterior via susceptible channels. Cold Pathogens tend to invade the *Tai-Yang* (Bladder and Small Intestine Channels) and Warm-induced Pathogens invade the *Yang-Ming* (Stomach and Large Intestine Channels). In addition, if the Pathogen continues to defeat the body's defenses, it penetrates deeper past the Exterior (*Shao-Yang*), and can further develop into an Interior Pattern (*Yang-Ming* or *Yin* Patterns).[1]

When the exogenous Pathogenic factors (*Xie-Qi*, Pathogens) invade the body, the Six Channels either fight against the *Xie Qi* or suffer disease due to the Pathogens. The disorders are summarized into the Patterns of the Six Channels, which include the *Tai-Yang*, *Shao-Yang*, *Yang-Ming*, *Tai-Yin*, *Shao-Yin*, and *Jue-Yin* Patterns. The first three, as *Yang* Patterns, represent conditions in which the body's resistance to disease (*Zheng-Qi*) is generally strong. In the last three (*Yin*) Patterns, overall, the *Zheng Qi* tends to be weak.

Important Chinese Herbal Medicines of the Cold Damage School

Xiao Chai Hu Tang (Minor Bupleurum)[3]

This formula is named for the King ingredient, *Chai*

Table 1: The Patterns of the Six Channels[1]

	Six Channels	Meridian Pair	Associations
YANG	*Tai-Yang*	Bladder Small Intestine	• Entry of Cold-Pathogens • Wind or Cold Associated • "Common cold" • Most Exterior
	Shao-Yang	Gallbladder Triple Heater	Half Exterior and Half Interior
	Yang-Ming	Large Intestine Stomach	Most superficial Interior
YIN	*Tai-Yin*	Lung Spleen	Interior Deficient Cold Pattern in the Middle *Jiao*
	Shao-Yin	Kidney Heart	*Yin* Deficiency or *Yang* Deficiency Pattern
	Jue-Yin	Liver Pericardium	The deepest Interior Pattern

Table 2: Ingredients and Actions of *Xiao Chai Hu Tang* (Minor Bupleurum)[3]

%	English Name	Chinese *Pin-Yin*	Actions
25	Bupleurum	*Chai Hu*	Relieves Liver *Qi* Stagnation
9.4	Scutellaria	*Huang Qin*	Clears Heat, dries Damp
25	Pinellia	*Ban Xia*	Resolves Phlegm
9.4	Zingiberis	*Sheng Jiang*	Warms the Middle *Jiao*
9.4	Ginseng	*Ren Shen*	Tonifies *Qi*
9.4	Glycyrrhiza	*Gan Cao*	Harmonizes, tonifies the Middle *Jiao*
12.4	Jujube	*Da Zao*	Harmonizes, tonifies the Middle *Jiao*

Actions: Harmonizes and releases *Shao Yang* disorders.

Hu (Bupleurum). The addition of *Xiao (*small or minor) in the name distinguishes *Xiao Chai Hu Tang* (Minor Bupleurum) from *Da Chai Hu Tang* (Major Bupleurum), (Table 2). Classical indications for this formula is the treatment of *Shao Yang* disease, or *a Shao Yang* level disorder where a pathogen lodges halfway between the Exterior and Interior.

The main clinical signs are alternating fever and chill, dry throat, chewing or lip-smacking as if there is a bitter taste in the mouth, irritability, mild resistance to thoracic and hypochondriac compression, nausea and vomiting, reduced appetite, normal or thin white tongue coating, and a tight or wiry pulse. The veterinary indications for this herbal formula are: Liver *Qi* Stagnation, Liver Heat along with *Qi* Deficiency and Stomach *Qi* Stagnation, Liver *Qi* Stagnation with Heat in acute viral hepatitis or acute otitis, chronic liver disease along with GI disorders, neoplasia or endocrine disorders (fever, *Qi* Deficiency and stress related Stagnation).

Shen Qi Wan (Kidney Qi Pill)[3]

Shen Qi Wan was originally described in the classical herbal book *Jin Gui Yao Lue Fang Lun* (Synopsis of Prescriptions from the Golden Cabinet) written by Zhang Zhongjing and published in 200-210 CE. The name *Shen Qi Wan* is derived from one of its primary functions which is tonifying Kidney *Qi*. Since it originates from *Jin Gui Yao Lue Fang Lun* (or *Jin Gui* for short), this herbal formula is also called *Jin Gui Shen Qi Wan*. Its ingredients have the primary actions of warming and tonifying Kidney Qi/ *Yang* (Table 3).

Indications for this Chinese herbal medicine includes Kidney *Qi* or Kidney *Yang* Deficiency with hindlimb paresis or pain, coldness in the hindlimbs and lumbar region, dysuria or polyuria, pale swollen tongue, deep and feeble pulse, Phlegm-retention, edema and diabetes. The most commonly used veterinary application is the treatment of chronic kidney disease or renal failure due to Kidney *Yang* Deficiency.

***Zhen Wu Tang* (True Warrior)**[3]

Zhen Wu Tang was originally described in the classical herbal book *Shang Han Za Bing Lun* (Treatise of Cold Damage and Miscellaneous Diseases) written by Zhang Zhongjing and published in 200-210 CE. According to Chinese folk culture, *Zhen Wu* represents the god located in the north that controls water and prevents flooding. *Zhen Wu* is needed when edema and ascites occur due to failure of osmotic regulation. *Zhen Wu Tang* translates to "True Warrior (or God that Controls the Water) Decoction."

The ingredients work together to warm Yang and drain Damp to resolve water retention (Table 4). Classical indications for the use of this Chinese herbal medicine is to treat water retention due to *Yang* Deficiency. Clinical signs of affected individuals include: chills, aversion to cold, stranguria or dysuria, palpitations, loss of balance

Table 3: Ingredients and Actions of *Shen Qi Wan* (Kidney *Qi* Pill)[3]

%	English Name	Chinese *Pin-Yin*	Action
25.9	Rehmannia Fresh	*Sheng Di Huang*	Clears heat, cools Blood and nourishes *Yin*
15.6	Dioscorea	*Shan Yao*	Strengthens the Spleen and tonifies *Jing*
15.6	Cornus	*Shan Zhu Yu*	Tonifies Liver and Kidney *Yin*, and nourishes *Jing* and Blood
11.7	Alisma	*Ze Xie*	Eliminates water retention and clears False Fire
11.7	Poria	*Fu Ling*	Strengthens the Spleen, promotes diuresis, excretes Dampness
11.7	Moutan	*Mu Dan Pi*	Cools Blood, resolves Blood Stagnation, clears Heat
3.9	Cinnamomum	*Gui Zhi*	Warms the channels to dispel Cold, tonifies *Yang*
3.9	Aconite	*Fu Zi*	Warms the Kidney, tonifies *Yang*, relieves pain and dispels Cold

Actions: Warms and tonifies Kidney *Yang.*

Table 4: Ingredients and Actions of *Zhen Wu Tang* (True Warrior)[3]

%	English Name	Chinese *Pin-Yin*	Actions
21.4	Aconite	*Fu Zi*	Warms *Yang-Qi*, dispels Cold, revives *Yang* for resuscitation
21.4	Poria	*Fu Ling*	Drains Damp, strengthens the Spleen
21.4	Paeonia	*Bai Shao Yao*	Tonifies *Yin* and harmonizes the Interior
21.4	Zingiberis	*Sheng Jiang*	Warms the Stomach to dispel Cold
14.4	Atractylodes	*Bai Zhu*	Tonifies Spleen *Qi*, dries Damp

Actions: Warms *Yang*, drains Damp to resolve water retention.

or disorientation, edema and pain of all four limbs, ascites, diarrhea, a pale and swollen tongue with tooth marks (indentations), a white, greasy coating of the tongue, and a deep, weak pulse. Veterinary use primarily includes treatment of ascites or edema associated with congestive heart failure due to *Yang* Deficiency. Cautions when using this formula include avoid feeding pork, peach and plum and it is contraindicated in cases with projectile vomiting or pregnancy.

***Si Ni Tang* (Four Frigid Extremities)**[3]

Si Ni meaning four and rebel refers to the fact that the four limbs are so cold that they are frigid and stiff (rebelling). *Si Ni Tang* was originally described in the classical herbal book *Shang Han Za Bing Lun* (Treatise of Cold Damage and Miscellaneous Diseases) written by Zhang Zhongjing and published in 200-210 CE. The ingredients warm the Middle Burner to dispel Cold and revives Yang for resuscitation (Table 5). The classical indication for this herbal formula is *Yang* exhaustion or collapse. The primary clinical signs are cold extremities, severe depression or loss of consciousness, a grey or dark tongue, and a feeble pulse. Veterinary use is primarily for hypothyroidism and Cushing's disease due to severe *Yang* Deficiency. Cautions when using the formula include avoiding its use in cases with *Qi* Stagnation in the Interior caused by Blood Deficiency or any Heat condition. This formula is contraindicated in pregnancy.

THE FOUR SCHOOLS OF THE *JIN-YUAN* PERIOD (1115-1368 CE)[2]

Historical background

Following the foundation of the Cold Damage School, there were quite a few well-known physicians including Hua Tuo who used this system during the Three Kingdoms (220-280 CE) and physicians like Ge Hong during the Jin Southern-Northern Dynasties (420-589 CE) who wrote numerous textbooks. A well governed society emerged during the Tang (618-907 CE) and the Song Dynasties (960-1279 CE), with the Tang court government commissioning authors to write the *Xin Xiu Ben Cao* (Newly Revised Materia Medica). During these 2 dynasties, the Office of Imperial Physicians (OIP) was in charge of TCM administration and practice and the Song OIP published the official herbal formulary text book called *Tai Yi Ju Fang* in 1078. When the OIP was changed to *He Ji Ju* (Bureau of Safe Formulary), this book was renamed *He Ji Ju Fang* or *Tai Ping Hui Min He Ji Ju Fang* in 1148. A total of 788 formulas listed in the book were collected from the Han Dynasty to the Song Dynasty. This book deeply impacted the physicians during that time and many of its formulas are still used, such as *Si Jun Zi Tang* (Four Gentlemen) for global *Qi* Deficiency, *Shen Ling Bai Zhu San* (Ginseng, Poria and Atractylodes) for watery diarrhea due to Spleen *Qi* Deficiency, *Si Wu Tang* (Four Substances) for Blood Deficiency, and *Shi Quan Da Bu Tang* (Ten Large Tonification) for *Qi*, Blood and *Yang* Deficiency. Due to the popularity of this book and the governmental suppression of new ideas, a significant TCM innovation did not occur until the Jin-Yuan Period (1115-1368 CE) when dramatic political and social changes were made.

The Northern Song Dynasty (960-1127) was culturally one of the most brilliant eras in Chinese history with its capital located at Bianliang (modern Kaifeng) in the Northern part of China. The Song dynasty was defeated in 1125 by the Jurchen, a semi-nomadic people from northeast Asia. The Jurchen founded their own Jin Dynasty in the north while the Song court reestablished itself in the south in Hangzhou and was historically called the Southern Song Dynasty (1127-1279).The Jin Dynasty (in the north; 1115-1234) coexisted with the Southern Song Dynasty and then was ousted by the neighboring Mongolians who formally established the Yuan Dynasty (1271-1368) with Yuandadu (currently Beijing) as their capital. They took control of the rest of the country (eventually spreading west as far as Europe). The Yuan Dynasty was replaced by the Ming Dynasty in 1368.

During the Jin-Yuan Period (1115-1368 CE), there were 4 TCM Schools established: the Cooling School, the Pathogen-attacking School, the Spleen-Stomach (Earth-tonic) School and the *Yin*-tonic School. Approaches to disease treatment expanded during this period. It was a necessity due to the large number of sick people resulting from long-periods of war, rebellions, chaos, natural disasters and plagues such as Black Death. The Bubonic Plague or Black Death caused by *Yersinia pestis* was carried and spread along the Silk Road with the Mongol armies during this period. The plague resulted in the deaths of an estimated 75 to 200 million people world-wide and peaked in Europe in the years 1346-1353 CE. During

Table 5: Ingredients and Actions of *Si Ni Tang* (Four Frigid Extremities)[3]

%	English Name	Chinese *Pin-Yin*	Actions
37.5	Aconite	*Fu Zi*	Warms *Yang-Qi* to dispel Cold, revives *Yang* for resuscitation
37.5	Zingiberis	*Gan Jiang*	Warms the Middle Burner to dispel Cold
25	Glycyrrhiza	*Gan Cao*	Harmonizes the other herbs actions

Actions: Warms the Middle Burner to dispel Cold, revives *Yang* for resuscitation.

this period, Chinese medicine (and culture) survived, flourished and grew. It appears that this was both related to the large number of sick people with great need and that the foreign rule of the Mongols allowed for a certain amount of revelation and renewal in Chinese thought. In addition to new medical thought, a new philosophy of Neo-Confucianism took hold with the introduction of Daoism and Buddhism. These were huge changes as Confucianism had been the mainstream of belief of the Han people who had ruled China since its beginning. This was also the period a Venetian merchant traveler, Marco Polo (1254-1324 CE) became a Yuan court officer during his 17 year stay in China (1292 CE) and was the first European to leave a detailed chronicle of his experiences with the Chinese culture.

COLD/COOLING SCHOOL, *HAN LIANG PAI* [4]

Historical Perspective

The Cold/Cooling School was created by Liu Wansu or Liu Hejian (1110-1200 CE) and is also called the Hejian School since Liu was born in Hejian, Hebei province, China. Liu's followers and students advanced this school with systematic explanations of etiology, pathology, treatment methods and the herbology of Fire and Heat. It is sometimes called the Fire/Heat School, which became the main foundation of the later Warm/Heat School.

Disease Pathology Philosophy

Liu Wansu's fundamental theory was based on his observations that "all Six Pathogens can be transformed into Fire" from *Su Wen* (General Question). During this unstable society and war period, the primary diseases Liu Wansu had seen were associated with fever and inflammation, therefore, the cooling herbals and foods were his primary materia medica. Pungent and cool herbals (such as *Bo He* Mentha) or sweat and cold diaphoretic herbals (such as *Hua Shi* Talcum) were used for Heat/Fire in the Exterior. Cold purgative herbals including *Da Huang* Rheum were used for Heat/Fire in the Interior and herbal formulas such as *Fang Feng Tong Sheng San* were designed to treat Fire/Heat in both the Exterior and Interior.

Table 6: Ingredients and Actions of *Fang Feng Tong Sheng San*[3]

%	English Name	Chinese *Pin Yin*	Action
4.1	Ledebouriella	*Fang Feng*	Expels Wind, eliminates Damp, relieves pain
4.1	Schizonepeta	*Jing Jie*	Disperses Wind-Cold
4.1	Forsythia	*Lian Qiao*	Clears Heat and toxins, subdues inflammatory swelling
4.1	Ephedra	*Ma Huang*	Promotes sweating, relieves asthma
4.1	Mentha	*Bo He*	Clears Wind-Heat, relieves sore throat, eliminates Heat and toxins
4.1	Ligusticum	*Chuan Xiong*	Moves *Qi* and Blood, relieves pain
4.1	Angelica	*Dang Gui*	Nourishes and moves Blood
4.1	Paeonia	*Bai Shao*	Nourishes Blood, restrains *Yin*, pacifies the Liver
4.1	Atractylodes	*Bai Zhu*	Strengthens the Spleen, dries Damp, tonifies *Qi*
4.1	Gardenia	*Zhi Zi*	Clears Heat and Fire away from the Three Burners (*San Jiao*)
4.1	Rheum	*Da Huang*	Purges the Large Intestine, clears Heat
4.1	Mirabilitum	*Mang Xiao*	Purges Fire, loosens the bowels
8.2	Gypsum	*Shi Gao*	Clears Heat or Fire in the Lung and Heat in the muscles
8.2	Scutellaria	*Huang Qin*	Clears Heat in the Lung
8.2	Platycodon	*Jie Geng*	Transforms Phlegm, guides other herbs to the upper part of the body
8.2	Glycyrrhiza	*Gan Cao*	Harmonizes
18	Talcum	*Hua Shi*	Drains Damp-Heat

Actions: Relieves Exterior Wind-Heat and purges Interior Heat.

Table 7: Ingredients and Actions of *Liu Yi San*[3]

%	English Name	Chinese *Pin Yin*	Action
85.71	Talcum	*Hua Shi*	Drains Fire and Damp-Heat, induces diuresis
14.29	Glycyrrhiza	*Gan Cao*	Harmonizes

Actions: Removes Summer-Heat, drains Damp, inducing diuresis.

Important Chinese Herbal Medicines of Cold/Cooling School

***Fang Feng Tong Sheng San* (Ledebouriella Passing the Sage)**[3]

Fang Feng Tong Sheng San was originally described in the classical herbal book *Xuan Ming Lun Fang* (Formula from the Discussion Illuminating) written by Liu Wansu, and published in 1172 CE. The herbal formula translation comes from *Fang Feng* (Ledebouriella), one of the King herbs with *Tong* (pass), *Sheng* (Sage) and *San* (powder) which literally means "Ledebouriella Passing the Sage".

Its ingredients promote the relief of Exterior Wind-Heat and purge Interior Heat (Table 6). Indications for this Chinese herbal medicine includes treating Excess Patterns of both the Interior and Exterior due to Wind invasion and internal accumulation of Heat. This is manifested as aversion to cold, high fever, constipation, dark yellow urine, papules, pustules, eczema, pruritus, a red dry tongue, and a fast pulse. The most common veterinary use is treatment of upper airway infections, influenza, and dermatitis due to both Exterior and Interior Excess Heat. This formula is contraindicated in patients with constitutional *Qi* and *Yin* Deficiency, in geriatric patients, and during pregnancy.

***Liu Yi San* (Six One Powder)**

Liu Yi San was originally described in the classical herbal book *Shang Han Zhi Ge* written by Liu Wansu. It is a simple formula consisting of *Hua Shi* (Talcum) and *Gan Cao* (Glycyrrhizae) and the ingredients remove Summer-Heat, induce diuresis and drain Damp (Table 7). Indications for this Chinese herbal medicine include treatment of Summer-Heat with Dampness marked by fever, lethargy, intense thirst, diarrhea and short urination. Other uses include mild heat/sun stroke, cystitis, urinary calculi and topically for heat rash. Its primary veterinary application is heat stroke.

PATHOGEN-ATTACKING SCHOOL, *GONG XIA PAI*[5]

Historical Perspective

The Pathogen-attacking School was created by Zhang Zihe (1151-1231 CE). He was also known as Zhang Congzheng and was one of Liu Wansu's students. He believed that the medicines and strategies of *Shang Han* School (Han Dynasty) were not designed or useful for present-day illnesses.

Disease Pathology Philosophy

His best known theory is the "Six Doors and Three Methods". Six Doors refers directly to the Six Pathogens (Wind, Summer-Heat, Dampness, Fire, Dryness and Cold) that are the main cause of any disease. The three methods are: sweating (diaphoresis), vomiting (emesis) and purging (purgation).The body versus disease is like a nation experiencing an attack. If the foreign invaders are kept away from the frontier, the nation will be safe. Pathogens (the cause of diseases) if attacked and kept from entering the body will result in the body remaining intact. Zhang Zihe's main treatment strategies, therefore, were to attack and get rid of any pathogens before they gained access to the body by using sweating, vomiting and purging. Thereby attacking the pathogens can protect *Zheng*.

Sweating can eliminate the pathogens on the body's surface. Zhang Zihe had about 40 favorite herbs including *Jing Jie* (Schizonepeta) and *Bai Zhi* (Angelica Dahurica), which are still used today. He used other methods including moxibustion, acupuncture, massage, steaming, washing and exercising to induce diaphoresis. This method was useful for an Exterior Pattern and also a combination of Exterior and Interior Patterns.

Zhang's vomiting method (emesis) included increasing salivation, lacrimation and sneezing in addition to inducing vomiting. Two commonly used herbs were

Table 8: Ingredients and Actions of *Mu Xiang Bing Lang Wan*[3]

%	English Name	Chinese *Pin-Yin*	Actions
5	Saussurea	*Mu Xiang*	Regulates *Qi*
5	Areca	*Bing Lang*	Expels Parasites/Regulates *Qi*
15	Rheum	*Da Huang*	Purgative
20	Pharbitis	*Qian Niu Zi*	Harsh Expellent
5	Citrus	*Qing Pi*	Regulates *Qi*
5	Citrus	*Chen Pi*	Regulates *Qi*
20	Cyperus	*Xiang Fu*	Regulates *Qi*
5	Zedoaria	*E Zhu*	Invigorates Blood
5	Coptis	*Huang Lian*	Clears Heat, dries Damp
15	Phellodendron	*Huang Bai*	Clears Heat, dries Damp

Actions: Promotes the movement of *Qi*, guides Stagnation out, purges accumulation, and drains Heat.

