Vaccine 37 (2019) 1889–1896

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

The serological response in dogs inoculated with canine distemper virus vaccine at the acupuncture point governing vessel-14: A randomized controlled trial

John A Perdrizet [a,*], Deng-Shan Shiau [b], Huisheng Xie [b,c]

[a] The Sanctuary Animal Clinic, 210 Linden St., Holyoke, Ma. 01040, USA
[b] Chi Institute of Traditional Chinese Veterinary Medicine, Reddick, FL. 32686, USA
[c] Department of Comparative, Diagnostic, and Population Medicine, University of Florida, Gainesville, FL. 32610, USA

ARTICLE INFO

Article history:
Received 31 May 2018
Received in revised form 4 October 2018
Accepted 19 October 2018
Available online 21 February 2019

Keywords:
Acupuncture
Canine distemper virus vaccine
Randomized controlled trial
Serum Neutralization (SN) titers

ABSTRACT

The improvement of immunity to vaccination has historically focused on manipulation of antigen presentation rather than the host. Immune modulation by stimulating specific acupuncture points along the Meridian System has been practiced in Traditional Chinese Medicine. The purpose of this study was to quantitatively determine whether acupoint vaccination, in which vaccine is administered at an acupuncture point in dogs, has the potential to enhance the immune response.

A randomized controlled trial was conducted to compare the effectiveness of acupoint vaccination versus a conventional method, based on humoral immune response in dogs given Canine Distemper Vaccine (CDV). One hundred client-owned dogs were admitted to the study with following characteristics: (1) passed a routine physical exam, (2) aged between 1 and 10 years old, (3) had no history of chronic disease, and (4) were not on immunomodulating medications. Dogs were randomly assigned to either the Acupuncture group inoculated at the acupoint Governing Vessel (GV)-14, or to the Control group inoculated conventionally at a non-acupuncture site. Mean changes from Day0 to Day14 of the response to CDV vaccination, measured by serum neutralization (SN) titers with log-transformation for reducing outlier effects, were compared between groups. No significant difference was found between groups in age, weight, or sex (all $p > 0.2$). Both groups had significant increases of CDV SN titer post-vaccination ($p < 0.001$). The mean increase in Acupuncture group (0.72; SD = 0.79) was significantly greater than that of the Control group (0.36; SD = 0.67); $p = 0.019$. Inference on percentage of change in raw SN titer data further revealed that the effects in the Acupuncture group was significantly greater than the Control group (242% vs. 83%; $p = 0.02$).

This study demonstrated that Acupoint vaccination at GV-14 resulted in a significantly elevated humoral immune response to CDV vaccine compared to Controls, which suggests the potential of acupoint vaccination to enhance the immune response.

Canine Distemper Virus (CDV), which attacks the respiratory, gastrointestinal, and nervous systems of puppies and dogs, is an RNA virus of the family *Paramyxoviridae* in the genus *Morbillivirus* [1]. It is a significant and persistent pathogen in domestic and wild members of the Canidae family, and has an even greater range of hosts, including many species within the families Felidae (cats), Hyaenidae (hyenas), Mustelidae (weasels, ferrets, otter, etc.), Mephitidae (skunks), Procyonidae (raccoons), Ursidae (bears), Viverridae (civets), and Phocidae (seals) [2].

The control and prevention of CDV rely on the pillars of infectious disease prevention: good nutrition, sanitation, environmental control (impervious or well drained surfaces, good ventilation, adequate stocking rates), preventive vaccination, and therapeutic drugs in exposed animals [3]. CDV continues to be a persistent and virulent pathogen even in the face of modern medical advances including vaccination [4,5]. The resurgence of distemper can still be seen in large populations of inadequately vaccinated dogs [6].

As with most viral induced diseases, prevention is critical for stopping the spread of disease and mitigating morbidity/mortality. The salient method to accomplish this goal in conventional western medicine is vaccination [3,4]. Immunity to CDV after an initial

Abbreviations: CDV, canine distemper virus; GV, governing vessel; MLV, modified live virus; PoC, percentage of change; SN, serum neutralization.