Chang Shan (Dichroa Root) and the stalk of *Gua Di* (Cucumis Melo). Acupoint SP-8 (*Di Ji*) also encouraged vomiting. This method proved useful for food poisoning and food stasis. It was also used for any Excessive Patterns in the Upper *Jiao* including headaches and Wind-Phlegm leading to stroke and pain in the chest.

Zhang's purging method included enhancing parturition, producing milk yield, softening the hardness, expelling water (diuretic purgatives), breaking up Blood Stasis and moving *Qi* in addition to the traditional purgative effects (laxative). It is often used for exogenous climatic pathogens and emotional stress factors that lodge in the chest or abdomen and cause *Qi*-Blood Stagnation leading to intense pain. The common herbs used with this method include *Qian Niu Zi* (Pharbitis), *Ba Dou* (Croton), *Gan Sui* (Euphorbia), *Yuan Hua* (Genkwa) and *Da Huang* (Rhubarb).

Important Chinese Herbal Medicines of the Pathogen-attacking School

Zhang Zihe's main contribution to TCM was to use three attacking therapeutic principles for most Excess Patterns. He was primarily rebelling against the classical tonifying and warming therapies from Zhang Zhongjing's *Shang Han* School. Even though he is not known for any specific herbal formulas, there are a few herbal formulas created by him that are still used today. One of these formulas is *Mu Xiang Bing Lang Wan* (Saussurea and Areca).

***Mu Xiang Bing Lang Wan* (Saussurea and Areca),**

Mu Xian Bing Lang Wan was originally described in the classical herbal book *Ru Men Shi Qin* (Confucians Duties to Their Parents) written by Zhang Zihe and published in 1228 CE. This formula was named for the two King ingredients, *Mu Xiang* (Saussurea) and *Bing Lang* (Areca). *Wan* means pill, the form of original herbal administration. *Mu Xiang Bing Lang* literally translates to "Saussurea and Areca".

The ingredients promote the movement of *Qi*, guide Stagnation out, purges accumulation and drains Heat (Table 8). Indications for this Chinese herbal medicine include focal or generalized distention, pain and fullness in the abdomen with constipation or malodorous diarrhea. Clinical signs demonstrated by patients usually are a reddish tongue with a greasy, yellow coating, and a slippery, full or rapid pulse. Veterinary use is primarily for the treatment of acute onset colitis or dysentery due to Damp-Heat in the gastrointestinal tract in horses, dogs and cats. This formula is contraindicated in cases of Deficiency and pregnancy.

SPLEEN-STOMACH (EARTH-TONIC) SCHOOL, *BU TU* (*PI WEI*) *PAI*[6]

Historical Perspective

This school was founded by Li Gao (1180-1251), later known as Dongyuan who was a native of Zhengding, Hebei Province during the Jin Dynasty.

Disease Pathology Philosophy

Li Gao was best known for his theory that the majority of diseases are caused by injury to the Spleen and Stomach (Earth System). All diseases, therefore, are related to the Spleen and Stomach, especially when deficient. There are three things that can damage the Spleen and Stomach: improper food intake (especially consumption of excess amounts of cold, raw, fatty, or unclean foods); overwork/overstrain (exhaustion, fatigue during the middle of the day) and mental irritation (excessive emotions that agitate the Heart Fire and thereby damage its Child Element-Spleen).

Li Gao believed "Any improper diet can cause Stomach and Spleen diseases. If the patient feels fatigue, he or she has a Spleen Deficiency". "Internal injuries of the Spleen and Stomach actually damage the body's *Qi*, while the External invasion of Wind and Cold attacks the body surface and can cause an Excess Pattern". His approach was that the Spleen and Stomach are the key organs for treatment and prevention of any disease. Since diseases of the Spleen and Stomach tend to be more deficient in

Table 9: Ingredients and Actions of *Bu Zhong Yi Qi Tang*[3]

%	English Name	Chinese *Pin-Yin*	Action
29	Astragalus	*Huang Qi*	Tonifies *Qi* to invigorate *Yang*, benefits the Lung to strengthen the body, promotes diuresis and relieves edema
14.5	Glycyrrhiza	*Gan Cao*	Harmonizes the effects of other herbs
9.7	Ginseng	*Ren Shen*	Replenishes the Source (*Yuan*) *Qi*, tonifies the Spleen and Lung
4.8	Angelica	*Dang Gui*	Nourishes Blood and activates circulation
8	Citrus	*Chen Pi*	Regulates Spleen *Qi*, dries up Dampness, transforms Phlegm
9.7	Cimicifuga	*Sheng Ma*	Clears Heat and toxins and lifts up Spleen *Qi*
9.7	Bupleurum	*Chai Hu*	Disperses Stagnant Liver *Qi* and raises *Yang Qi*
14.6	Atractylodes	*Bai Zhu*	Strengthens the Spleen, dries up Dampness, tonifies *Qi*

Actions: Replenish*es Qi* in the Middle Burner, raises Spleen *Yang* to treat any prolapsed organs.

nature, tonification should be used which underlies an additional name given to the school, The Earth-tonic School. Unlike the Cold/cooling School and the Pathogen-attacking School with which tonics and warming therapies were generally disliked, the Spleen-Stomach school relied on warming and tonifying remedies and became known as the School of Warm Tonification.

Important Chinese Herbal Medicines of the Spleen-Stomach School

Li Gao believed that the main pathology in Spleen Deficiency was the sinking of *Zhong Qi* (Middle *Jiao Qi*), so his treatment strategies included raising the Spleen *Qi*. Two of his most important formulas were *Sheng Yang Yi Wei Tang* and *Bu Zhong Yi Qi Tang*. Both formulas include *Ren Shen* (Ginseng), *Huang Qi* (Astragalus), and Cang *Zhu* (Atractylodes) to tonify the *Qi*, and both utilized *Chai Hu* (Bupleurum) to raise *Qi*. He also formulated *Sheng Mai San* (Pulse Generating Powder), a formula that is widely used in modern Chinese hospitals, and *Dang Gui Bu Xue Tang*, a combination of *Huang Qi* (Astragalus) and *Dang Gui* (Angelica) in a ratio of 5:1; used to nourish the *Qi* and Blood following extensive blood loss.

***Bu Zhong Yi Qi Tang* (Tonifying the Middle and Reinforcing *Qi*)**

Bu Zhong Yi Qi Tang was originally described in *Nei Wai Shang Bian Huo Lun* (Clarifying Doubts about Injury from Internal and External Causes) written by Li Gao and published in 1247. This formula was named after its functions: *Bu* (tonifying), *Zhong* (middle referring to Middle Burner), *Yi* (reinforcing or to benefit) and *Tang* (decoction, the form of herbal administration). Literally *Bu Zhong Yi Qi* can be translated as tonifying the Middle and reinforcing *Qi*.

The ingredients combine to replenish *Qi* in the Middle Burner and raises Spleen *Yang* to treat any prolapsed organ (Table 9). Indications for this Chinese herbal medicine includes prolapse due to Deficient *Qi* which is marked by poor appetite, lassitude, loose stool, and thirst with a preference for heated liquids. The formula is also used for rectal and uterine prolapse, prolonged diarrhea, protracted dysentery, a pale large tongue with thin white coating, and a deep weak pulse. The most common veterinary applications include fecal incontinence due to Spleen *Qi* Deficiency and chronic diarrhea in geriatric patients. This formula is contraindicated for cases with fever due to *Yin* Deficiency.

***Dang Gui Bu Xue Tang* (Angelica to Tonify Blood)**

Dan Gui Bu Xue Tang was also described in *Nei Wai Shang Bian Huo Lun* (Clarifying Doubts about Injury from Internal and External Causes) written by Li Gao in 1247. The formula name translates to *Dang Gui* (Angelica) which is the King herb, *Bu Xue* (tonify Blood) and *Tang* (decoction) as the form of herbal administration.

The ingredients combine to Tonify *Qi* and to promote generation of Blood (Table 10). Indications for this Chinese herbal medicine include Blood Deficiency with *Yang* floating marked by fever, polydipsia, a large weak pulse when pressed or acute blood loss after parturition or trauma. Veterinary use is primarily for anemia due to blood loss as a result of surgery or parturition. Combined with *Gui Pi Tang*, this herbal formula can be used for the treatment of immune-mediated thrombocytopenia. This formula is contraindicated for patients with *Yin* Deficiency and recurrent fever typically with afternoon spikes.

YIN-TONIC SCHOOL (NOURISHING *YIN* SCHOOL), *YANG YIN PAI*

Historical Perspective

The *Yin*-tonic School was created by Zhu Danxi (also known as Zhu Zhenheng), 1280-1358 CE. Danxi means Red Stream, a stream near where he was born in Yiwu, Zhejiang. Late scholars and people respected him as Master Danxi and Mr. Danxi. Master Danxi had studied the books of Liu Wansu, Zhang Zihe and Li Gao, all of who lived in Northern China where the majority of people were taller and had a strong constitution. He also understood the essences of the Cold/cooling, Pathogen-attacking and Earth-tonic Schools. He insisted that "when studying medicine one must read the *Nei Jing* and *Nan Jing*". His *Yin*-tonic teaching was the last of the four TCM schools originating during the Jin-Yuan Period. Danxi said "when people use previous formulas to treat various current diseases, it is to look for the sword by marking the boat". Zhu Danxi wrote several books, some of his most important teachings were gathered and published as *Dan Xi Xin Fa* (The Essential Methods of Danxi) and *Ge Zhi Yu Lun* (Extra Treatises of Investigation).

The Nourishing *Yin* School gained more followers around the end of the Ming Dynasty as the result of work by Zhang Jingyue, a competent proponent of using *Shu Di Huang* (Rehmannia) and other *Yin*-nourishing herbs.

Table 10: Ingredients and Actions of *Dang Gui Bu Xue Tang* [3]

%	English Name	Chinese *Pin-Yin*	Action
83	Astragalus	*Huang Qi*	Replenishes *Qi* to invigorate *Yang*, benefits the Lung to strengthen the body
17	Angelica	*Dang Gui*	Nourishes Blood and activates Blood circulation

Actions: Tonifies *Qi* to promote the generation of Blood.

During the Qing Dynasty, the famous Rehmannia Six Formula, originally a pediatric prescription, became the subject of much interest, including the development of numerous modifications. The Rehmannia Six Formula and its modifications are the basis for much of the modern application of the Nourishing *Yin* School's basic tenets.

Disease Pathology Philosophy[7,8]

Danxi lived in Southern China where the majority of people were smaller and had a weak constitution. They also lived an overindulgent lifestyle. He had observed that people suffered from chronic disease mainly due to overindulgence in pleasurable things and activities (too much sex and alcohol consumption), which damaged *Yin* Essence. He proposed his famous theory "*Yang* often is plentiful while *Yin* always tends toward Deficiency". He favored using tonic formulas, especially those that nourished the Kidney and Liver. Unlike the warming and tonic herbal formulas (*Shi Quan Da Bu*, *Si Wu Tang* and *Si Jun Zi Tang*) from *He Ji Ju Fang*, he believed *Yin* Tonics were crucial in treating patients because their *Yang* is already over abundant.

In addition, Master Danxi proposed the difference between *Jun Huo* (Emperor Fire) and *Xiang Huo* (Minister Fire). The Emperor Fire is associated with the Heart, and keeps a person alive, and without it, people will die. On the other hand, the Minister Fire is stored in the Kidney, it keeps a person warm and provides the individual with vitality and energy. This Fire is also a moving *Yang* that gives rise to human desires. If this Minister Fire becomes too strong because those desires are excessive, it will pathologically impair the *Yin* Essence (*Yin*, Blood and Kidney Essence), leading to False Heat (False Fire) and causing Fire symptoms. Indulgent alcohol consumption, excessive sexual desires and activity induce the Minister Fire to flare up consequently damaging the *Yin* Essence leading to *Yin* Deficiency and False Heat. Master Danxi believed that one must use caution using Zhang Zihe's Pathogen-attacking teaching for Excess conditions. He also warned that the use of warm, dry or spicy herbs that Li Gao's Earth-tonic School relied upon, could harm the *Yin* if improperly used. Master Danxi believed that the best treatments were cooling herbs to tonify Kidney *Yin* and help anchor the Minister Fire in addition to temperance in sex and diet to restore the balance of *Yin* and *Yang*.

Table 11: Ingredients and Actions of *Da Bu Yin Wan*[3]

%	English Name	Chinese *Pin-Yin*	Action
30	Rehmannia Prepared	*Shu Di Huang*	Nourishes Blood and *Yin*
30	Testudinis Prepared	*Gui Ban Jiao*	Nourishes *Yin* to suppress Excess Liver *Yang*, reinforces the Kidney to strengthen the bones
20	Phellodendron Parched	*Huang Bai*	Nourishes *Yin* and clears Kidney False Fire
20	Anemarrhena Parched	*Zhi Mu*	Moistens dryness through nourishing *Yin*

Actions: Nourishes *Yin* and clears Fire.

Table 12: Ingredients and Actions of *Hu Qian Wan*[3]

%	English Name	Chinese *Pin-Yin*	Action
25	Phellodendron	*Huang Bai*	Clears Fire from the Kidney and resolves Damp-Heat
20	Testudinis (Prepared)	*Gui Ban Jiao*	Nourishes *Yin* to soothe Liver *Yang*, tonifies the Kidney, strengthens the bones, nourishes Blood
5	Anemarrhena (baked)	*Zhi Mu*	Clears Lung and Stomach Heat and moistens dryness through nourishing *Yin*
10	Rehmannia (Prepared)	*Shu Di Huang*	Nourishes Blood and *Yin*
10	Citrus	*Chen Pi*	Regulates Spleen *Qi*, dries up Dampness
10	Paeonia	*Bai Shao*	Nourishes Blood, pacifies the Liver and relieves pain
7.5	Cynomorium	*Suo Yang*	Warms *Yang* and replenishes Essence
10	Cyathula	*Chuan Niu Xi* *	Strengthens the Kidney and benefits the knees
2.5	Zingiberis	*Gan Jiang*	Disperses Spleen and Stomach Cold for the treatment of abdominal pain

Actions: Nourishes *Yin* and purges Fire, strengthens the tendons and bones.

***** Tiger bone *Hu Gu* (Os tigris) was originally used in this formulation. *Chuan Niu Xi* (Cyathula) is substituted because the tiger is endangered and can no longer be used.

Important Chinese Herbal Medicines of the *Yin* Tonic School

Da Bu Yin Wan (Greatly Replenishing *Yin*)

Da Bu Yin Wan was originally described in the classical book *Dan Xi Xin Fa* (The Essential Methods of Danxi) written by Zhu Danxi and published in 1481. *Da* (great or big), *Bu* refers to tonify or replenish and *Wan* refers to pill, the form of the original herbal administration. *Da Bu Yin Wan* was thus named after its function of greatly replenishing the *Yin*. This formula is a representative formula of Zhu Danxi and consists of 4 ingredients: *Shu Di Huang* (Rehmannia), *Huang Bai* (Phellodendron), *Zhi Mu* (Anemarrhena), and *Gui Ban* (Tortoise Shell), (Table 11). The first three ingredients became the main ingredients of the most widely used formula for a *Yin* Deficiency with False Fire, *Zhi Bai Di Huang Wan*.

The ingredients of *Hu Qian Wan* work together to Nourish *Yin* and clear Fire. Indications for this Chinese herbal medicine formula is to treat severe False Fire due to *Yin* Deficiency accompanied by what is described in humans as a steaming sensation in the bones, recurrent fever with afternoon spikes, cough, hemoptysis, feverish sore knees and feet, a red tongue with little coating and a rapid forceful pulse. The most commonly used veterinary application is the treatment of severe Deficiency Patterns with False Fire (Heat). Clinical signs include a red and dry tongue, fast strong pulse, panting, cool-seeking behavior, and warm ears and body. It also is effective in geriatric conditions including hyperthyroidism, hyperadrenocorticism and *Wei* Syndrome. *Da Bu Yin Wan* can be used when *Zhi Bai Di Huang Wan* is not strong enough to treat False Heat due to *Yin* Deficiency. Both herbal formulas may be used interchangeably in clinical settings. This formula should not be used too frequently or for a prolonged period of time due to its likelihood of promoting Dampness.

Hu Qian Wan (Hidden Tiger)

Hu Qian Wan was also described in the classical book *Dan Xi Xin Fa* (The Essential Methods of Danxi) written by Zhu Danxi in 1481. The name translated as *Hu* (tiger), *Qian* (hidden) and *Wan* (pill) refers to the hidden tiger lying on the ground. In Chinese culture, as the tiger lies on the ground it becomes even stronger and more powerful as it receives the *Yin* from both heaven and earth. *Hu Qian* is named for its action of tonifying *Yin* leading to enhancing the strength of the bones and tendons.

Its ingredients work together to Nourish *Yin*, purge Fire and strengthen the bones and tendons (Table 12). This formula is indicated for soreness and weakness of the lumbar spine and stifles, tendonopathies, osteopenia, muscle atrophy of the limbs, altered ambulation or ataxia, a red tongue with little coating, and a thready weak pulse. Veterinary application is usually for hindlimb paresis (*Wei* Syndrome) due to *Yin* Deficiency. This Chinese herbal medicine may also be appropriate for clinical patterns resulting from degenerative myelopathy. The use of this formula is contraindicated in paresis of the hindlimbs due to Dampness or invasion by exogenous pathogenic factors.

FOUR SCHOOLS FOUNDED DURING THE MING-QING DYNASTY (1368-1912 CE)

WARMING TONIC SCHOOL, *WEN BU PAI*[9]

Historical Background

The Warming Tonic School was founded by Zhang Jingyue (1563-1640 CE), also called Zhang Jiebin. He was born in Shaoxing, Zhejiang during the late Ming Dynasty. He had an immense impact on the development and advancement of TCM. He was respected as "the only physician 1,000 years later after Zhang Zhongjing". Zhang's two major works are the *Lei Jing* (a study of the *Nei Jing*) and *Jing Yue Quan Shu* (Collected Treatises of Zhang Jingyue).

Disease Pathology Philosophy

Due to Liu Wansu's Cooling School and Zhu Danxi's *Yin*-tonic school being dominant in TCM practice during

Table 13: Ingredients and Actions of *You Gui Wan*

%	English Name	Chinese *Pin-Yin*	Action
21.1	Rehmannia	*Shu Di Huang*	Nourishes *Yin* and Blood
10.5	Dioscorea	*Shan Yao*	Tonifies the Spleen and Lung
7.9	Cornus	*Shan Zhu Yu*	Tonifies the Liver and Kidney *Yin*, and nourishes *Jing*
10.5	Lycium	*Gou Qi Zi*	Nourishes the Liver and Kidney *Yin*, improves eyesight
10.5	Cuscuta	*Tu Si Zi*	Tonifies the Liver and Kidney *Yin*, strengthens Kidney *Yang*
10.5	Cervus	*Lu Jiao Jiao*	Nourishes the Kidney and Blood
10.5	Eucommia	*Dang Gui*	Tonifies the Liver and Kidney *Yang*, strengthens the tendons and bones
7.9	Angelica	*Du Zhong*	Nourishes Blood and activates circulation
5.3	Cinnamomum	*Rou Gui*	Warms and tonifies the Kidney and Spleen *Yang*, disperses Internal Cold
5.3	Prepared Aconite	*Fu Zi*	Warms and tonifies *Yang*, relieves pain and dispels Cold

Actions: Warms and nourishes Kidney *Yang*, and replenishes *Jing* and Marrow.

the Ming Dynasty, Zhang Jingyue observed the abuse of Cold/cooling herbs which often damaged the Spleen and Stomach causing a *Yang* Deficiency. He believed that the body's vitality and *Qi* originated from *Yang* which is hard to generate but easy to lose and very difficult to recover when lost. He supported Danxi's theory with which *Yin* always tends toward Deficiency. He proposed that "*Yin* tends to be Deficiency while *Yang* is not sufficient". He therefore favored tonifying both *Yin* and *Yang*, especially tonifying the Kidney. He called this *Yin* and *Yang* as *Yuan Yang* and *Yuan Yin* which are stored in the Kidney. Zhang Jingyue stated: "For the treatment of a Deficiency and Excess, the general rule is that Excess patients accept Cold while Deficiency patients accept Heat. Tonification therefore must be warming, and sedation must be cooling". His favorite warming herbs included *Fu Zi* (Aconite), *Rou Gui* (Cinnamon), *Gan Jiang* (Dry ginger) and *Ren Shen* (Ginseng).

Important Chinese Herbal Medicines of the Warming Tonic School

Zhang Jingyue's formulas are specific and simple with each formula consisting of an average of 6 ingredients. Out of 186 of Zhang's formulas, 146 (78%) contain 8 or fewer ingredients, and only 13 (7%) consist of 10 or more ingredients. His favorite herbs were *Shu Di Huang* (Rehmannia), *Dang Gui* (Angelica) and G*ou Qi Zi* (Lycium). He was respectfully called Master Di Huang because of his frequent use of Rehmannia *Shu Di Huang*. There are 47 formulas (25%) containing Rehmannia *Shu Di Huang* among his 186 recipes. He created 29 special herbal formulas, 21 of them (72%) contained Rehmannia *Shu Di Huang*. Many of his formulas are modified ancient classical formulas. For example, *Zuo Gui Wan* and *You Gui Wan* both are built on *Liu Wei Di Huang Wan*.

***You Gui Wan* (Restoring the Right)**[3]

You Gui Wan was originally described in the classical herbal book *Jing Yue Quan Shu* (Collected Treatises of Zhang Jingyue) written by Zhang Jingyue and published in 1624. *You* (right) and *Gui* (returning or restoring) literally means restoring the right. The Kidney is divided into Kidney *Yin* and Kidney *Yang*. According to the TCM pulse theory, Kidney *Yin* is associated with the left side of pulse while the Kidney *Yang* is related to the right side of the pulse. *You Gui Wan* thus refers to Restoring Kidney *Yang*.

The ingredients work together for the common action of warming and nourishing Kidney *Yang* and replenishing *Jing* and Marrow (Table 13). Indications for this Chinese herbal medicine formula are to treat Kidney *Yang* Deficiency and a decline in Fire of the *Ming Men* (Gate of Life), as well as physical and mental fatigue from chronic illness in geriatric patients. The main clinical

Table 14: Ingredients and Actions of Z*uo Gui Wan*[3]

%	English Name	Chinese *Pin-Yin*	Action
22.9	Rehmannia (Prepared)	*Shu Di Huang*	Enriches Blood and nourishes *Yin*, tonifies *Jing*
11.4	Dioscorea (baked)	*Shan Yao*	Tonifies Spleen *Qi* and secures Kidney *Jing*
11.4	Lycium	*Gou Qi Zi*	Nourishes Liver *Yin* and Blood
11.4	Cornus	*Shan Zhu Yu*	Tonifies Liver and Kidney *Yin*
8.7	Cyathula	*Chuan Niu Xi*	Strengthens the bones and joints, and relieves pain
11.4	Cuscuta	*Tu Si Zi*	Tonifies Liver and Kidney *Yin*, strengthens Kidney *Yang*
11.4	Cervus	*Lu Jiao Jiao*	Nourishes the Kidney and Blood
11.4	Testudinis	*Gui Ban Jiao*	Tonifies the Kidney *Jing* to strengthen the bones, nourishes Blood to nurture the Heart

Actions: Nourishes *Yin* and reinforces the Kidney, replenishes the Essence to benefit the Marrow.

Table 15: Ingredients and Actions of *Sheng Mai Yin*[3]

%	English Name	Chinese *Pin-Yin*	Action
37.5	Ginseng	*Ren Shen*	Replenishes the Source (*Yuan*) *Qi*, tonifies the Spleen and Lung, promotes the production of Body Fluid, and calms the mind
37.5	Ophiopogon	*Mai Men Dong*	Nourishes *Yin*, clears False Heat and promotes the production of Body Fluid to relieve thirst
25	Schisandra	*Wu Wei Zi*	Restrains the dissipation of Lung *Qi*, promotes the production of Body Fluid, preserves *Jing* and restrains excessive sweating

Actions: Tonifies *Qi* and promotes the production of Body Fluid, astringes *Yin* and stops coughing.

signs include warm-seeking behavior, cold back and limbs, soreness and paresis of the back and rear limbs, or infertility due to *Yang* Deficiency. Clinical signs include a pale tongue with a white coating, and a deep and slow pulse. Veterinary applications include the treatment of renal failure, infertility and hyperadrenocorticism due to Kidney *Yang* Deficiency in horses, dogs and cats.

***Zuo Gui Wan* (Restoring the Left)**[3]

Zuo Gui Wan was described in the classic text, *Jing Yue Quan Shu* (Collected Treatises of Zhang Jingyue), written by Zhang Jingyue in 1624. The translation *Zuo* (left), *Gui* (restore/return), *Wan* (pill) refers to restoring Kidney *Yin*. According to the TCM pulse theory, Kidney *Yin* is associated with the left side of pulse while the Kidney *Yang* is related to the right side of the pulse. *Zuo Gui Wan* thus refers to Restoring Kidney *Yin*.

The ingredients work together for the common action of Nourishing *Yin* and to reinforce the Kidney along with replenishing the Essence and to benefit the Marrow (Table 14). Indications for the use of this Chinese herbal medicine is to treat *Yin* Deficiency with signs of soreness or weakness of the back and knees, premature ejaculation, and a dry red tongue with little coating. Veterinary applications include treating a *Yin* Deficiency Pattern with some degree of *Yang* Deficiency (panting, cool-seeking, warm ears and body, a pale purple tongue, and a deep weak pulse) in geriatric conditions including chronic renal failure, endocrine disorders, and *Wei* Syndrome. This formula should not be used with Spleen Deficiency, *Qi* Stagnation and Damp-Heat since the ingredients are likely to generate Dampness.

***Sheng Mai Yin* (Generating the Pulse)**[3]

Sheng Mai Yin was described in the classical book *Jing Yue Quan Shu* (Collected Treatises of Zhang Jingyue) written by Zhang Jingyue in 1624. *Sheng* meaning generating and *Mai* meaning pulse literally means "generating the pulse". This formula was named because of its main function of tonifying *Qi* to activate the pulse.

The ingredients of this Chinese herbal medicine work together to perform the actions of Tonifying *Qi*, promote the production of Body Fluid, astringe *Yin* and stops coughing (Table 15). Indications for its use include treatment of both *Qi* and *Yin* Deficiency. Clinical signs may include lethargy, shortness of breath, physical and mental fatigue, thirst, prolonged cough with little, but sticky sputum, a red dry tongue, and a feeble rapid pulse. The use of this formula is contraindicated in cases with Excess Heat or for those with a cough with profuse sputum. The most common uses in veterinary medicine include treatment of heart failure, behavioral problems and chest discomfort due to Heart *Qi* and *Yin* Deficiency.

FIRE-SPIRIT (FIRE GOD) SCHOOL, *HUO SHEN PAI* OR *YANG*-WARMING (*YANG*-SUPPORTING) SCHOOL[10]

Historical Background

Zheng Qinan or Zheng Shouquan (1824-1911) was born in Qióng Lái, Sichuan. In 1874 at the age of 50, Zheng established the *Huo Shen Pai* at Cheng Du. It was derived from the *Shang Han* School. Its main herbs were those from Zhang Zhongjing.

Disease Pathology Philosophy

Zheng Qinan studied *Nei Jing*, *Shang Han Lun* and *Yi Jing*. After having practiced several decades, he questioned the use of *Si Ni Tang* which contained *Fu Zi* (Aconite) created by Zhang Zhongjing for the emergency treatment of collapsed *Yang* and wondered why not use the *Yang* tonic much earlier to prevent *Yang* collapse. Zheng also discovered that *Fu Zi* (Aconite) was more effective in restoring *Ming Men* Fire and tonifiying *Yang* than other *Yang* tonics including *Lu Jiao Jiao* (Cervi Colla Cornu) and *Yin Yang Huo* (Epimedium). Zheng believed that *Gan Cao* (Glycyrrhiza) and *Fu Zi* (Aconite) can help *Yang* reach the 12 Channels. In addition to its strong *Yang* tonification, *Fu Zi* (Aconite) can also dispel Excessive *Yin* Patterns including Cold and Dampness. The top 3 herbs favored by Zheng Qinan were *Fu Zi* (Aconite), *Gan Jiang* (Dry Ginger) and *Rou Gui* (Cinnamon). All are extremely hot and pungent, generating warm energy to support *Yang*, thus he is called *Huo Shen Zheng* (Fire Spirit or Fire God Zheng). His main emphasis is to use these *Yang* herbs at higher doses (30 to 200 grams) as early as possible, specifically for *Yang* Deficiency.

Important Chinese Herbal Medicines of Fire Spirit School (Yang Warming School)

The main herbs are *Fu Zi* (Aconite) and *Gan Jiang*

Table 16: The Four Stages (Four Phases or Four Levels)[1]

Stage	Level		Location
1	*Wei*	Defense	Superficial, Body surface, Muscle and Joints
2	*Qi*	*Qi*	Internal organs: Lung, Stomach, Large Intestine
3	*Ying*	Nutrient	CNS, *Shen*, Heart, Pericardium
4	*Xue*	Blood	Hemorrhage, Sepsis, Blood dyscrasias, Hemo-parasites

(Dry Ginger). The main herbal formula *Si Ni Tang* is composed of *Fu Zi* (Aconite), *Gan Jiang* (Zingiberis) and *Gan Cao* (Glycyrrhiza). Oftentimes *Fu Zi* (Aconite) alone is used for many severe conditions including heart failure with fever, measles and any *Yin* Fire (False Heat) in the Upper *Jiao* due to Kidney *Yang* Deficiency. Examples include chronic laryngitis, gingivitis, stomatitis, halitosis, headaches, uveitis and otitis. It was also considered effective for any other *Yin* Fire (False Heat*)* due to *Yang* Deficiency during the course of diabetes, hypertension, cancer, and prostatitis.

SCHOOL OF WARM DISEASES, *WEN BING XUE*[1]

Historical Background

The school of Warm Diseases was developed by a group of physicians in the Ming and Qing Dynasties (1368–1911). The main representatives and works include Wu Youke and his book *Wen Yi Lun* "Treatise on Warm Epidemic" in 1642, Ye Tianshi and his book "Treatise on Exogenous Epidemic Fevers" in 1746 and Wu Jutong and his book *Wen Bing Tiao Bian* (Systematic Difference of Warm Diseases) in 1798.

Disease Pathology Philosophy

Wei, Qi, Ying and Xue, or the Four Stages is a diagnostic and treatment system for Warm Diseases, in which the initial signs are acute febrile conditions (Excess Heat). *Wei* correlates with Defense, *Ying* is associated with Nutrient, and *Xue* with Blood and are associated with common signs of acute, febrile diseases observed in practice. These types of conditions tend to usually occur when the *Zheng Qi* is not strong enough to prevent the *Xie Qi* from invading the body. These diseases are characterized by an abrupt onset of clinical signs, through their tendency to injure *Yin*, Body Fluid, and Blood and by

Table 17: Ingredients and Actions of *Yin Qiao San* [3]

%	English Name	Chinese *Pin Yin*	Action
17.9	Lonicera	*Jin Yin Hua*	Clears Heat and toxins
17.9	Forsythia	*Lian Qiao*	Clears Heat and toxins, subdues swelling
10.7	Platycodon	*Jie Geng*	Opens the Lung *Qi*, relieves cough, dissolves Phlegm
10.7	Mentha	*Bo He*	Disperses Wind and Heat
10.7	Arctium	*Niu Bang Zi*	Disperses Wind-Heat in the Lung channel, relieves sore throat
7.1	Lophatherum	*Dan Zhu Ye*	Clears Heat, relieves restlessness, promotes diuresis
7.1	Schizonepeta	*Jing Jie Sui*	Opens the surface to relieve Exterior pathogens
8.95	Soja	*Dan Dou Chi*	Relieves the Exterior
8.95	Glycyrrhiza	*Sheng Gan Cao*	Harmonizes

Actions: Relieves exogenous pathogenic factors, clears Heat, and eliminates toxins.

Table 18: Ingredients and Actions of *Qing Ying Tang*[3]

%	English Name	Chinese *Pin-Yin*	Actions
3.3	Rhinoceros*	*Xi Jiao*	Cools Blood, removes toxins
19.7	Rehmannia	*Sheng Di Huang*	Nourishes *Yin*, eliminates Heat, cools Blood
14.8	Scrophularia	*Xuan Shen*	Clears Heat and nourishes *Yin*
4.9	Bambusa	*Zhu Ye*	Clears Heat, promotes diuresis
14.8	Lonicera	*Jin Yin Hua*	Clears Heat and removes toxins
9.8	Forsythia	*Lian Qiao*	Clears Heat and removes toxins
8.2	Coptis	*Huang Lian*	Clears Heat, expels Damp
9.8	Salvia	*Dan Shen*	Cools Blood
14.7	Ophiopogon	*Mai Men Dong*	Nourishes *Yin* and clears Heat

* The original formulation used *Xi Jiao* (Rhinoceros horn). Its use is now prohibited because the rhinoceros is endangered. Today, water buffalo horn, or *Shui Niu Jiao* (Bubali) is used instead.
Actions: Cools Blood and detoxifies, clears Heat and nourishes *Yin*.

their frequent changes.

The Defense, *Qi*, Nutrient, and Blood represent four different stages of a disease's pathological development because they are suggestive of the depth and severity of a disease. In addition, each stage has associations with particular body tissues and *Zang-Fu* organs (Table 16). The most superficial is the Defense Stage (*Wei*), which includes the body surface, muscles, and joints. The next level is the *Qi* Stage and deeper still is the Nutrient (*Ying*) Stage. The deepest and most severe level is the Blood Stage (*Xue*) that includes Pathogens that affect the Blood. Generally, the superficial Defense (*Wei*) and *Qi* Patterns are more mild and easily resolved, whereas the Nutrient (*Ying*) and Blood (*Xue*) Patterns are much deeper, more severe, and harder to treat and bring into balance.

Important Chinese Herbal Medicines for the School of Warm Diseases

The majority of herbs for the School of Warm Diseases are Cold or cooling and very light such as *Jin Yin Hua* (Lonicera), *Lian Qiao* (Forsythia), *Sang Ye* (Mulberry Leaf), *Bo He* (Mentha) and *Ju Hua* (Chrysanthemum Flower). Unlike the Fire Spirit School, the hot herbs including *Ma Huang* (Ephedra) and *Fu Zi* (Aconite) are never used.

***Yin Qiao San* (Lonicera and Forsythia)**[3]

Yin Qiao San was originally described in the classical book *Wen Bing Tiao Bian* (Systematic Difference of Warm Diseases) written by Wu Jutong and published in 1798. This herbal formula was named after the two King herbs which are *Yin* referring to *Jin Yin Hua* (Lonicera) and *Qiao* referring to *Lian Qiao* (Forsythia) and *San* meaning powder. *Yin Qiao San* is thus translated as "Lonicera and Forsythia Powder."

The ingredients of this Chinese herbal medicine work together to perform the actions of relief of exogenous pathogenic factors, clearing Heat and eliminating toxins (Table 17). Its indications for clinical use are to treat the early stage of an Exterior Pattern due to Wind-Heat invasion. The most common veterinary application is treatment and prevention of upper airway infections and influenza in horses, dogs and cats. This formula is not indicated for Exterior Patterns due to Wind-Cold.

***Qing Ying Tang* (Clearing *Ying* Heat)**[3]

Qing Ying Tang was originally described in the classical book *Wen Bing Tiao Bian* (Systematic Difference of Warm Diseases) written by *Wu Jutong* in 1798. *Qing* means to clear or remove, *Ying* refers to Heat in the *Ying* Stage (Nutrient Level) and *Tang* is a decoction, the original form of this formula. Together, *Qing Ying Tang* is used to clear *Ying* Heat (Heat in the *Ying* Level of the body).

The ingredients of this Chinese herbal medicine work together to perform the actions of "Cools Blood and detoxifies, clears Heat and nourishes *Yin*", (Table 18). Its indications are to treat pathologic Heat just entering the Ying Stage. Clinical signs include a high fever, increased thirst, faint macular eruptions, restlessness or coma, a red tongue, dry mouth and a fast slippery pulse. *Qing Ying Tang* is used primarily in veterinary medicine to treat Heat in the *Ying* Level/Stage. It is often used in treating encephalitis, meningitis, and other neurological disorders with Heat as well as behavioral changes occurring after an infectious disease including parvo and distemper viruses. This formula should be used with caution in cases with Spleen *Qi* Deficiency

INTEGRATIVE SCHOOL OF CHINESE AND WESTERN MEDICINE, *HUI TONG PAI* [11]

Historical Background

The Western Medicine (WM or *Yang Xi* of *Xi Yang Yi Xue* in Chinese) was brought to China in the late 16th century Ming Dynasty (1368-1644) by preachers and/or traders, and spread rapidly in the late 19th century in the Qing Dynasty (1644-1912). [12] In order to differentiate WM from their own medicine, the Chinese started to use the term Chinese Medicine or Traditional Chinese Medicine (TCM). In the meantime, the integrative school of Chinese and Western Medicine was started by a group of well-known physicians during the Qing Dynasty: Tang Rongchuan (1846-1897) from Peng county Sichuan, Zhu Peiwen (the middle of 19th century) from Foshan Guangdong, Yun Tieqiao (1878-1935) from Changzhou Jiangsu and Zhang Xichun (1860-1933) from Yanshan Hebei. These physicians recognized that WM and TCM had totally different approaches and each of them had some strengths and weaknesses. They thought that the best medicine would be to integrate them, recognizing the differences between the two approaches yet taking advantage of their strengths. For example, Tang Rongchuan stated that "Always be fond of ancient knowledge, but never be superstitious about it; Extensive learning of the west and east can gain the strength and avoid the shortness of each other". This school continues to be adopted and advanced by mainstream TCM and TCVM universities and hospitals.

SUMMARY

There are four classics that all TCM students are required to study: *Huang Di Nei Jing* (Yellow Emperor's Classic of Medicine), *Shang Han Lun* (Treatise of Cold Damage), *Jin Gui Yao Lue* (Synopsis of Prescriptions from the Golden Cabinet), and *Wen Bing Xue* (Warm Disease Theory), Figure 1. *Shang Han Lun* and *Jin Gui Yao Lue* are associated with the Cold Damage School while *Wen Bing Xue* is the root of the School of Warm Diseases. In addition, Li Gao's Earth-tonic, Zhu Danxi's *Yin*-tonic and Zhang Jingyue's Warm Tonic Schools extend the treatment strategies and methods for Deficiencies, especially for geriatrics. Liu Hejian's Cold/Cooling, Zhang Zihe's Pathogen-attacking, and Zheng Qinan's Fire-Spirit Schools have enhanced treatment options for

Excess patterns. The main ideas and difference of these schools are summarized in Table 19.

REFERENCES:

1. Xie H, Preast V. *Traditional Chinese Veterinary Medicine-Fundamental Principles* (2nd edition). Beijing: Beijing University of Aeronautics & Astronautics Printing House, Beijing, China. 2013:408-411.
2. Dharmananda S. The Jin-Yuan Medical Reforms. Institute for Traditional Medicine, Portland, Oregon, 2001. http://www.itmonline.org/arts/jinyuan.htm
3. Xie H, Preast V. *Xie's Chinese Veterinary Herbology*. Wiley-Blackwell, 2010: 305-434.
4. Wu Y, A medical line of many masters: a prosopographical study of Li Wansu and his disciples from the Jin to the Ming. Chinese Sciences, 11 (1993-94): 36-65.
5. Qian Y. Introduction to Zhang Zihe's Theory. Shanghai Journal of Traditional Chinese Medicine 1963; (10): 38-40.
6. Xu F, Xu J, Liu T et al. Analysis of <Treatise on the Spleen and Stomach> by Li Gao. Heilongjiang Journal of Traditional Chinese Medicine 2013; (6): 9-10.
7. Si J. Discussion about Xiang Huo Theory. Jiangxi Journal of Traditional Chinese Medicine 2010; (2): 15-17.
8. Yang B. Discussion about Zhu Danxi's Method of Nourishing Yin and Falling Fire. Journal of Anhui Traditional Chinese Medical College 1984; (4): 3-4.
9. Xie W. Analysis about Zhang Jingyue's Yin-Yang Concept. Henan Traditional Chinese Medicine 2003; 23(11): 9-12.
10. Li Z. Discussion about Huo Shen Pai in Traditional Chinese Medicine. Contemporary Medicine 2011; 17(7): 163.
11. Zheng H, Huang J, Zhou J et al. Overview of Integrative school of Chinese and Western Medicine. Journal of Traditional Chinese Medicine Literature 1996; (4): 38-41.
12. Zhao P. The spread of Western Medicine in China. History Study 1980; (3):37-48.
13. Yu X, Shi K, Song W et al. Clinical Experience of Treatment of SARS on Syndrome Differentiation. Shanghai Journal of Traditional Chinese Medicine 2003; 37(11): 3-5.

Table 19: Comparison of 9 Different TCM Schools

School	Founder(s)	Main Ideas	Examples
Cold Damage School (*Shang Han Pai*)	Zhang Zhongjing (150-219 CE)	Cold Pathogens initially induce a disease; Established Pattern Differentiation system	*Shen Qi Wan* for renal failure due to *Yang* Deficiency; *Zhen Wu Tang* for heart failure due to *Yang* Deficiency
Cold/Cooling School (*Han Liang Pai*)	Liu Wansu (Hejian) (1110-1200 CE)	"All Six Pathogens can be transformed into Fire"; Use cold/cooling herbs to combat the disease	*Fang Feng Tong Sheng San* can be used for Fire/Heat in both the Exterior and Interior
Pathogen-attacking School (*Gong Xia Fa*)	Zhang Zihe (Congzheng) (1151-1231 CE)	Pathogens are the main cause of diseases; The treatment strategy is to attack and get rid of pathogens	Sweating methods eliminate the pathogens on the body's surface; Purging methods break up Blood Stasis and cancer
Spleen-Stomach (Earth-Tonic) School (*Pi Wei Pai*)	Li Dongyuan (Gao) (1180-1251CE)	The majority of diseases are caused by injury to the Spleen and Stomach; Since diseases of the Spleen tend to be of a Deficient nature, tonification should be used	*Bu Zhong Yi Qi Tang* is used to treat fecal incontinence or chronic diarrhea due to Spleen *Qi* Deficiency; The Spleen is the key element for treatment and prevention of any diseases
Yin-tonic School (*Yang Yin Pai*)	Zhu Danxi (Zhenheng) (1280-1358 CE)	*Yang* often is plentiful while *Yin* always tends toward Deficiency; Indulgent alcohol consumption, excessive sexual desires and activity induce the Minister Fire to flare up leading to *Yin* Deficiency and False Heat	*Da Bu Yin Wan* is used to treat severe *Yin* Deficiency Patterns with False Fire (Heat); *Hu Qian Wan* is used for hindlimb paresis, degenerative myelopathy, or any *Wei* Syndrome due to *Yin* Deficiency

Table 19 continued.

School	Founder(s)	Main Ideas	Examples
Warming Tonic School (*Wen Bu Pai*)	Zhang Jingyue (Jiebin) (1563-1640 CE)	*Yin* tends to be Deficient while *Yang* is not sufficient; Excess patients accept Cold while Deficiency patients accept Heat; Tonification, therefore, must be warming and sedation must be cooling	*You Gui Wan* is good for renal failure, infertility and hyperadrenocorticism due to Kidney *Yang* Deficiency; *Sheng Mai Yin* is used for Heart failure, behavioral problems and chest discomfort due to Heart *Qi* and *Yin* Deficiency
School of Warm Diseases (*Wen Bing Pai*)	Wu Youke (1582-1652); Ye Tianshi (1666-1745); Wu Jutong (1758-1836)	Epidemics are the warm diseases; *Wei*, *Qi*, *Ying* and *Xue*, or the Four Stages is a diagnostic and treatment system for Warm Diseases	*Yin Qiao San* is for the treatment and prevention of upper airway infections and influenza; *Qing Ying Tang* is used to treat Heat in the *Ying* Level/Stage in encephalitis, meningitis, and behavioral changes occurring after an infectious disease including parvo and distemper viruses
Fire-Spirit (Fire God) School (*Huo Shen Pai*)	Zheng Shouquan (Qinan) (1824-1911)	It is too late to treat collapsed *Yang* using *Si Ni Tang*; Higher dosage of *Fu Zi* (Aconite) should be used much earlier to prevent *Yang* collapse	*Si Ni Tang* can be used for heart failure with fever due to *Yang* Deficiency; *Fu Zi* can be used for any *Yin* Fire (False Heat) in the Upper *Jiao* due to Kidney *Yang* Deficiency, such as gingivitis, stomatitis, uveitis and otitis
Integrative school of Chinese and Western Medicine (*Hui Tong Pai*)	Tang Rongchuan (1846-1897); Zhu Peiwen (mid-19th century); Yun Tieqiao (1878-1935); Zhang Xichun (1860-1933)	Western Medicine and TCM have totally different approaches, but both have strengths and weaknesses; The best medicine is to integrate both to allow the existence of differences between them, but take advantage of the strength of each and avoid the weaknesses	Zhang Xichun used Aspirin with Gypsum for febrile diseases with good results Subcutaneous fluid therapy + TCVM for renal failure

ABSTRACT

Jeon JH[1], Shin MS, Lee MS, Jeong SY, Kang KW, Kim YI, Choi SM
Acupuncture reduces symptoms of dry eye syndrome: a preliminary observational study
J Altern Complement Med. 2010 Dec;16(12):1291-4.

OBJECTIVE:
The aim of this study was to evaluate the effect of acupuncture treatment on dry eye syndrome.

DESIGN:
This was a prospective observational study.

SETTING:
The study was conducted at a clinical evaluation center for acupuncture and moxibustion of the Korean Institute of Oriental Medicine, Republic of Korea.

SUBJECTS:
The subjects were patients with dry eye disease (N = 36), defined by Schirmer test scores of <10 mm/5 min and tear film break-up times (BUTs) of <10 seconds.

TREATMENTS:
Participants were treated with acupuncture three times per week for 4 weeks. MEASURE OF EFFECTIVENESS: Schirmer test scores, BUTs, symptom scores, ocular surface disease index (OSDI) scores and dry eye symptom questionnaires were compared before and after treatment to evaluate the efficacy of acupuncture treatment.

RESULTS:
After treatment, symptom scores, OSDI scores and the number of dry eye symptoms were all significantly lower ($p < 0.0001$). Although tear wettings were significantly higher (left: $p < 0.0001$, right: $p = 0.0012$), there were no significant differences in BUTs.

CONCLUSIONS:
This study suggests that acupuncture treatment can effectively relieve the symptoms of dry eye and increase watery secretion.

ABSTRACT

Klide AM, Farnbach GC, Gallagher SM.
Acupuncture therapy for the treatment of intractable, idiopathic epilepsy in five dogs.
Acupunct Electrother Res. 1987;12(1):71-4.

Five epileptic dogs, nonresponsive to high levels of anticonvulsants were presented to the acupuncture clinic at the Veterinary Hospital of the University of Pennsylvania for treatment. Acupuncture was performed by placing small gold implants subcutaneously over the calvaria at acupuncture points on the Governing Vessel (GV), Gall Bladder (GB), and Bladder (B) meridians and left in place to provide constant stimulation to the points. Each of the five dogs treated showed a change in seizure patterns following gold implant placement. Two dogs had decreases in seizure frequency with their medication continued as before acupuncture, but they reverted to their previous pattern approximately five months after treatment. Three dogs continued to have decreased numbers of seizures and were maintained on decreased levels of anticonvulsants.

Retrospective Studies

A Retrospective Study of the Therapeutic Effect of Acupuncture in 9 Dogs with Neurologic Deficits From Suspected Canine Distemper Virus Infections

Weerapongse Tangjitjaroen DVM, PhD, Pranisa Mahatnirunkul DVM, MS

ABSTRACT

Medical records from 2011-2014 were reviewed for dogs with neurological signs associated with canine distemper virus (CDV) infections, referred for acupuncture and receiving 3 or more treatments. Of 9 dogs identified, limb paresis or paralysis was found in 8/9 (89%), myoclonus in 3/9 (33%) and seizures in 2/9 (22%). Duration of signs ranged from 1 month-4.5 years. All dogs with paresis were assigned a spinal deficit grade of 1-5 before and after treatment. TCVM patterns at the time of the 1st treatment included: 7/9 (78%) with *Qi/Yang* Deficiency, 1/9 (11%) with *Yin* Deficiency and 1/9 (11%) with *Jing* Deficiency. Internal Wind was diagnosed in 7/9 (78%) dogs (seizures, myoclonus or delirium). Dogs were treated with a combination of dry needle acupuncture (DNAP), electro-acupuncture (EAP) and aqua-acupuncture (Aqua-AP) weekly for 3-9 times then bi-monthly in 4 dogs for a total of 10-18 acupuncture treatments. The dogs were followed clinically until they were discharged (6 dogs) or died (3 dogs). The dogs died from pneumonia, automobile accident and cachexia. After acupuncture at discharge or prior to death, 7/8 (88%) dogs with paresis had improved 1-3 grade levels and 3/3 dogs with myoclonus had reduced strength and frequency of contractions. Seizures had resolved in 2/2 dogs by the 5th treatment. The results of these clinical cases suggest that a combination of DNAP, EAP and Aqua-AP treatments can improve paresis, reduce the severity of myoclonus and control seizures in dogs with acute and chronic neurological signs associated with CDV infection warranting further study.

Key words: Canine distemper, acupuncture, electro-acupuncture, aqua-acupuncture, paresis, paralysis, myoclonus, seizures, traditional Chinese Veterinary medicine

ABBREVIATIONS

CDV	Canine distemper virus
DNAP	Dry needle acupuncture
EAP	Electro-acupuncture
Aqua-AP	Aqua-acupuncture
TCVM	Traditional Chinese Veterinary Medicine
NT-3	Neurotrophin 3

Canine distemper virus (CDV) infection is one of the most ubiquitous and serious diseases affecting dogs and other members of the family Canidae.[1] In spite of effective vaccines, the disease remains endemic in many parts of the world. The virus belongs to the Paramyxoviridae family and is related to the measles virus in humans and rinderpest virus in cattle. Infection of dogs by the virus is pantropic with most tissues of the body demonstrating variable deleterious effects. In particular, the respiratory and gastrointestinal tracts are affected with clinical manifestations of pneumonia and diarrhea along with infection of the lymphoid tissues culminating in severe immune suppression.[1]

The CDV readily infects neurons and glial cells of the central nervous system resulting in both encephalomyelitis and chronic demyelinating disease.[1-3] Neurological signs can be sudden and quickly follow the onset of systemic signs or be delayed several weeks to months.[2-4] Common neurological signs include: 1) cerebral dysfunction manifesting as obtundation, dementia, seizures and compulsive circling, 2) cerebellar-vestibular dysfunction manifesting as ataxia of the head and limbs with head tilt, circling and disequilibrium and 3) spinal cord dysfunction manifesting as paresis or paralysis of the pelvic limbs alone or all 4 limbs.[2-5] The seizures can be focal or generalized and symmetrical or asymmetrical. "Chewing gum" seizures characterized by masticatory muscle contractions and salivation and psychomotor seizures (complex partial seizures) characterized by hysterical behavior and aimless running are common.[2-3] Myoclonus or the rhythmic contraction of specific muscle groups, continuous even

From: Department of Companion Animals and Wildlife Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Thailand

during sleep, is considered almost pathognomonic for CDV infection.[2,3] Myoclonus is hypothesized to be due to abnormal pacemaker activity of neurons of the brain stem or spinal cord in the area of the lower motor neurons to affected muscles.[1-3] Dogs that survive CDV infections may go on to suffer from chronic encephalitis referred to as old dog encephalitis (ODE) with many of the same signs as the acute infection.[2-3] Treatment is usually symptomatic and supportive as there is no specific treatment. Although some dogs can recover from systemic and some neurological deficits, neurological signs often persist.[2,3]

From the traditional Chinese veterinary medicine (TCVM) perspective, the CDV virus itself is considered to be Heat Toxin.[5] The invasion of Heat Toxin leads to the generation of Internal Fire that damages Body Fluids, gradually leaving *Yin* Deficiency. Deficient *Yin* fails to restrain the Liver *Yang*, leading to Liver *Yang* Rising, consequently causing Internal Wind (seizures, myoclonus and abnormal movements including circling). Heat Toxin can also overwhelm and consume *Zheng Qi*, gradually leading to *Qi* Deficiency that can cause generalized weakness, ataxia and incoordination. Common TCVM Patterns associated with CDV infection are Heat Toxin, Liver *Yin* Deficiency and *Qi* Deficiency. As the TCVM patterns change during the recovery process, repeated evaluations are necessary to effectively treat the changing pattern, as chronic *Yin* and *Qi* Deficiency can lead to *Yang* Deficiency.[6,7]

Dry needle acupuncture (DNAP) and electro-acupuncture (EAP) have been used to improve neurologic deficits resulting from compression of the spinal cord secondary to intervertebral disc disease.[7-11] The authors have noted in neurological clinical cases presented to them that improvement in spinal cord deficits can be best obtained with EAP. The aim of this study was to investigate whether EAP along with DNAP and aqua-acupuncture (Aqua-AP) would improve function in dogs with acute and chronic neurological deficits associated with CDV. To the authors' knowledge, acupuncture treatment of post-CDV neurological signs has not been before reported. A retrospective study, therefore, was undertaken to evaluate the efficacy of acupuncture with the objective that the results might be useful to veterinary practitioners working with this otherwise untreatable disease.

MATERIALS AND METHODS

Medical records from 2011-2014 were reviewed for dogs that had survived an episode of CDV infection and referred to the Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand, for acupuncture treatment of neurological signs. All referred CDV survivors were included in this retrospective study. The primary veterinarian made the diagnosis of the CDV infection based on lack of vaccination history, previous typical clinical signs of CDV infection and current neurological signs.

Study dogs were placed into 3 categories based on presenting clinical signs and history: dogs with paresis, dogs with myoclonus and dogs with seizures. Dogs could be in more than 1 category depending on their presenting clinical signs. For dogs in the paresis category the following grading system of spinal cord deficits was used: 0 = normal, 1 = pain but no deficits, 2 = ambulatory paraparesis, 3 = non-ambulatory paraparesis, 4 = paraplegia with preserved deep pain and 5 = paraplegia and loss of deep pain (paraparesis = some voluntary movement and paraplegia = no voluntary movement).[2] Information regarding type, severity, duration and frequency of seizures was obtained from caretakers and documented for dogs having seizures. Each dog presenting with myoclonus had clinical signs recorded which usually included myoclonus frequency (contractions per minute), intensity, location, and severity at rest or with movement. In addition, stimulation that exacerbated myoclonus such as stress, light, noise and movement was recorded. Along with the neurological examination, study dogs also received a TCVM examination that included ear temperature and tongue color. A TCVM Pattern(s) was assigned to each dog.

Two veterinarians certified in veterinary acupuncture performed all DNAP, EAP, and Aqua-AP treatments. Needles used for DNAP and EAP were 25-mm, 34-gauge sterile stainless steel disposable acupuncture needles[1]. Acupoints consistently treated included: GV-20, GB-20, BL-18, BL-15, SP-10 and ST-36. Other acupuncture points that were used in some dogs included: KID-1, BL-40, LI-18, LI-4, BL-20, BL-21, BL-23, BL-35 and *Shen-shu* (1.5 cun lateral the lumbosacral junction). Stimulation of GV-20 was accomplished *via* DNAP. Acupoints that were consistently stimulated with EAP in the dogs included: GB-20, BL-18, and ST-36. During each treatment a total of 14 acupoints were electrically stimulated, while the other acupoints were stimulated by DNAP. An overview of acupoints treated and their indications are listed in Table 1.[12]

For the EAP, acupuncture needles were bilaterally connected (same acupoints on the left and right sides) to the electropuncturoscope[2]. Selected acupoints were electrically stimulated with 2-4 Hz, 3-4 mA and 6 volts of direct current for 15 minutes. After the first 15 minutes, the stimulation parameters were adjusted to 80-120 Hz, 3-4 mA, 6 volts of direct current for another 15 minutes. All of the dogs were physically restrained and comforted by the caretaker throughout the treatments.

After each EAP session, Aqua-AP was performed by injecting a sterile B12 and phosphorus solution[c] into *An-shen* (on side of head, caudal to base of ear, halfway between TH-17 and GB-20), BL-18, BL-15, SP-10, and GB-39 acupoints with a 1-inch, 25-gauge hypodermic needle attached to a syringe. The volume of solution injected into each acupoint depended on the size of the dog and ranged from 0.2 to 0.5 ml per location. The

treatment aim was to perform acupuncture once weekly for 9 treatments and then reduce to once every 2 weeks until discharge. Other treatments administered during the study, including conventional pharmaceuticals or Chinese herbal medicines were documented.

Clinical response of dogs to experimental treatment was based on improvement of neurological deficits when comparing pre-treatment and post-treatment clinical signs. Treatment success for dogs placed in the paresis category was an improvement of 1 or more deficit grade levels during the treatment period, while success for the seizure category was seizure-free status for a period of more than 1 month. The authors subjectively evaluated the myoclonus category after reviewing the medical records and noted any improvement in myoclonus clinical signs for each individual dog post-treatment.

Demographic data were analyzed using descriptive statistics in a computer spreadsheet.[d] Alterations in paraparesis, myoclonus, and seizures were analyzed using descriptive statistics.

RESULTS

A total of 9 dogs were enrolled in the study and included: 1 Labrador Retriever, 2 Beagles and 6 mixed-breed dogs. There were 5 males and 4 females with ages ranging from 3.5 months to 5 years with a mean plus or minus standard deviation (M ± SD) of 29.6 ± 23.5 months. Their body weights ranged from 1.5-25 kg. At the time of referral the duration of the neurological signs ranged from 1 month to 4.5 years with a M ± SD duration of 18.8 ± 21.1 months. For 3/9 (33%) dogs, post-CDV neurological signs had been present for more than a year. An overview of the signalment and clinical details of the dogs are presented in Table 2. Limb paresis or paralysis was found in 8/9 (89%) dogs with 2 dogs in lateral recumbency unable to lift their heads. Myoclonus was present in 3/9 (33%) dogs. Seizures occurred in 2/9 (22%) dogs.

On TCVM examination, 8/9 (89%) dogs had cold ears and 1/9 (11%) had a normal ear temperature. A pale tongue was present in 6/9 (67%), a red tongue was present in 2/9 (22%.) dogs and 1/9 (11%) had a pale red tongue. Heat Toxin was the initial etiology associated with CDV disease but TCVM patterns at the time of the 1st treatment, had changed to: 7/9 (78%) dogs with *Qi/Yang* Deficiency, 1/9 (11%) with *Yin* Deficiency and 1/9 (11%) with *Jing* Deficiency (poor development and swimming puppy syndrome). Internal Wind was diagnosed in 7/9 (78%) of dogs associated with seizures, myoclonus or delirium. Two dogs (Dog 5 and 6) required 2 people to hold them during the EAP treatment, but the remaining 8 dogs tolerated acupuncture well. Dog 4 received amoxicillin from the primary veterinarian for the first 5 days of the acupuncture treatment and Dog 9 received a multivitamin[e] until her death. None of the other dogs received any treatment other

Table 1: Acupoints and indications for treatment in traditional Chinese veterinary medicine stimulated during treatment of 9 dogs with neurological signs following canine distemper virus infections

Acupoint	Indications[12]
GV-20	*Shen* disturbances, epilepsy, sleep disorders
LI-4	Master point of face and mouth, fever, immunodeficiency, general pain
LI-18	Cough, dyspnea
SP-10	Blood Heat, Blood Stagnation, Heat toxin, fever, pelvic limb paresis or paralysis
BL-15	Sleep disorders, *Shen* disturbance, cognitive dysfunction, epilepsy
BL-18	Epilepsy, irritability
BL-20	Spleen Deficiency, Dampness
BL-21	Loss of appetite, vomiting, constipation, generalized weakness
BL-23	Kidney *Qi* and *Yin* Deficiency, renal disease, and intervertebral disc disease
BL-35	Bloody diarrhea, hematuria, sacrocaudal pain, tail paralysis
BL-40	Dysuria, urinary incontinence, intervertebral disc disease, diarrhea, pelvic limb paresis or paralysis
ST-36	General *Qi* tonic
GB-20	External Wind, Internal Wind, cervical pain, intervertebral disc disease, nasal discharge or congestion, epilepsy
GB-39	Neurological disorders, cervical pain, intervertebral disc disease, pelvic limb paresis or paralysis
Shen-shu	Kidney *Qi* and *Yin* Deficiency
An-shen	Behavioral problems, *Shen* disturbance, Internal Wind, epilepsy

than acupuncture.

Four dogs completed the initial 9 weekly treatments (Dogs 2, 3, 8, 9) and continued bimonthly for a total of 18, 10, 13 and 12 treatments, respectively (Table 3). Four dogs only completed 6-8 initial weekly treatments (Dogs 1, 4, 5, 7). One of these dogs died of pneumonia (Dog 5), while the other 3 dogs had abbreviated therapy due to owner financial and time constraints. Dog 6 died from a car accident after only 3 treatments. Dog 9 died after 4 months of treatment. She began the study in an emaciated condition, partly due to having been fed strictly as a vegetarian since a puppy. Even following supplementation of the diet with plant-based protein such as soybean products, the dog was still unable to gain muscle mass. No necropsy was performed at the owner's request.

An overview of the pre and post treatment spinal cord severity scores are outlined in Table 3 in the 8 dogs with spinal cord deficits. At study termination, 7/8 (88%) dogs had improved scores and only 1/8 (12.5%) dog had no change, but had died after receiving only 3 treatments. At the initiation of the study 6/8 (75%) dogs were non-ambulatory and after treatment 5/8 (62%) had

Table 2: Signalment, history, neurological and TCVM findings in 9 dogs with neurological sequelae from canine distemper virus infection

Dog	Signalment	Body weight (kg)	Duration of Signs	Neurological signs	Tongue Color	Ear Temp	TCVM pattern
1	Beagle 5 yrs Fe	7.5	4.5 yrs	Grade 3 paraparesis, proprioceptive deficits in all limbs, dementia, delirium, stumbling and rolling if assisted to stand, incoordination	Pale	Cold	*Qi*/Yang Deficiency Internal Wind
2	Mixed 3 yrs M	12	2.4 yrs	Grade 2 paraparesis, seizures, dysmetria, dementia, depression, circling, ataxia	Pale	Cold	*Qi*/Yang Deficiency, Internal Wind (seizures)
3	Labrador Retriever 4 yrs M	25	5 mos	Grade 3 paraparesis, proprioceptive deficits of pelvic limbs, depression	Pale red	Normal	*Qi*/Yin Deficiency
4	Beagle 5 yrs M	7	4.5 yrs	Grade 3 paraparesis, proprioceptive deficit of all limbs, dementia, delirium;	Pale	Cold	*Qi*/Yang Deficiency Internal Wind
5	Mixed 4 mos M	1.5	3 mos	Grade 4 paraparesis, proprioceptive deficits of all limbs; dementia, delirium, unable to lift head	Pale	Cold	*Jing*, *Qi*/Yang Deficiency Internal Wind
6	Mixed 3.5 mos M	3	1 mos	Grade 3 paraparesis; proprioceptive deficits of all limbs; depression	Pale	Cold	*Qi*/Yang Deficiency
7	Mixed Mos Fe	5	5 mos	Grade 2 paraparesis, myoclonus over the neck and forelimbs; dementia, ataxia, depression	Red	Cold	*Qi*/Yang Deficiency Internal Wind (myoclonus)
8	Mixed 1 yr Fe	11	8 mos	Myoclonus of head, neck, and back region, seizures ("chewing gum fit"), dementia,	Red	Cold	*Yin* Deficiency, Internal wind (myoclonus, seizures)
9	Mixed 3 yr Fe	8	1 yr	Grade 5 paraparesis, proprioceptive deficits of all limbs, myoclonus pelvic limbs, depression, obtunded, unable to lift head, no voice	Pale, white coat	Cold	*Qi*/Yang Deficiency Internal Wind (myoclonus)

Spinal cord Deficit Grades: 0 = normal, 1 = pain but no deficit, 2 = ambulatory paraparesis, 3 = non-ambulatory paraparesis, 4 = paraplegia, preserved deep pain, and 5 = paraplegia, loss of deep pain (paresis defined as some voluntary movement and

become ambulatory. The 5 dogs that improved from non-ambulatory status took on average 6.2 treatments to become ambulatory.

An overview of myoclonus and response to therapy in all 3 affected dogs is found in Table 4. At the time of referral the myoclonus in Dog 7 consisted of strong continuous contractions over the neck and both front limbs at a rate of 30 times/minute. There was an increase in intensity and severity of contractions when the dog was excited and decreased severity during sleep. After discharge following 7 treatments, there were no contractions during sleep and while awake myoclonus had decreased to mild continuous contractions over the cervical area and front limbs at a rate of 10 times/minute. During normal walking there were no visible contractions. Similar to before treatment, the frequency and intensity of the myoclonus still increased when the dog became excited.

Myoclonus in Dog 8, at the time of referral, consisted of moderate continuous contractions over the head, neck and back regions at a rate of 78 times/minute (Table 4). There was lessening of the intensity of myoclonus during sleep. After 13 treatments, at the time of discharge there were no contractions during sleep. While the dog was awake, contractions were still continuous, but were now mild and just located over the neck and back regions. The intensity of contractions still increased when the animal became excited or was stressed.

Myoclonus in Dog 9, at the time of referral consisted of strong continuous contractions at 45 times/minute over the gluteal, bicep femoris, and gastrocnemius muscles of both pelvic limbs (Table 4). The intensity and severity of contractions did not change during sleep. After 12 treatments at the time of discharge the contractions occasionally abated during sleep. While awake there were

Table 3: Pre-treatment and post-treatment spinal cord severity scores and number of treatments in 8 dogs with paraparesis or paraplegia from canine distemper virus infections

Dog	Breed Age	Duration of Signs	Severity Score Pre-treatment	Severity Score Post-treatment	Number of Treatments
1	Beagle 5 yrs	4.5 yrs	Grade 3	Grade 1	8
2	Mixed 3 yrs	2.4 yrs	Grade 2	Grade 0	18
3	Labrador Retriever 4 yrs	5 month	Grade 3	Grade 0	10
4	Beagle 5 yrs	4.5 yrs	Grade 3	Grade 2	8
5	Mixed 4 mos	3 mo	Grade 4	Grade 3	6 (died- pneumonia)
6	Mixed 3.5 mos	1 mo	Grade 3	Grade 3	3 (died- auto accident)
7	Mixed 7 mos	5 mos	Grade 2	Grade 0	7
9	Mixed 3 yrs	1 yr	Grade 5	Grade 3	12 (died- cachexia)

Spinal cord Deficit Grades: 0 = normal, 1 = pain but no deficit, 2 = ambulatory paraparesis, 3 = non-ambulatory paraparesis, 4 = paraplegia, preserved deep pain and 5 = paraplegia, loss of deep pain (paraparesis = some voluntary movement and paraplegia = no voluntary movement); mos= months, yr = year

Table 4: Myoclonus changes of 3 dogs associated with canine distemper virus infections, before and after acupuncture treatment

Dog	Breed Age	Duration of Signs	Location Before Tx	Location After Tx	Strength Frequency Before Tx	Strength Frequency After Tx	Number Tx
7	Mixed 7 mos	5 mos	Neck, thoracic limbs	Neck, thoracic limbs	Strong 30/min	Mild 10/min	7
8	Mixed 1 yr	8 mos	Head, neck, back	Neck, back	Moderate 78/min	Mild 32/min	13
9	Mixed 3yr	1 yr	Pelvic limbs	Pelvic limbs	Strong 45/min	Mild 28/min	12

Tx= acupuncture treatment; mos= months, yr = year

mild continuous contractions at the rate of 28 contractions/minute over the gluteal, bicep femoris, and gastrocnemius muscles of both pelvic limbs and the frequency and intensity of contractions increased when excited (Table 4).

At the time of referral, 2 dogs (Dogs 2 and 8) suffered from seizure episodes that lasted 1-2 minutes every 4-5 days (Table 2). After the 5th treatment (5 weeks), the dogs were seizure-free until discharge. Dog 2 was discharged after the 18th treatment, which was 11 months following the first treatment and Dog 8 was discharged after the 13th treatment, which was 3.5 months after the first treatment.

DISCUSSION

Neurological signs can vary widely in CDV infection and reflect the distribution of viral damage to tissues of the central nervous system.[13-17] Both neurons and glial cells (astrocytes) are major cell targets and clinical signs associated with central nervous system infection can be debilitating resulting in untreatable conditions.[13] Often these affected dogs are euthanized.[16]

In the present case series 8/9 (88%) dogs had paresis with loss of ambulatory ability and 3/9 (33%) dogs had myoclonus. In a review of 100 dogs with neurological sequellae of CDV infections, 33% of the dogs had paresis or ataxia and 40% had myoclonus.[24] Conventional treatments for limb paresis or paralysis following CDV infection are usually ineffective.[2-5] Seizures can usually be controlled with anticonvulsant drugs, but these may contribute to depression and ataxia already present.[2,3] Myoclonus is usually unaffected by conventional treatments. In a case report of a dog, 2 injections of botulinum toxin administered into the affected muscle reduced the severity of myoclonus.[18] Other specific treatment methods aimed to treat chronic neurologic deficits associated with CDV infection have not been documented.

In this case series, treatment with a combination of DNAP, EAP and Aqua-AP improved the ambulatory ability, reduced myoclonus and resolved seizure activity in dogs with signs ranging from 1 month to 4.5 years. Seven of the 8 dogs (87.5%) that had paraparesis before treatment improved 1-3 grade levels following acupuncture treatment. The only dog in the study with paresis not improved at least 1 grade level was Dog 6, which was killed in a car accident after only 3 therapy sessions. Paraparesis for over a year is considered a poor prognosis and unlikely to improve, but all 4 cases with paraparesis for 1-4.5 years improved 1 or more grades with acupuncture treatment.[2-5] In the author's experience, even if the dog does not regain normal ambulatory abilities, if it can still function independently, euthanasia is less likely. In this case series, the improved paraparesis score in Dogs 1, 4 and 7 might be the reason the caretakers discontinued further treatment after 6-8 acupuncture sessions. Acupuncture has been recommended for the treatment of limb paresis and paralysis from many causes.[7]

In 3/3 (100%) dogs with myoclonus, acupuncture reduced the strength and frequency of contractions. While trying to improve myoclonus is helpful for ambulation and quality of life, as this study demonstrates, the myoclonus did not completely resolve. For 2/2 (100%) dogs seizures had resolved by the 5th acupuncture treatment. Acupuncture has been recommended for the treatment of seizures from many causes.[7]

The mechanism by which acupuncture improves long-term neurological deficits associated with CDV infection is currently not known. There are several experimental studies on the neuroregenerative and neuroprotective effects of EAP.[19-23] One experimental study of gerbils documented a reduction in the expression of neuroinflammatory proteins undergoing EAP treatment at KID-3.[19] In other studies neurotrophin 3 (NT-3), a nerve growth factor important for the growth, survival and differentiation of neurons was upregulated by EAP.[20] In another study of rats with a transected cord (T9-T10), those undergoing EAP at acupoints along the Governing Vessel had higher levels of NT-3 in the spinal cord and improved functional recovery, when compared to untreated controls.[21] Rats with experimentally induced ototoxicity treated with EAP had increased NT-3 in the inferior colliculus nuclei and improved auditory brainstem response.[22] Finally an experimental study using an Alzheimer's disease transgenic mouse model demonstrated EAP upregulated expression of NT-3 and reduced amyloid β-peptide in the cerebral cortex in treated mice.[23] The neuroprotective and neuroregernerative effect created by upregulation of NT-3 induced by EAP was further documented with immunohistochemistry which demonstrated greater numbers of survival neurons and newly proliferated cells in treated animals.

In-vivo investigation of the mechanism of acupuncture modification of neurologic deficits that occur as a sequelae to CDV infection would be difficult. This is due partly to the fact that sampling CNS tissue in live animals is problematic and would have humane considerations in dogs. It is possible, however, based on the acupuncture mechanistic research in the experimental models just discussed that the mechanism in dogs is similar and that EAP may exert its effects of neuroprotection and neuroregeneration by increasing levels of endogenous NT-3, and promoting neurogenesis.

In summary, it was found that acupuncture (EAP, DNAP, Aqua-AP) treatment of the neurological sequelae in dogs recovering from naturally occurring infection with CDV resulted in improved ambulatory ability, reduction in severity of myoclonus and resolution of seizure activity. There were no adverse effects observed associated with any of the acupuncture treatments. Although further studies in more dogs are warranted to determine the short-term and long-term efficacy of DNAP, EAP and Aqua-AP treatments to improve neurological deficits caused by CDV infections, clinicians are encouraged to try acupuncture even if neurological deficits have been present for more

than 1 year.

FOOTNOTES

a. *Zhong yan tai he,* sterile acupuncture needle, Beijing Zhong yan Tai he Medicine Co., Ltd, Beijing, China
b. WQ-108, Electronic Acupunctoscope, Donghua Electronic Equipment Factory, Beijing, China
c. Catosal, B12 and phosphorus, Bayer®, Germany
d. Microsoft Excel for Mac 2011, version 14.1.0 (110310), Microsoft Corporation
e. Neurobian, Merck, Germany

REFERENCES

1. Dungworth DL. The respiratory system, viral diseases. Pathology of Domestic Animals 3rd Ed. Jubb K, Kennedy P, Palmer N (ed). Orlando, FL: Academic Press 1985:476-481.
2. Dewey CW. A Practical Guide to Canine and Feline Neurology 2nd Ed. Ames, IA:Wiley Blackwell 2008:179.
3. DeLahunta A, Glass E. Veterinary Neuroanatomy and Clinical Neurology 3rd Ed. St. Louis, Mo:Elsevier 2009:213.
4. Amude AM, Alfieri AA Alfieri AF. The nervous form of canine distemper. Vet e Zootec 2006; 13(2):125-136. In Portugese
5. Xie H, Priest V, Atria S. Etiology and Pathology. Traditional Chinese Veterinary Medicine 2nd Ed, Xie H, Priest V (ed). Reddick, FL: Chi Institute Press 2013:157-172.
6. Xie H. Personal communications
7. Chrisman C. Practical Guide to Traditional Chinese Veterinary Medicine. Xie H, Wedemeyer L, Chrisman C (eds). Reddick, FL: Chi Institute Press 2014:41-256.
8. Joaquim JG, Luna SP, Brondani JT et al. Comparison of decompressive surgery, electroacupuncture, and decompressive surgery followed by electroacupuncture for the treatment of dogs with intervertebral disk disease with long-standing severe neurologic deficits. J Am Vet Med Assoc 2011; 236(11):1225-1229.
9. Hayashi A, Fonseca Pinto A, Cortopassi S et al. S100beta Levels in CSF of Nonambulatory Dogs with Intervertebral Disk Disease Treated with Electroacupuncture. J Vet Med 2013; 2013:1-8.
10. Hayashi AM, Matera JM, Fonseca Pinto AC. Evaluation of electroacupuncture treatment for thoracolumbar intervertebral disk disease in dogs. J Am Vet Med Assoc 2007; 231(6):913-918.
11. Yang JW, Jeong SM, Seo KM et al. Effects of corticosteroid and electroacupuncture on experimental spinal cord injury in dogs. J Vet Sci 2003; 4(1):97-101.
12. Chrisman C, Xie H. Xie's Veterinary Acupuncture. Xie H, Preast, V.(ed) Ames, Iowa: Blackwell 2007:129-216, 217-234.
13. Mutinelli F, Vandevelde M, Griot C et al. Astrocytic infection in canine distemper virus-induced demyelination. Acta Neuropathol 1989; 77:333-335.
14. Headley SA, Amude AM, Alfieri AF et al. Molecular detection of Canine distemper virus and the immunohistochemical characterization of the neurologic lesions in naturally occurring old dog encephalitis. J Vet Diagn Invest 2009; 21(5):588-597.
15. Koutinas AF, Polizopoulou ZS, Baumgaertner W et al. Relation of clinical signs to pathological changes in 19 cases of canine distemper encephalomyelitis. J Comp Pathol 2002;126(1):47-56.
16. D'Intino G, Vaccari F, Sivilia S et al. A molecular study of hippocampus in dogs with convulsion during canine distemper virus encephalitis. Brain Res 2006; 1098:186-195.
17. Galan A, Gamito A, Carletti BE et al. Uncommon acute neurologic presentation of canine distemper in 4 adult dogs. Can Vet J 2014; 55(4):373-378.
18. Schubert T, Clemmons R, Miles S, et al. The use of botulinum toxin for the treatment of generalized myoclonus in a dog. J Am Anim Hosp Assoc 2013;49(2):122-127.
19. Yang EJ, Cai M, Lee JH. Neuroprotective effects of electroacupuncture on an animal model of bilateral common carotid artery occlusion. Mol Neurobiol 2016; 53(10):7228-7236.
20. Hempstead BL. Brain-Derived Neurotrophic factor: Three ligands, many actions. Trans Am Clin Climatol Assoc 2015; 126:9-19.
21. Ding Y, Yan Q, Ruan JW et al. Electro-acupuncture promotes survival, differentiation of the bone marrow mesenchymal stem cells as well as functional recovery in the spinal cord-transected rats. BMC Neurosci 2009; 10:35.
22. Zhou P, Ma W, Sheng Y et al. Protective effects of acupuncture against gentamicin-induced ototoxicity in rats: possible role of neurotrophin-3. Med Sci Monit 2017; 23:446-451.
23. Li X, Guo F, Zhang Q et al. Electroacupuncture decreases cognitive impairment and promotes neurogenesis in the APP/PS1 transgenic mice. BMC Complement Altern Med 2014; 14:37.

ABSTRACT

Ling Jun Kong, Min Fang, Hong Sheng Zhan, Wei An Yuan, Jiang Hui Pu, Ying Wu Cheng, and Bo Chen
Academic Editor: Andreas Michalsen
***Tui-na*-Focused Integrative Chinese Medical Therapies for Inpatients with Low Back Pain: A Systematic Review and Meta-Analysis**
Evidence-Based Complementary and Alternative Medicine; Volume 2012 (2012), Article ID 578305, 14 pages

OBJECTIVE:
To evaluate the effectiveness of Tui-na-focused integrative Chinese medical therapies (TICMT) on inpatients with low back pain (LBP).

METHODS:
Six English and Chinese databases were searched for randomized controlled trials (RCTs) of TICMT for in-patients with LBP. The methodological quality of the included RCTs was assessed based on PEDro scale. Meta-analyses of TICMT for LBP on pain and functional status were conducted.

RESULTS:
There were 20 RCTs included. The methodological quality of the included RCTs was poor. The meta-analyses' results showed that TICMT had statistically significant effects on pain and functional status, especially Tui-na plus Chinese herbal medicine (standardized mean difference, SMD: 1.17; 95% CI 0.75 to 1.60 on pain; SMD: 1.31; 95% CI 0.49 to 2.14 on functional status) and Tui-na plus acupuncture (SMD: 0.94; 95% CI 0.38 to 1.50 on pain; SMD: 0.53; 95% CI 0.21 to 0.85 on functional status). Tui-na plus moxibustion or hot pack did not show significant improvements on pain and the long-term evidence for TICMT was far from sufficient.

CONCLUSIONS:
The preliminary evidence from current studies suggests that TICMT might be effective complementary and alternative treatments for in-patients with LBP, however, the poor methodological quality of the included RCTs means that high-quality RCTs with long follow-up are warranted.

Case Series

Treatment of 15 Cases of Coxofemoral Luxation with *Tui-na* Massage Manipulation and Cahyono's Modified Figure-8 Bandage

Tatang Cahyono DVM, MS

ABSTRACT

Coxofemoral (CF) luxation may be caused by hip dysplasia, degenerative joint disease or external trauma and is diagnosed by palpation and radiography. When there are no complicating factors, most simple luxations can be reduced closed if they are treated within the first 4 to 5 days after the injury. Failure rates of 47% to 65% have been reported for single attempts at closed reduction and the presence of degenerative joint disease or hip dysplasia can significantly lower the chance of success even further. If closed reduction is unsuccessful, surgeries such as femoral head osteotomy (FHO) or total hip replacement (THR) may be recommended. Not all patients are candidates for surgery due to concurrent illness, age or caretaker financial constraints. *Tui-na* manipulation and Cahyono's modified figure-8 bandage was instituted as an alternative treatment for CF luxation over a 6 month period at the author's clinic. A total of 15 clinic cases underwent the alternative treatment. Recovery time using this treatment method had a mean of 3.5 days (3.5 + 4.57) with an 80% success rate.

Key words: Chinese herbal medicine, traditional Chinese veterinary medicine, coxofemoral luxation, hip joint, *Tui-na*, canine, bandage

ABBREVIATIONS

CF	Coxofemoral
FHO	Femoral head osteotomy
THR	Total hip replacement
TCVM	Traditional Chinese Veterinary Medicine

Coxofemoral (CF) luxations in dogs and cats are generally the result of external trauma, with 59% to 83% related to vehicular trauma.[1] The clinical signs of CF luxation are lameness, pain, decreased abduction of the flexed hip, hesitation to sit, stiff gait in the pelvic limbs and difficulty rising from lying position. Craniodorsal is the most common direction of CF luxation, seen in 78% of affected dogs and 73% of cats.[1] Caudodorsal luxation is a rare condition and may simply be a craniodorsal luxation with more severe instability, allowing the femoral head to move caudally. Ventral luxation is relatively rare (1.5%-3.2% in reported case studies) and may occur as a separate entity or may be associated with an impaction fracture of the acetabulum.[1] Diagnosis of CF luxation is by palpation and radiography. Typically, CF luxation is treated through either closed or open reduction. Closed reduction encompasses reposition of the femoral head into the hip joint capsule without surgery while open reduction involves surgery.

In the case of craniodorsal luxation, the joint capsule can theoretically rupture in three places: midway between the acetabulum and neck of the femur (type A), avulsion from the acetabulum (type B), or avulsion from the femoral neck (type C).[1] Type A is probably the most common type and usually responds best to closed reduction. Type B results in marked hip instability due to loss of the fibrous lip or labrum of the acetabulum that normally aids femoral head coverage. When type C craniodorsal luxation is encountered, the joint capsule lies across the acetabulum "like a hammock," preventing deep-seated reduction.

When there are no complicating factors, most simple luxations can be reduced closed if they are treated within the first 4 to 5 days after the injury.[1] It is best to attempt closed reduction as soon as general anesthesia can be administered safely since good relaxation is essential for the reduction process. For successful closed reduction to occur, the hole in the joint capsule and torn muscle if present must be found and the femoral head returned through the tissue defect to seat into the acetabulum.[1] In most cases, after closed reduction it is appropriate to apply an Ehmer sling. Failure rates of 47-65% have been reported for single attempts at closed reduction and the

From: Praktek Dokter Hewan Bersama, Drh Cucu Kartini S. dkk Sunter, Jakarta, Indonesia

presence of degenerative joint disease or hip dysplasia can significantly lower the chance of success even further.[1]

In traditional Chinese veterinary medicine (TCVM), coxofemoral luxation, which is commonly associated with external trauma presents as an acute or excess condition accompanied by clinical findings such as a purple tongue, wiry pulse and hip pain. Stagnation is the predominant TCVM pattern presented and is associated with reduced *Qi* and Blood flow through the coxofemoral joint. The TCVM pattern diagnosis, therefore, is local *Qi* and Blood Stagnation in the hip joint area.

Tui-na is a form of traditional Chinese veterinary medicine (TCVM) that addresses patterns of disharmony in the body and facilitates the flow of *Qi* through specific massage techniques. Stagnation is particularly well suited to treatment with *Tui-na* manipulation as this modality is very effective at removing Stagnation by creating normal *Qi* and Blood flow again. The objective of this study was to develop a simple method of closed reduction to successfully allow reposition of the hip joint when open reduction was not feasible due to age, comorbidities and/or financial constraints of the caretaker. The hypothesis for the study was that *Tui-na* massage accompanied by a specialized bandage could be successfully used to reduce coxofemoral luxation in dogs and cats with a quick return to normal mobility. This would be augmented by the use of 2 Chinese herbal medicines that invigorate *Qi* and Blood, resolve Stagnation and relieve pain.[2,3]

Materials and Methods

Between January 2015 and June 2016, dogs and cats with suspected coxofemoral luxation that presented to Praktek Dokter Hewan Bersama, Drh Cucu Kartini S. dkk (PDHB Small Animal Clinic), Jakarta, Indonesia were used to select the study population. PDHB Small Animal Clinic is organized into 3 clinics which are open 24 hours a day, 7 days a week. Daily case load between the 3 clinics averages 100 to 150 animals per day. Clinical cases that presented for evaluation of hip pain and diagnosed radiographically with craniodorsal, caudodorsal or ventral coxofemoral luxation were enrolled in the study (Figures 1-3). There were no comorbities that would exclude an animal from participation. An owner permission sheet and a study enrollment examination sheet, which included clinical signs along with TCVM diagnosis and treatment, was completed for each animal enrolled in the study.

Treatment for all cases was consistent. Animals were maintained under general anesthesia during the application of *Tui-na* techniques which were applied until the femoral head was repositioned into the acetabulum

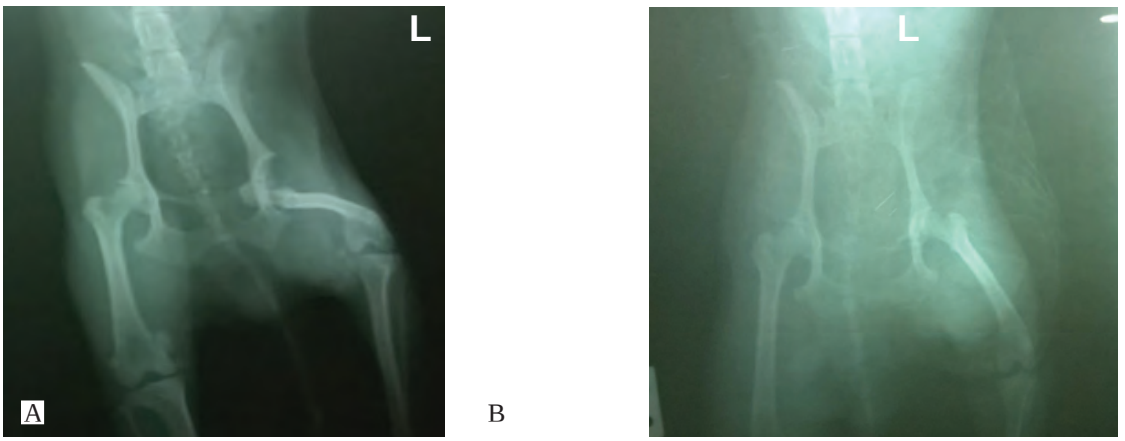


Figure 1: Radiographs of a 2-year-old Pomeranian (Case 5) showing left coxofemoral luxation (A) that was subsequently repositioned using *Tui-na* and Cahyono's modified figure-8 bandage (B).

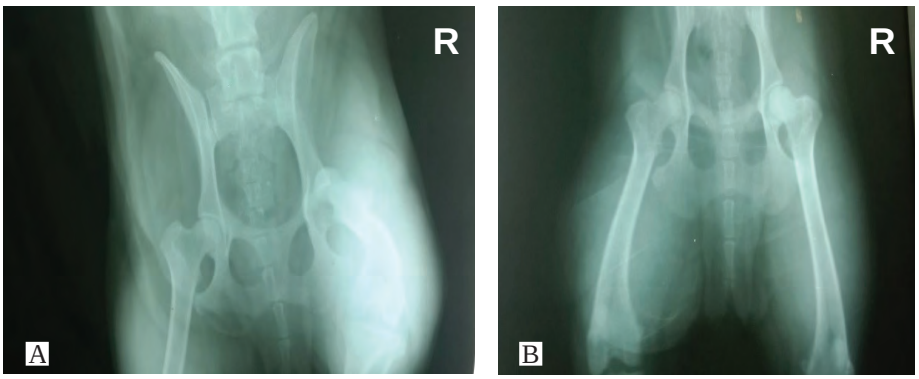


Figure 2: Radiographs of a 3-year-old mixed breed dog (Case 3) showing right coxofemoral luxation (A) that was subsequently repositioned using *Tui-na* and Cahyono's modified figure-8 bandage (B).

(Table 1). Radiography was then performed to verify successful positioning of the femur. Using Cahyono's modified figure-8 bandage, a gauze roll bandage is started dorsal to the greater trochanter of the femur and is applied in a figure-8 pattern by crossing the hip, the abdomen, dorsal back with return to the femur several times to stabilize the coxofemoral joint. A pre-prepared large gauze roll bent into a "C" shape is then placed dorsal to the hip joint with the 2 arms of the "C" placed caudal and cranial to the greater trochanter. This roll additionally stabilizes the coxofemoral joint and prevents dorsal luxation. The bandage is completed by additional gauze applied in a crisscross pattern around the hip joint and back and finished by applying strips of tape to protect the gauze and give the bandage additional strength (Figure 4). Following bandaging and recovery from anesthesia, study animals were restricted to complete cage rest with a bandage change every 7-10 days for 1 month. After bandage removal, animals were allowed limited exercise which was slowly increased over the next month.

Two Chinese herbal medicines were given to the animals during recovery. Body Sore[a] (modified *Shen Tong Zhu Yu Tang*) was orally administered twice daily at 0.5g/10 lb body weight (BW) to relieve pain and alleviate Blood Stagnation along with *Di Gu Pi*[a] at 0.5g/10 lb BW which treats Bony *Bi* syndrome due to Kidney *Yin/Qi* Deficiency to also assist in invigorating Blood, resolving Stagnation and for pain relief in the hip area. The herbal medicines were given for 1 month.

Criteria for treatment success was based on return to normal ambulatory ability (walking, running, jumping) within 1 month of experimental therapy without surgery. Treatment failure was based on the inability of a dog or cat to use the leg normally within 1 month after *Tui-na* and the

Table 1: *Tui-na* massage techniques to reposition femoral head into acetabulum

***Tui-na* Technique**	**Actions**
Dou-fa (shaking)	Regulates *Qi* and Blood and smoothes the joint
Ba-shen-fa (stretching)	Stretches the tendons and regulates the Channels
Yao-fa (Rocking) Craniodorsal luxation: performed counter-clockwise	Unblocks the Channels and smoothes the joint
Yao-fa (Rocking) Caudodorsal luxation: performed clockwise	Unblocks the Channels and smoothes the joint
Combination of *Ba-shen-fa, Yao-fa* and *An-fa*	

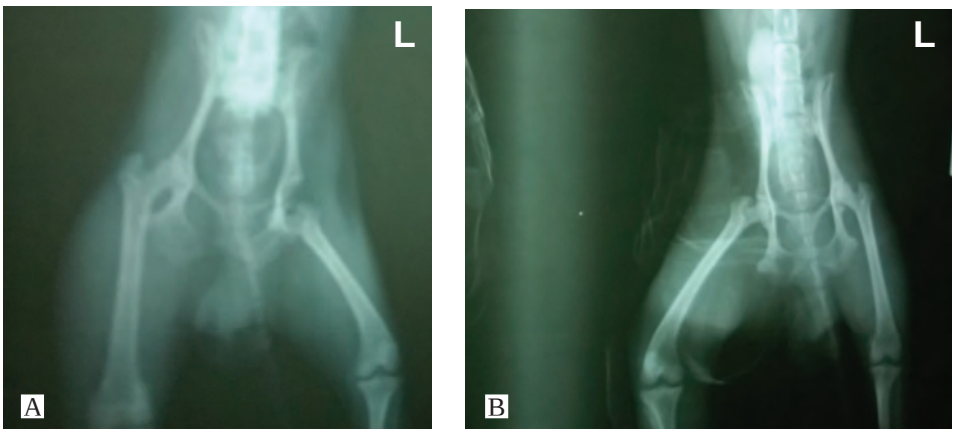
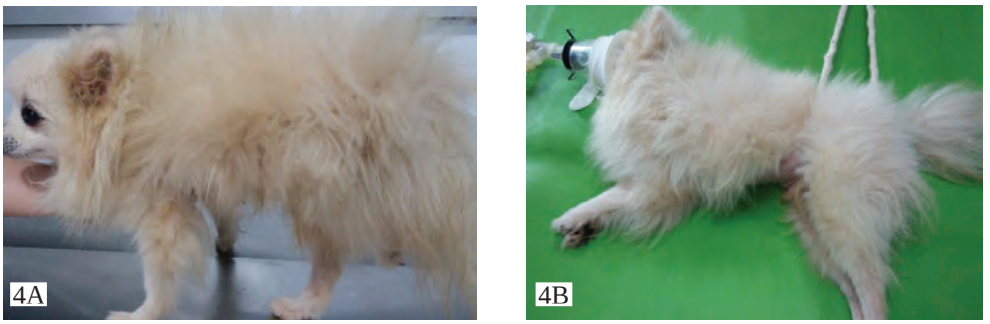


Figure 3: Radiographs of a 2-year-old female Pomeranian (Case 7) showing left coxofemoral luxation (A) that was subsequently repositioned using *Tui-na* and Cahyono's modified figure-8 bandage (B).

Figure 4: Stepwise application of Cahyono's modified figure-8 bandage for hip luxation (Figure 4A-M).



Figures 4A and 4B: Clinical presentation of dog (Case 5) with left coxofemoral luxation with typical shortened affected limb and non-weight-bearing lameness (4A). The animal is sedated and inhalation anesthesia is administered. To start the *Tui-na* reposition process, a soft rope is placed in the inguinal area and gently pulled dorsally (4B).

Figure 4C: Prepare bandage piece that will be placed over the coxofemoral joint: Bend cotton/ gauze roll into a "C" position and place tape across it to hold in bent position. This bandage applies pressure to the greater trochanter of the femur.

Figures 4D and 4E: Start bandage dorsal of greater trochanter of femur (4D) and then wrap gauze back and forth in a figure-8 pattern which includes the back and hip joint (4E).

Figures 4Fand 4G: The prepared pressure roll is placed over the greater trochanter of femur to prevent dorsal luxation of the coxofemoral joint. Use the tape to make sure that the pressure roll stays in the correct position (4F). After the roll is stabilized, continue to figure-8 the gauze around the area to hold all parts of the bandage in place (4G).

Figures 4H and 4I: Place tape along the back area in front of the bandage to keep it in place. Please make sure with your finger that the bandage is not tight and the dog can still urinate and defecate normally (4H). Next start a strip of tape at the medial femur and pull it upwards across the rump to the back area to stabilize the bandage (4I).

use of Cahyono's modified figure-8 bandage or continued coxofemoral luxation and the need for surgery.

Results

A total of 15 animals (14 dogs and 1 cat) met study inclusion criteria and were enrolled (Table 2). There was a wide range of ages which included the youngest at 5 months to the oldest at 13 years of age. Craniodorsal was the most common type of CF luxation and was diagnosed in 86.66% (13/15) of study animals, followed by caudodorsal luxation at 6.67% (1/15) and ventrodorsal luxation 6.67% (1/15) (Table 3). *Tui-na* manipulation and Cahyono's modified figure-8 bandage produced an overall success rate of 80% (12/15) (Table 4). All successful cases walked in 7 days or less with the exception of the 13 year old dog which took 20 days. Normal ambulation in successful cases had a mean of 3.5 days recovery time (mean $\pm$ SD = 3.5 $\pm$ 5.47).

There were 3 therapy failures, cases 4, 9, 10. Case 4 was a 2 yr. old female cat with ventral coxofemoral luxation. Twenty-one days had elapsed before treatment and the luxation was complicated by a damaged acetabulum which would not support reposition attempts. There was no recovery at 28 days and FHO surgery was performed at 60 days. Case 9 was a 7 month old male mixed-breed dog with right craniodorsal coxofemoral luxation. The dog was presented 12 days after injury with a severely damaged joint. Despite several reposition attempts, the femoral head would not remain in place after 7 days, therefore, the dog required FHO surgery. Case

Figures 4J and 4K: Prepare several strips of tape approximately the same length and apply strips in the following order to cover gauze and strengthen bandage:

1st step: place a strip along the dorsal back area

2nd step: place the next strip of tape at the tubercoxae.

3rd step: place a strip at the femur dorsal to greater trochanter.

4th step: Next start a strip of tape from the greater trochanter and wrap towards the medial femur, then caudal to femur and finally return to the back area. Repeat this tape placing 2-3 times.

5th step: Start a strip of tape from the caudal femur and pull it towards the medial femur, then cranial to the femur and then extend tape to back area. Repeat this tape placement up to 2-3 times.

Finally tape again across the femur to the back area. The tape strips will be in a crisscross pattern covering the gauze and adding strength to the bandage.

Figures 4L and 4M: Tape across the dorsal back area again to stabilize the bandage. Make sure the hair in the femur region is free from tape to allow easy movement. Check the hip joint again to make sure that the femur is still well seated in the acetabulum after the bandaging process. The dog in this picture (Case 5) walked within several hours of *Tui-na* massage and bandage application.

Table 2: Treatment of CF luxation in 14 dogs and 1 cat with *Tui-na* and the Cahyono modified figure-8 bandage system between January 2015 and June 2016

Case	Species, Breed	Sex	Age (years)	Direction of CF Luxation	Days Elapsed Before Treatment	Treatment Successful (S) or Failed (F)	Recovery Time (days before walking normally)
1	Dog, Maltese	F	12	Left, craniodorsal	12	S	3
2	Dog, Pomeranian	M	13	Right, craniodorsal	5	S	20
3	Dog, Mixed Breed		3	Right, craniodorsal	2	S	2
4	Cat, Mixed Breed	M	2	Left, ventral	21	F	No recovery at 28 days, FHO surgery at 60 days
5	Dog, Pomeranian	M	2	Left, craniodorsal	3	S	1
6	Dog, Pomeranian	F	5	Right, craniodorsal	3	S	6
7	Dog, Pomeranian	F	2	Left, caudodorsal	2	S	1
8	Dog, Mixed Breed	F	7	Right, craniodorsal	4	S	1
9	Dog, Mixed Breed	M	0.7	Right, craniodorsal	12	F	No recovery at 7 days and CF luxation still present, FHO surgery
10	Dog, Pomeranian	M	3	Right, craniodorsal	3	F	Subsequent ventral luxation at 30 days leading to FHO surgery
11	Dog, Poodle	M	0.5	Right, craniodorsal	7	S	1
12	Dog, Pomeranian	F	8	Right, craniodorsal	5	S	1
13	Dog, Pomeranian	M	2	Left, craniodorsal	1	S	1
14	Dog, Pomeranian	M	4	Left, craniodorsal	1	S	1
15	Dog, Shih-tzu	M	12	Right, craniodorsal	4	S	1

Table 3: Coxofemoral luxation type and percent of occurrence in study animals

Type of Coxofemoral Luxation	Number of Animals	Percentage
Craniodorsal	13	86.66%
Caudodorsal	1	6.67%
Ventral	1	6.67%

Table 4: Outcome of *Tui-na* Manipulation and Dr. Cahyono's modified figure-8 bandage

Treatment Outcome	Number of Animals	Percentage
Success	12	80%
Failure	3	20%

10 was a 3 year old male Pomeranian dog with a right craniodorsal coxofemoral luxation that was first presented for therapy 3 days after the inciting incident. There was successful repositioning of the joint at that time and the dog was walking normally in 7 days. One month later the dog was running up and down stairs and reinjured himself and presented with a ventral coxofemoral luxation of the same leg. The joint had severe injury and was unstable requiring FHO surgery.

Case Example: Craniodorsal CF luxation

A 2-year-old female Pomeranian (Case 7) was diagnosed with caudodorsal CF luxation of the left hip. The primary care veterinarian recommended surgery, specifically a femoral head osteotomy (FHO), but the caretaker declined this procedure. One week after the diagnosis the dog was presented to PDHB Small Animal clinic for TCVM treatment. On examination, the left pelvic limb was limp with a caudodorsal CF luxation and severe pain in the coxofemoral joint. The tongue was purple and the pulse rapid and wiry. The TCVM diagnosis was local *Qi* and Blood Stagnation in hip area. After meeting inclusion criteria, the dog was enrolled in the study and then anesthetized for confirmation of CF luxation and *Tui-na* treatment. The *Tui-na* treatment consisted of *Dou-fa* first to relax the pectineus, gluteal, biceps femoris and vastus lateralis muscles. *Ba-shen-fa* was then used to stretch out the biceps femoris tendon and patellar ligaments. *Yao-fa* (rocking) was applied to unblock the channels and smoothes the joint. Since this was a caudodorsal luxation, *Yao-fa* was then applied in a clockwise direction. *An-fa* (pressing) was the next movement to invigorate the Blood and *Qi*, and unblock obstructions. Finally *Tui-na* massage with combinations of *Ba-shen-fa, Yao-fa* and *An–fa* was continued until the femoral head was seated in the acetabulum. Cahyono's modified figure-8 bandage was then applied to stabilize the joint. Following recovery from anesthesia, additional sedation was used to assist with a smooth anesthetic recovery and provide immediate pain relief following the procedure (Figure 4).

The dog's hip remained bandaged for a total of 1 month, with the leg examined and the bandage replaced on a weekly basis. Two Chinese herbal medicines were given during recovery. Body Sore[a] (modified *Shen Tong Zhu Yu Tang*) was used to relieve pain and Blood Stagnation along with *Di Gu Pi*[a] designed to treat Bony *Bi* syndrome due to Kidney *Yin/Qi* Deficiency and to also assist in invigorating Blood, resolve Stagnation and provide pain relief in the hip area (Tables 5 and 6). The herbal medicines were dosed orally at 0.5g/ 10 lb. body weight twice daily for 1 month. A few hours after treatment, the dog regained the ability to walk on the injured hip. The dog was restricted to cage rest for 1 month while bandaged. After removal of the bandage, the dog returned to normal activity.

Discussion

The results of this study demonstrate that *Tui-na* accompanied by the modified figure-8 bandage developed by the author was successful in 80% of CF luxation cases presenting to a typical busy small animal practice in animals spanning a wide range of ages. Successful cases demonstrated normal ambulation quickly (mean of 3.5 days) and 5 cases walked within 1 day.

Almost all coxofemoral luxations (86.66%) in this study were in the craniodorsal direction and is similar to literature values of 78% of dogs and 73% of cats.[1] Ventral luxation is considered quite rare at 1.5-3.2% and only occurred in 1 animal in the study. It usually indicates severe injury such as an impaction fracture of the acetabulum and one of the 3 case failures was ventral luxation.

Tui-na is effective for repositioning simple CF dislocations, but less effective when CF is complicated by damage to the pelvic muscles and ligaments, hip dysplasia or hip osteoarthritis.[3,4] Repositioning with *Tui-na* should be performed within 1-7 days after the initial dislocation. When dislocation persists beyond this time frame, further musculoskeletal damage can occur and complicate repositioning. Two of the case failures substantiated this in that they were well beyond the 7 day period at 21 days (case 4) and 12 days (case 9). The third case failure was presented in 3 days and had a successful outcome initially but luxated the coxofemoral joint again 1 month later and sustained considerable joint damage at that time. Only 1 dog in the study (case 1) had a successful outcome with a delay of greater than 7 days.

The modified figure-8 bandage developed by the author stabilizes the affected hip while allowing the animal to walk. This is different from the usual bandage used for CF luxation (ehmer sling) which suspends the injured leg off the ground and holds it near the body wall.[5] With the modified figure-8 bandage and the ability to walk within days versus non-use for 4-6 wks with the ehmer sling, considerable muscle atrophy is avoided.[5] It is important, however, that post-repositioning, a dog must be crated with a maximum of only 10-20 minutes of exercise twice daily during the first month. Since the patient is allowed to use the leg, unsupervised exercise and stairs must be absolutely avoided. CF luxation may occur again if the dog over-exercises, jumps, uses the stairs or suffers further trauma in the immediate post-repositioning period.

Although there was excellent success with this conservative therapy in this study without exclusion for size of dog or injury type; in general, success will be lower in dogs with hip damage due to severe trauma and in medium to large dogs, in which *Tui-na* is more difficult to perform effectively. This treatment does, however, provide an effective alternative to surgery in suitable patients, which are smaller dogs without additional hip trauma. It can also be used to reduce luxation before surgery to stabilize the hip.

When patients are not candidates for surgery due to concurrent illness, age or caretaker financial constraints, the advantages of *Tui-na* and Cahyono's modified figure-8 bandage should be considered as it is low cost and its

simplicity makes it easy for practitioners to incorporate into a veterinary practice. It is usually less painful and has a faster recovery time than surgical treatment. The results of this study demonstrate the use of *Tui-na* and a joint stabilizing bandage (Cahyono's modified figure-8 bandage) can be used to reduce coxofemoral luxations in dogs and cats and provide an effective alternative to open reduction in selected clinical cases.

FOOTNOTES

[a.] Jing Tang Herbal, Reddick, FL http//:www.tcvmherbal.com

REFERENCES

1. DeCamp C, Johnston S, DeJardin L, Schaffer S. Brinker, Piermattei and Flo's Handbook of Small Animal Orthopedics and Fracture Repair, 4th ed. St. Louis, MO, Elsevier Publishing 2006: 456-511.
2. Xie H. Chinese Veterinary Herbal Handbook 2nd edition. Reddick, FL: Chi Institute of Chinese Medicine Publishing 2008: 134-185.
3. Xie H, Ferguson B, Deng X. Application of *Tui–Na* in Veterinary Medicine 2nd edition. Reddick, FL: Chi Institute of Chinese Medicine Publishing 2007:57-126.
4. Xie H, Wedemeyer L, Chrisman C *et al*. Practical Guide To Traditional Chinese Veterinary Medicine: Small Animal Practice. Reddick, FL: Chi Institute Press 2014: 30-33.
5. Roush JK, Renberg WC. The ehmer sling in canine orthopedic surgery. Clinician's Brief.com, Aug 2015: 29-32.

Table 5: Ingredients and actions of the Chinese herbal medicine Body Sore[a]

English Name	*Pin Yin* Name	Actions[2]
Ligusticum	*Chuan Xiong*	Relieves pain and activate Blood
Notopterygium	*Qiang Huo*	Relieves pain and activates Blood
Angelica	*Dang Gui*	Activate Blood, resolves Stagnation and relieves pain
Epimedium	*Yin Yang Huo*	Tonifies Kidney *Yang* and *Yin*
Achryanthes	*Niu Xi*	Strengthens bones and limbs
Angelica	*Du Huo*	Relieves pain and eliminates Wind-Damp
Cuscuta	*Tu Su Zi*	Nourishes Kidney Liver
Cordyalis	*Yan Hu Suo*	Moves *Qi*/Blood, resolves Stagnation and relieves pain
Paeonia	*Chi Shao*	Relieves pain and cools Blood
Eucommia	*Du Zhong*	Strengthens bone and tonifies *Yang*
Psoralea	*Bu Gu Zhi*	Strengthen bone and tonifies *Yang*
Myrrh	*Mo Yao*	Moves Blood, relieves pain
Olibanum	*Ru Xiang*	Moves Blood, relieves pain
Milletia	*Ji Xue Teng*	Nourishes Blood
Persica	*Tao Ren*	Breaks down Blood Stasis, relieves pain
Carthamus	*Hong Hua*	Breaks down Blood Stasis, relieves pain

Table 6: Ingredients and actions of Chinese herbal medicine *Di Gu Pi*[a]

English Name	*Pin Yin* Name	Actions[2]
Lycium	*Di Gu Pi*	Nourishes *Yin* and clears deficient Heat
Moutan	*Mu Dan Pi*	Cools Blood, clears Heat, resolves Stagnation
Rehmania, processed	*Shu Di Huang*	Nourishes Blood and *Yin*
Rehmania, unprocessed	*Sheng Di Huang*	Clears Heat, nourishes *Yin*
Gentiana	*Qin Jiao*	Clears Wind-Damp, nourishes *Yin*
Psoralea	*Bu Gu Zhi*	Tonifies Kidney *Yang* and *Yin*
Drynaria	*Gu Sui Bu*	Tonifies Kidney *Yang* and strengthens the bone
Eucommia	*Du Zhong*	Strengthens the back
Alisma	*Ze Xie*	Drains Damp and benefits urination
Salvia	*Dan Shen*	Invigorates Blood and resolves Stagnation
Angelica	*Du Huo*	Dispels Wind, Cold and Dampness, relieves pain
Angelica	*Dang Gui*	Nourishes Blood and relieves pain
Phellodendron	*Huang Bai*	Nourishes *Yin* and clears Heat

Brief Communication

Topical Treatment of a Non-Healing Wound Infected with Multi-Drug Resistant *Enterobacter cloacae*, Methicillin-resistant *Staphylococcus schleiferi* and *Escherichia coli* using the Chinese Herbal Medicine Golden Yellow Powder

Michael D. Bartholomew DVM, MS

ABSTRACT

A geriatric neutered male canine with pre-existing multi-systemic disease was presented with a non-healing wound of the right rear paw. After culture and sensitivity testing it was discovered that the wound contained multi-drug resistant bacteria (*Enterobacter cloacae*, methicillin-resistant *Staphylococcus schleiferi* and *Escherichia coli*) and conventional antibacterial therapy was discontinued. The topical Chinese herbal medicine, Golden Yellow Powder, was then selected as a primary wound treatment. Several of the herbs in this formula have exhibited antibacterial and anti-inflammatory properties in human clinical studies. These properties correspond to the treatment of Damp Heat and Toxic Heat patterns according to Traditional Chinese Veterinary Medicine (TCVM). This case demonstrates the potential use and effectiveness of topical Golden Yellow Powder in the treatment of wounds with multiple drug resistant bacteria.

Key words: Golden Yellow Powder, *Ru Yi Jin Huang San*, methicillin-resistant *Stapholococcus spp, Escherichia coli*, MRSA, *Enterobacter cloacae,* antibiotic resistant bacteria

ABBREVIATIONS

TCVM	Traditional Chinese Veterinary Medicine
BID	Twice daily
MRSS	Methicillin-resistant *Staphylococcus schleiferi*
BPM	Beats per minute
RPM	Respiration per minute
CBC	Complete blood count
WBC	White blood cells
RBC	Red blood cells

A 13-year-old, 46 pound, neutered male coonhound mix initially presented for assessment of a 2 cm spherical ulcerated right rear paw wound of unknown origin. The patient had a complicated medical history that included diabetes mellitus for 6 years, chronic keratoconjunctivitis sicca (KCS), as well as a 4 x 2.8 cm splenic mass diagnosed by ultrasound 10 months previously. Current therapies included insulin[a], *Yunnan Bai Yao*[b], and a nutraceutical[c].

After clinical examination of the wound, a standard wound treatment protocol was initiated that combined a 2-week course of an antibiotic, Baytril[d] [136 mg tab divided twice daily (BID)] and daily topical treatment with Vetericyn[e]. At recheck 1 month later, the wound appeared to be healing without incident, however, 18 days later the dog re-presented due to sudden worsening of the wound.

Upon re-examination, the wound on the paw had enlarged to 4 cm and now extended from the base of the fifth digital pad into the central pad where it replaced approximately 30-40% of the normal surface including the adjoining haired skin. Close examination revealed a deeply ulcerated area filled with purulent hemorrhagic exudate and necrotic tissue. The wound was encircled by a raised erythematous border and covered by a cracked and crusted surface. General physical examination of the dog was normal with a body weight of 46.2 pounds, temperature of 100.2° F, pulse of 120 BPM (beats per minute) and respiratory rate of 40 RPM (respiration per minute). The pet was eating well but the owner had noted increased thirst while on the antibiotic regimen. The wound was cleaned with chlorhexadine and the dog was placed on another course of antibiotics Baytril (136 mg tab split BID) and Cephalexin (500 mg BID) for 2 weeks.

From: The Animal Hospital of Dunedin, Dunedin, Florida USA

Diagnostics performed at this appointment included a complete blood count (CBC) and cytology of the wound surface. The cytological sample was obtained by swabbing the wound surface with a soft cotton swab that was rolled across a slide. The sample was heat fixed and stained with DipQuick[f] and examined microscopically by a trained technician. The cytology revealed modest numbers of red blood cells (RBC) combined with a predominately neutrophilic white blood cell (WBC) population accompanied by numerous cocci bacteria and was considered consistent with a septic exudate. At this time a culture was obtained using a Copan Swab[g] from the wound site and was submitted to a commercial laboratory for culture and antibiotic sensitivity[h].

One week later, the dog was returned for a recheck and to discuss laboratory results. The wound culture results had revealed 3 bacteria with heavy antibiotic resistance profiles, *Enterobacter cloacae*, *Escherichia coli* (*E. coli*) and methicillin-resistant *Staphylococcus schleiferi* (MRSS). All three organisms tested resistant to the antibiotics Baytril and Cephalexin that were being used in the patient. The CBC revealed a mildly elevated total WBC count at 18,300 a differential of 10,797 neutrophils, 6,039 lymphocytes and monocytes at 1,098.

The treatment strategy for the dog was changed based on these results. Conventional oral antibiotic therapy was stopped and only topical wound treatment with Golden Yellow Powder was initiated. In TCVM, moist pyodermas are generally considered a combination of the patterns Damp-Heat, Heat-Toxin and local Stagnation. The wound displayed these patterns of heat, swelling, erythema and pain with a purulent-hemorrhagic exudate. With this TCVM pattern in mind, the Chinese herbal medicine, Golden Yellow Powder[j] (modified *Ru Yi Jin Huang San)* was selected for topical use. Golden Yellow Powder has the actions of clearing Damp-Heat and Heat-Toxin along with moving Blood to resolve pain and stagnation. In addition, Golden Yellow Powder has been shown in pharmacological studies to have both antibacterial and anti-inflammatory effects on such pathogenic organisms as MRSA, *Salmonella typhimurium, E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus anthracis*.[1, 2]

The paw lesion was cleaned with chlorhexadine solution and packed with Golden Yellow Powder. After packing, a sterile Telfa Pad was placed over the wound and a bandage was applied using cast padding and Co-flex bandaging[k] (Figure 1). For 10 days, the wound was cleaned daily with chlorhexidine, re-packed with Golden Yellow Powder and re-bandaged. By day 5, the wound had dramatically improved with almost complete resolution of swelling and central filling of the legion with a healthy bed of granulation tissue. At day 10, the wound had improved so much that the frequency of Golden Yellow Powder application and bandage changes were reduced to every other day (Figure 2). There was complete filling of the wound defect by day 14 with healthy granulation tissue, surface epithelization and a 75% wound size decrease as the edges continued to contract (Figure 3). On day 18, the wound demonstrated such marked improvement that bandaging and wound care was further reduced to every 3 days. This treatment protocol was continued and healing gradually progressed until the dog made a full recovery approximately 90 days following the start of topical treatment with Golden Yellow Powder. (Figure 4).

DISCUSSION

Bacterial resistance is of increasing concern, not only in human medicine but now also in veterinary medicine. As bacterial resistance to common antibiotics renders them ineffective, the veterinarian is forced to employ new and stronger antibacterials thereby perpetuating the cycle of resistance. This not only creates a problem in choosing proper therapies for veterinary patients with a compromised skin surface or immune system, but also causes concern for public health.[3] Acquired resistance is possible by a variety of mechanisms some of which include chromosomal mutation, phage transduction and selection of resistant clones.[3]

Golden Yellow Powder is a Chinese herbal medicine first mentioned in Wai Ke Zheng Zhong written by Chen Shi-gongin 1617.[4] The English translation of the Chinese name is "Golden Yellow Powder that will bring healing".[4] This formula is for topical use only and is traditionally applied after being mixed with honey or vinegar to form a paste.[4] For this clinical case, the ground raw herbal powder was applied directly to the wound without mixing it into a paste and a bandage was applied to the area around the lesion to avoid self-trauma and ingestion of the power.

There is some variation of ingredients contained in this Chinese herbal formula depending on source. The ingredients of the classical Golden Yellow Powder are *Tian Hua Fen* (Tricosanthes), *Huang Bai* (Phellodendron), *Da Huang* (Rheum), *Bai Zhi* (Angelica), *Jiang Huang* (Curcuma), *Tian Nan Xing* (Arisaema), *Cang Zhu* (Atractylodes), *Hou Po* (Magnolia), *Chen Pi* (Citrus), and *Gan Cao* (Glycyrrhiza). The formula from Jing Tang Herbal (source used for this study) does not include *Tian Nan Xing* (Arisaema) but does include *Huang Lian* (Coptis). In Chinese herbal medicines, each herb interacts with the others to produce a synergistic effect. The herbs are assigned as King, Minister, Adjuvant or Messenger function within the formula. The King herb, *Tian Hua Fen* (Tricosanthes), exerts the main action of this formula. The Minister herbs are the *Huang Bai* (Phellodendron), *Jiang Huang* (Curcuma), and *Da Huang* (Rheum). These herbs are there to enhance the effect of the King herb. The Adjuvant herbs of the formula include *Tian Nan Xing* (Arisaema), *Cang Zhu* (Atractylodes), *Hou Po* (Magnolia), *Chen Pi* (Citrus), and *Bai Zhi* (Angelica). The Adjuvant has a number of tasks including suppressing toxic or extreme actions of the King and treating adjunct

Figure 1: The right rear paw wound present in a 13-year-old dog as it appeared 3 days after starting treatment with Golden Yellow Powder. The 4 cm lesion includes an ulcerated central pad containing a purulent-hemorrhagic exudate and extends to the base of the fifth digital pad.

Figure 2: Lateral surface of the right rear paw wound on the 9th day of treatment with Golden Yellow Powder. A healthy granulation tissue bed now fills the central ulcerated area. The wound has contracted to at least 50% of its original size.

Figure 3: The wound on the 14th day of treatment demonstrates continued healing with epithelization of the wound surface and further decrease in wound size and depth.

Figure 4: The appearance of the right rear paw approximately 90 days after starting topical therapy with Golden Yellow Powder. There has been complete resolution of the wound with regrowth of hair and normal pad surface.

patterns. The Messenger *Gan Cao* (Glycyrrhiza) has the task of harmonizing the actions of the herbs in the formula to achieve the overall therapeutic effects of the formula.[4] Herbs that have strong antibacterial effect in the formula include Coptis (*Huang Lian*) and Rheum (*Da Huang*) while the Philodendron (*Huang Bai*) is considered to be similar to Coptis, but slightly weaker in effect.[4] The ingredients and actions of each herb within Golden Yellow Powder are listed in Table 1.

This case demonstrates the potential wound healing benefits of the topical Chinese herbal medicine, Golden Yellow Powder. As veterinarians search for new ways to treat antibacterial resistant conditions, options other than antibiotics continue to be explored and utilized. These results suggest that dogs with antibiotic resistant wounds with few to no options for conventional antibacterial therapy may benefit from the topical use of Golden Yellow Powder. It is hoped that more research can be done to further assess the effects of Golden Yellow Powder to validate these case findings.

FOOTNOTES

a. NPH Insulin, R Insulin; Eli Lilly and Company, Indianapolis, Indiana 46285 USA
b. *Yunnan Baiyao*; Yunnan Baiyao Group Co. Ltd., Kunming, Yunnan, PR China
c. CAS Options; Garmon Corp.Vetresources, Temecula, Ca 92590 USA
d. Baytril; Bayer Healthcare LLC, Animal Health Division, Shawnee Mission, KS, 66201 USA
e. Vetericyn; VF-Innovacyn, Inc., Rialto, CA 92377 USA
f. Dip Quick 3 Step Stain; Jorgenson Labratories, Loveland, CO 80538 USA
g. Copan Swab; Copan Diagnostics, Inc, Murrieta, CA 92562 USA
h. Antech Laboratories, Irvine, CA, 92614 USA
i. Cephalexin, generic; local drug store by prescription
j. Golden Yellow Powder; Jing Tang Herbal, Reddick, FL, 32686 USA
k. Co Flex Elastic Bandage; Andover Healthcare, Salisbury, MA 01952 USA

REFERENCES

1. Ma A. Clinical Manual of Chinese Veterinary Herbal Medicine. Gainesville, FL: Ancient Art Press 2016: 301.
2. Chen J, Chen J. Chinese Medical Herbology and Pharmacology. City of Industry, CA:Art of Medicine Press, Inc. 2004: 143.
3. Moriello K. Overview of Pyoderma. Merck Manual of Veterinary Medicine, 9th Edition. Whitehouse Station, NJ: Merck & Co., Inc. 2005: 2053.
4. Xie, Huisheng. Xie's Veterinary Herbology. Ames, Iowa: Wiley-Blackwell 2010: 11, 12, 133-135, 156-159, 539, 540.

Table 1: Ingredients of the Chinese herbal medicine Golden Yellow Powder[j] and their actions

***Pin Yin* Name**	**English Name**	**Actions[1]**
Tian Hua Fen	Tricosanthes	Clear Heat/Promote Body Fluid
Da Huang	Rheum	Clear Heat/Dissipate Swelling
Huang Bai	Phellodendron	Clear Heat/Detoxify
Jiang Huang	Curcuma	Activate Blood/Relieve Pain
Bai Zhi	Angelica	Relieve Pain/Clear Wind Cold
Cang Zhu	Atractylodes	Dry Damp
Chen Pi	Citrus	Move *Qi*/Relieve Pain
Gan Cao	Glycyrrhiza	Harmonize
Hou Po	Magnolia	Dry Damp/Move *Qi*
Huang Lian	Coptis	Clear Heat/Detoxify

AJTCVM INSTRUCTIONS TO AUTHORS
Part II

Judith E Saik, DVM, DACVP

OVERVIEW OF ARTICLE TYPES

- Manuscripts for *AJTCVM* are classified as:
  - Clinical Studies
  - Basic Science Studies
  - Retrospective Studies
  - Reviews
  - Case Series
  - Pearls from TCVM Practice
  - Brief Communications
  - Epidemiology
  - Business Economics of TCVM Practice
  - Editorial
  - Letters to the Editor, Comments and Other Reader Feedback

FORMAT FOR CLINICAL STUDIES

- Please refer to the **Basic Format and Style of the Text of All Manuscripts** section to ensure all information is included in the appropriate format and style.
- Please refer to the link **Reporting Guidelines for Randomized Controlled Clinical Trials in Traditional Chinese Veterinary Medicine** at: *www.ajtcvm.org* for details regarding *specific* information to be included in each section.
- Text length *must not exceed 6000 words excluding* footnotes, references, tables and figure legends.
- **Title:** Add randomized, controlled, blinded (if done) and a clear succinct statement of species, disease and what was done
- **Abstract:** a 250 word summary of what was done, results and conclusions
- **Key Words:** All words others may use to search databases
- The manuscript should be divided into *four* sections only.
- There will be *no subsections* unless deemed necessary by the Editor-in-Chief and any subsections must contain two or more paragraphs
- The four sections are:
  - **Introduction**
  - **Materials and Methods**
  - **Results**
  - **Discussion**
- **Introduction:**
  - Statement of problem prevalence (backed by epidemiological studies if possible with most current references, easy for most readers to find)
  - Clinical signs and causes, conventional diagnosis and treatments (few pertinent current references)
  - Disease from a TCVM perspective
  - Basis for proposed TCVM treatment (no exhaustive literature review)
  - Objective and a hypothesis of the study
- **Materials and Methods**
  - Source of animals and animal care and use guidelines followed
  - State whether a natural occurring disease
  - Randomization method
  - Inclusion criteria
  - Exclusion criteria
  - What was done (specific details so other research clinicians can repeat)
  - Outcomes measure
  - Statistics used to evaluate outcomes (keep simple for readers)
- **Results**
  - Number of animals included
  - Any other pertinent information about animals (age, breed, sex, weight etc.)
  - Provide just the statistical results with no comments about the results
  - Show all your data including means plus and minus standard deviations and *p*-values in Tables so others can check your results for themselves
- **Discussion**
  - Comments on the results and acceptance of the hypothesis
  - Comparison of results with the results of other studies if available
  - Review of animal and human studies on the TCM treatment studied
  - Review literature of mechanisms of action for

the TCM treatment studied
- Pitfalls and problems with the current study
- Cost effectiveness, availability and ease of administration of treatment
- Conclusion and recommendations regarding treatment
- What future studies are needed

- For more detailed guidance please refer to the following AJTCVM articles: Chrisman C, Xie H, Ma A et al. 2014 Reporting Guidelines for Randomized Controlled Blinded Clinical Trials in Traditional Chinese Medicine. AJTCVM 2014; 9(1):33-38; Mayo E. Clinical Trials for Acupuncture and Other Traditional Chinese Veterinary Medicine Treatments. AJTCVM 2014; 9(1):23-31.

FORMAT FOR ORIGINAL SCIENTIFIC REPORTS, RETROSPECTIVE AND EPIDEMIOLOGY STUDIES

- Please refer to the **Basic Format and Style of the Text of All Manuscripts** section to ensure all information is included in the appropriate format and style.
- Please refer to the link **Reporting Guidelines for Randomized Controlled Clinical Trials in Traditional Chinese Veterinary Medicine** at: *www.ajtcvm.org* for details regarding *specific* information to be included in each section.
- Text length *must not exceed 6000 words excluding* footnotes, references, tables and figure legends.
- The manuscript should be divided into *four* sections only.
- There will be *no subsections* unless deemed necessary by the Editor-in-Chief and any subsections must contain two or more paragraphs
- The four sections are:
  - **Introduction**
  - **Materials and Methods**
  - **Results**
  - **Discussion**
- **Introduction**
  - *No title* for the section is necessary; just *begin text as a first paragraph indented by 0.5*.
  - Present a brief review of the literature and other background information on the topic being studied using pertinent references.
  - Conclude the section with a clear statement of the objective(s) and rationale of the study and the research question.
- **Materials and Methods**
  - A title is needed for the section, *aligned left, all capitals and bolded* (Example: **MATERIALS AND METHODS)**.
  - Present a concise, clear description of the experimental design, subjects and statistical methods, *beginning under the title as a first paragraph indented by 0.5*.
  - Provide enough information that another researcher could perform the identical study.
- **Results**
  - A title is needed for the section, *aligned left, all capitals and bolded* (Example: **RESULTS)**.
  - The results of the study should be stated concisely and in a logical sequence summarizing important observations, *beginning under the title as a first paragraph indented by 0.5*.
  - Refer to tables that summarize the data (place actual tables at the end of the text, after the list of references).
  - Show the *exact p* value for all data (Example: $p = 0.02$; $p = 0.74$).
  - Do not simply use $p < 0.05$ or $p > 0.05$ as the exact *p* value is important for anyone using your data to calculate confidence limits or using your data in a meta-analysis.
- **Discussion**
  - A title is needed for the section, *aligned left, all capitals and bolded* (Example: **DISCUSSION**.
  - First present a brief synopsis of the key findings *beginning under the title as a first paragraph indented by 0.5*).
  - Next provide a discussion of the possible mechanisms and explanations of findings.
  - Include anomalous results, even if there is no explanation or reason for them, as others might benefit.
  - Then compare the present study's findings with relevant findings from other published studies and when possible formulate a brief systematic review combining the results of the current study, with the results of all previous relevant studies.
  - Provide logical reasons for the discrepancy, if the results differ from other published results.
  - Include a critical description of the limitations of the study and methods used to minimize and compensate for those limitations.
  - Write a brief section that summarizes the clinical and research implications.
  - Conclude with a paragraph that summarizes findings and provides recommendations and new possibilities or investigations, based on the results; *do not use* a title like "Conclusions" or "Summary".

FORMAT FOR REVIEW ARTICLES

- Please refer to the **Basic Format and Style of the Text of All Manuscripts** section to ensure all information is included in the appropriate format and style.

- A review article should provide readers with an overview of the scientific literature in a specialized area of TCVM research.
- Review articles may be solicited by the Editor-in-Chief or submitted unsolicited by the author(s).
- Manuscripts *should not generally exceed 6,000 words*, excluding footnotes, references, tables and figure legends.
- The Editor-in-Chief reserves the right to increase the manuscript length for some topics.
- The manuscript may have several sections with titles determined by the author and Editor-in-Chief.
- The use of illustrations, line drawings and figures is encouraged.

FORMAT FOR BRIEF COMMUNICATIONS

- The AJTCVM no longer publishes case reports unless they address something very unusual or important for TCVM practitioners. Articles containing this information will be referred to as a Brief Communication.
- Contact the Editor-in-Chief for approval
- The format is similar to the CASE SERIES described below except is usually about 1500 words.

FORMAT FOR CASE SERIES

- Please refer to the **Basic Format and Style of the Text of All Manuscripts** section to ensure all information is included in the appropriate format and style.
- Case Series include a detailed description of the diagnosis, management and clinical outcome of 3-9 animals. Use a Table relating information for greater than 3 animals
- For more than 5 animals, the report should follow the format of a clinical study, as described above.
- The manuscripts *should not exceed 2500 words excluding* footnotes, references, tables and figure legends.
- Case series should include information not previously reported, findings that are new or unique, new treatments or diagnostic methods.
- Begin with a 250 word abstract summarizing the cases (Example: signalment, primary complaint, clinical signs, TCVM examination findings, TCVM diagnosis, conventional treatments (include dose, frequency and duration), TCVM treatment (include acupuncture points, frequency of treatments, number of treatments and any herbal formulas with doses, frequency and duration), rehabilitation activities (if applicable), outcome and length of follow-up.
- *Do not begin with an introduction.*
- *Begin the manuscript with the case descriptions*
    - For each case begin with the signalment, primary complaint and duration (Example: A ten year old male neutered Beagle dog was presented for a history of intermittent seizures of one year duration).
    - Next provide a pertinent history to include the conventional history as well as a TCVM history (Example: seeks heat, seeks cool, personality characteristics to determine constitution and such).
    - Include conventional diagnostic test results and conventional diagnosis if applicable.
    - Current conventional and TCVM examination findings (include tongue, pulse, acupoint palpation and ears and body surface temperature) should be presented next
    - Add a Table if more than 3 animals.
- The TCVM diagnosis and findings that support the diagnosis should be clearly stated next.
- The TCVM treatment strategy and rationale should be presented next.
- The TCVM treatments, outcomes and duration of follow-up are then presented
    - **Acupuncture treatment-** List *acupoints chosen* and their *actions, type* of acupuncture, the *duration of each treatment, number and frequency* of treatments, *total time period* treated and if *ongoing, how long it has been* (Put information in a table if needed for clarity; see **Tables**).
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    - **Food Therapy**- List food to add to the diet *with reasons* to include; list *foods to avoid* in the diet with *reasons* to exclude.
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- Conclude the article with how the information in this case study can be useful for similar cases in the future.
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"the horse, dog, cat, bird etc".
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ABSTRACT

Schlager A.; Siedentopf, Golaszewski, S.; Felber S.
Effects of Laser Acupuncture on the Visual Cerebral Cortex: A Functional MRI Study
Dept. of Magnetic Resonance, Radiology, University of Innsbruck, Austria, Dept. of Anaesthesia, University of Innsbruck, Austria Abstracts of the ICMART Symposium, June 14-17, 2001, Berlin, Germany

The aim of this study was to investigate the effect of laser acupuncture on the cerebral cortex and to compare the results with needle acupuncture on the same acupoint performed in a study by Cho et al.1998. The study used 10 healthy male volunteers (ages: 18-38).

The acupoint BL-67 was used and compared the laser acupuncture with a placebo acupuncture at the same point. For placebo the laser was put on the skin with same tactile stimulus as verum, but the laser was not switched on. Subjects were not able to differentiate between verum and placebo acupuncture. All experiments were performed on a 1.5 Tesla MR scanner. For post-processing SPM99 software (Friston et al. 1995) was used.

A statistical parametric activation map was calculated for the whole subject group as well as single subject analysis was performed using a p-value of 0.001 and a cluster size of 4. For comparison of results between verum and placebo, the placebo group result was subtracted from the verum group result. A statistically significant difference was found between the two groups in the Brodmann area 18, 19 and 37 of the left visual cortex with the dominant activation focus within the BA 19 and 37 area and in the Pons. A similar activation pattern to Cho et al was demonstrated.

The results demonstrate that laser acupuncture elicits a similar response within cerebral cortex to needle acupuncture and thus gives further evidence for the therapeutic potential of laser acupuncture.

Herbal Formula Spotlight

Gui Pi Tang (Restore the Spleen Decoction)

Gui Pi Tang (Restore the Spleen Decoction) is a renowned blood tonic used to treat Heart (Blood) and Spleen (*Qi*) deficiencies. The original source of the formula is from the *Ji Sheng Fang* (Formulas to Aid the Living) by Yan Yong-He in 1253.[1] The original formula of *Gui Pi Tang* is composed of the following individual herbs: 6 g *Ren Shen* (Radix et Rhizoma Ginseng), 12 g *Huang Qi* (Radix Astragali), 9 g *Bai Zhu* (Rhizoma Atractylodis Macrocephalae), *3 g Zhi Gan Cao* (Radix et Rhizoma Glycyrrhizae Praeparata), 9 g *Dang Gui* (Radix Angelicae Sinensis), 12 g *Long Yan Rou* (Arillus Longan), 9 g *Fu Shen* (Poria Pararadicis), 12 g *Yuan Zhi* (Radix Po.lygalae), 12 g *Suan Zao Ren* (Semen Ziziphi Spinosae), and 6 g *Mu Xiang* (Radix Aucklandiae).[1] The Chinese therapeutic actions of this formula are to tonify *Qi* and Blood, strengthen the Spleen and nourish the Heart. *Gui Pi Tang* is composed of *Si Wu Tang* (the base formula for building Blood) plus additional herbs to tonify Spleen and nourish the Heart. The king herbs in this formula are *Ren Shen* (Radix et Rhizoma Ginseng) and *Huang Qi* (Radix Astragali). These two herbs serve to strongly tonify Spleen. In addition *Bai Zhu* (Rhizoma Atractylodis Macrocephalae), and *Zhi Gan Cao* (Radix et Rhizoma Glycyrrhizae Praeparata cum Melle) warm and tonify Spleen *Qi*. *Dang Gui* (Radix Angelicae Sinensis) and *Long Yan Rou* (Arillus Longan) nourish the Liver, tonify Heart blood, and regulate blood circulation. *Fu Shen* (Poria Paradicis), *Yuan Zhi* (Radix Polygalae) and *Suan Zao Ren* (Semen Ziziphi Spinosae) nourish the Heart to calm the *shen* (spirit). *Mu Xiang* (Radix Aucklandiae) moves qi and moderates the stagnating nature of the tonic herbs. *Sheng Jiang* (Rhizoma Zingiberis Recens) and *Da Zao* (Fructus Jujubae) harmonize the middle *jiao*.

Clinical signs of Heart (Blood) deficiency include arrhythmia, tachycardia, and inability to concentrate and follow commands, anxiety and insomnia. Clinical signs of Spleen (*Qi*) Deficiency include poor appetite, lethargy and bleeding disorders. A pale, dry tongue and a thready, weak pulse indicate *Qi* and blood deficiencies. This condition can be caused by overwork and chronic illness that injure the Spleen leading to Spleen *Qi* Deficiency. The Spleen is the source of production of Blood. In this case, Deficient Spleen fails to generate Blood that is governed by the Heart, consequently leading to Spleen *Qi* Deficiency and Heart Blood Deficiency. [2] *Gui Pi Tang* is also indicated for disorders in which the Spleen fails to hold the blood within the vessels due to Spleen qi deficiency. Symptoms of bleeding, such as petechiae and ecchymoses, hematochezia, hematuria, hematemesis, epistaxis and hemoptysis, may occur. *Gui Pi Tang* is frequently used to treat dogs with immune-mediated hemolytic anemia (IHA) and immune-mediated thrombocytopenia (ITP). This herbal formula can help to decrease the high mortality rate seen. *Gui Pi Tang* has been given with transfusions and/or in combination with steroidal, antimicrobial (doxycycline, enrofloxacin, cephalexin) and immunosuppressive drugs (azathioprine, cyclosporine, cytoxan), without adverse effects. Generally, the dose of steroids is gradually reduced over time, and the hematocrit and other blood parameters are closely monitored as the animal is weaned off steroids. Herbal formulas to treat hemolytic and platelet disorders typically require treatment for a minimum of three months, with modifications as needed. Treatment should be started as soon as possible for best results and it is important to use an adequate amount of the formula to achieve the best therapeutic results. *Gui Pi Tang* can be used alone, but may require modification, depending on the case. To stop bleeding, add *E Jiao* (Colla Corii Asini), *Pu Huang* (Pollen Typhae), *Xian He Cao* (Herba Agrimoniae), *Mo Han Lian* (Herba Ecliptae) and *Ou Jie (Nodus Nelumbinis Rhizomatis)*; with damp-heat, add *Dan Shen* (Radix et Rhizoma Salviae Miltiorrhizae), *Huang Lian* (Rhizoma Coptidis), *Zhi Zi* (Fructus Garde- niae), *Xuan Shen* (Radix Scrophulariae) and *Di Huang* (Radix Rehmanniae); with Spleen yang deficiency and cold, add *E Jiao* (Colla Corii Asini) and *Fu Zi* (Radix Aconiti Lateralis Praeparata). *Gui Pi Tang* can be used to treat non-regenerative anemia seen in dogs with congestive heart failure with *Qi* and Blood deficiencies. For the treatment of cardiac arrhythmia, *Long Gu* (Os Draconis) and *Dang Shen* (Radix Codonopsis) can be added for improved clinical effects. *Gui Pi Tang* can address cases of chronic nasal bleeding in horses. These patients are typically not racehorses with exercise-induced pulmonary hemorrhage (EIPH), but instead are sport/performance horses that have been overworked and have Spleen *Qi* deficiency that compromises the Spleen's ability to hold blood in the vessels. These nosebleeds may or may not be associated with exertion and can result from both pulmonary and ethmoid hemorrhage.

Horses and Cattle: 15–60g, twice daily. Camels: 30–75g, twice daily. Llamas, Alpacas, Pigs, Goats, and Sheep: 5–15g, twice daily. Dogs: 0.5–5g (or 0.1g per kg body weight), twice daily. Cats and Rabbits: 0.2–0.5 g (or 0.1 g per kg body weight), twice daily. Birds: 0.1–0.2g per kg body weight, twice daily.[2] *Gui Pi Tang* is relatively neutral in property and usually does not produce side effects even with long-term use. The pharmacological effects of *Gui Pi Tang* include anti-ulcer and cognitive effects.

The use of *Gui Pi Tang* was effective in preventing stress-induced ulcer in mice. The mechanism of action was attributed to the regulatory effect of the herbs on the central nervous system to reduce the production and secretion of gastric acid. [3] In another study *Gui Pi Tang* was shown to be effective in improving cognitive function in mice with

impaired memory due to drugs, electric shock, or aging.[4] Human clinical studies have shown *Gui Pi Tang* to be effective in the treatment of hepatitis with thrombocytopenia, anemia and thrombocytopenia purpura. Nine patients with hepatitis and thrombopenia were treated with modified *Gui Pi Tang* with recovery in 5 patients (asymptomatic with no recurrence within 2 years), improvement in 2 patients, and no benefit in 2 patients. Most patients began to notice improvement within 14-21 days of treatment.[5] In another study, 19 patients with anemia due to iron deficiency were treated with 79% effectiveness within one month.[6] In another study, 20 patients with anemia due to hemorrhage were treated for an average of 24 doses (ranges from 5-49 doses) of decoction with good results.[7] Lastly, its effectiveness was documented in patients with impaired renal function with anemia and leukopenia.[8]

In a study on the treatment of thrombopenic purpura, 46 patients with primary thrombopenic purpura were treated with modified *Gui Pi Tang* with good results. The treatment protocol was to administer the herbs in decoction one time daily for one month per course of treatment. The average length of treatment was three courses. Out of 46 patients, the study reported marked effect in 11 patients (asymptomatic with normal platelet count for at least 3 months), improvement in 15 patients (asymptomatic with normal platelet count for at least 2 months), slight improvement in 11 and no benefit in 9 patients. The overall rate of effectiveness was 80.4%.

Signe E Beebe DVM

Table 1: The ingredients of the Chinese herbal medicine *Gui Pi Tang* (Restore the Spleen Decoction) and their actions.[1,2]

***Pin Yin* Name**	**English Name**	**Amount** (g)	**Actions**
Ren Shen	Ginseng	6g	Tonifies *Yuan Qi* and Spleen *Qi*
Huang Qi	Astragalus	12g	Tonifies Spleen *Qi*
Bai Zhu	Atractylodis	9g	Warm and tonify Spleen *Qi*, and dry Dampness
Zhi Gan Cao	Licorice	3g	Warm and tonify Spleen *Qi*
Dang Gui	Angelica	9g	Nourish the Liver, tonify Heart blood, and regulate blood circulation
Long Yan Rou	Longan	12g	Nourish the Liver, tonify Heart blood, and regulate blood circulation
Fu Shen	Poria	9 g	Strengthen the Spleen, calm *Shen*, drain damp
Yuan Zhi	Polygala	6 g	Nourish the Heart to calm the *Shen*
Suan Zao Ren	Ziziphus	12 g	Nourish the Heart to calm the *Shen*
Mu Xiang	Aucklandia	6 g	Moves *Qi*

REFERENCES

1. Beebe, S. Salewski, M, Chen J, Chen T. Chinese Herbal Formulas for Veterinarians. City of Industry, CA., Art of Medicine Press: 2012:587-589.
2. Xie H, Preast V. Xie's Chinese Veterinary Herbology. Ames, IA:Wiley-Blackwell 2010: 320-322.
3. *Tian Jin Zhong Yi* (Tianjin Chinese Medicine), 1995; 4:28
4. *Zhong Cheng Yao Yan Jiu* (Research of Chinese Patent Medicine), 1987; 5:20.
5. *Zhong Yi Za Zhi* (Journal of Chinese Medicine), 1985; 5:32.
6. *Shan Xi Zhong Yi Xue Yuan Xue Bao* (Journal of Shanxi University School of Chinese Medicine), 1988; 1:29.
7. *Guo Wai Yi Yao Zhi Wu Yao Fen Ce* (Monograph of Foreign Botanical Medicine), 1993; 15(3):44.
8. *Bei Jing Zhong Yi Za Zhi* (Beijing Journal of Chinese Medicine), 1993; 3:227.

***Gou Teng* (Uncaria)**

Common Name: Uncaria Stem
Botanical Name: Uncaria Rhynchophylia
Part Used: Stem with hooks
Channel/Organ: Liver and Pericardium
Taste: Sweet
Energy: Cool
Energetic Functions:

1. Eliminate Endogenous Wind and stop spasm and seizure
2. Clear Heat and pacify the Liver

Comments:

1. May cause bradycardia, dizziness and allergic skin reaction in patients with hypertension
2. Do not decoct for long time
3. Contraindication for Excess-fire patient

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World Association of TCVM

The Chi Institute has donated space in their new teaching facility to raise support for the WATCVM. Commemorative bricks will be added to the school's entrance to support the WATCVM's cause.
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Excess Patterns Within the Earth Element

Invasion of either Damp-Cold or Damp-Heat into the Spleen
Invasion of Heat or Cold into the Stomach and Food Stasis

Food Therapy to Treat Excess Patterns

Stomach Heat
Excess Heat is often due to too much Hot/Dry food or the environment. Heat rises resulting in halitosis, gingivitis, oral ulcers, dry nose and dry feces. Clinical signs include: a red tongue, superficial fast pulse, excessive thirst, pica, grass-eating, vomiting/regurgitation.

Duck: Lightly cooked so as to avoid Damp associated with raw meat; ½ cup for dogs, ¼ cup for cats

Clams: canned or fresh; 1 tbs for dogs, 1 tsp for cats

Green beans: lightly steamed or peas; ½ cup for dogs, 1 tbs for cats

Apples: Diced; ¼ cup for dogs, 1 tbs for cats

300mg calcium for a 30# dog, 100mg for cats
For: 30# adult dog, or 10# adult cat

Spleen Damp-Heat
Damp is pervasive, therefore hard to treat. Chronic intestinal upset, or intestinal inflammation (Heat), also leads to Damp accumulation. Symptoms include lethargy, inappetance, and malodorous diarrhea. Clinical signs include: a red tongue, often with yellow/greasy coating, and a pulse which is fast yet soft. A few small meals help tonify the Spleen.

Crab: Lightly steamed in water with dandelions; ½ cup for dogs, ¼ cup for cats

Yellow squash: Lightly steamed in water with dandelions; ¼ cup for dogs, 1 tbs for cats

Shitake mushrooms: Lightly steamed in water with dandelions; ¼ cup for dogs, 1 tbs for cats

300mg calcium for 30# dog, 100mg for cats
* This recipe is a smaller portion than normal to help tonify the Spleen by avoiding overeating.

Cucumber or apples: Served as snacks for those pets who will eat them

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