* Corresponding author.
E-mail addresses: jperd@verizon.net (J.A Perdrizet), xieh@ufl.edu (H. Xie).

https://doi.org/10.1016/j.vaccine.2018.10.076
0264-410X/

vaccine series of 2 or 3 doses appears to be long lasting, and the consensus in the veterinary community is that booster vaccination every 3–4 years, or serological testing with vaccination as needed, is adequate for protection in the field [4,7].

In human medicine, the causal relationship between vaccination and immune-mediated disease has been suspected for some time [8–11]. In dogs, immune-mediated thrombocytopenia, hemolytic anemia and other adverse reactions have been associated with many antigens including CDV [12–17]. Based on the potential adverse consequences of vaccination, and the perception by the public that vaccines are a risk to their health and wellbeing, it is imperative to develop protocols that extend the duration of immunity and mitigate the negative side effects of vaccination [18]. This goal has historically focused on antigen presentation [19,20] and addition of adjuvants to vaccines [21,22]. However, the host's innate immunity and complex biological response are critical components of vaccine success that must be considered [23].

Traditional Chinese Medicine incorporates its view of immunity and homeostasis into the use of acupuncture for both infectious disease prevention and therapy [24]. Acupuncture is known to be immunomodulating, and in both human and veterinary medicine its use has been reported for immune stimulation [25–34]. The acupoint GV-14 has been used for immunomodulation in humans [29,32,35–37] and animals [30,31,33]. Although the immunomodulating qualities of GV-14 have been known for decades, to the authors' knowledge, there has never been any controlled clinical trial in which vaccine is inoculated directly into the GV-14 acupoint published in the English language.

The purpose of this study was to quantitatively evaluate the hypothesis of enhanced immunity with acupoint vaccination, by comparing the immune response of dogs vaccinated with CDV vaccine between two treatment groups: dogs vaccinated by a conventional protocol and dogs vaccinated at the acupuncture point GV-14. The immune response was calculated as a change in Serum Neutralization (SN) titer to CDV vaccination in subjects over a two-week period. We hypothesized that dogs given vaccine at the acupoint GV-14 would have a greater change in CDV SN titer than dogs conventionally vaccinated at a non-acupoint.

1. Materials and methods

1.1. Animals

One hundred (100) client-owned dogs were enrolled at the time of yearly wellness visits at the Sanctuary Animal Clinic in Holyoke, Massachusetts. The time for enrollment and collection of samples covered an eleven-month period, ending in September 2017. All patients had a physical exam just prior to vaccination and were ascertained to be disease free. Dogs with a history of chronic complicating diseases such as Cushing's, Addison's, atopy, heart disease, renal disease, liver disease, or on long-term steroidal or immune-suppressing therapy, were excluded. All dogs had been previously vaccinated within the past 1–3-year period, and the time since previous vaccination was recorded. The age range was 1–10 years. At the time of the yearly examination clients were given a single page description of the study and an informed consent form was signed.

1.2. Experimental design and procedure

A randomized controlled trial was designed for this study. At the time of enrollment, each dog was randomly assigned to one of the two treatment groups: Control and Acupuncture groups. A simple randomization process was used to assign individuals to one of the two groups. Specifically, 50 identically sized pieces of paper with the word 'control' and 50 with the word 'case' were folded to hide their designated group and placed in a box. At the time of enrollment, the owner chose a paper from the box; their dog was then assigned to the group specified on that paper. The owners were present during the inoculation, and subsequently were aware of the treatment group to which their dog had been allocated.

The dogs in the Control group were inoculated subcutaneously with the standard 1 ml dose of commercially available MLV multivalent vaccine[1] (Distemper-Adenovirus Type 2-Parainfluenza-Parvovirus), which includes CDV, at a location away from any known acupoint on the right caudolateral side of the neck. The exact vaccination site for the control group was 2 *Cun* cranial to the cranial edge of the scapula, between the Gallbladder (GB) Channel ventrally and the Bladder (BL) Channel dorsally, and the needle was inserted to the hub at a 25° angle to the skin subcutaneously. A '*Cun*', also called the 'Chinese inch', is a proportional unit of measurement used in acupuncture to accurately locate a specific anatomical location. For example, there are always 12 *Cun* between the carpus and the elbow (cubital crease) of every dog, so the proportional distance between the carpus and elbow is the same regardless of the animal's size. The dogs in the Acupuncture group were inoculated with the same dose of vaccine as the controls but at the acupoint GV-14. The exact location for GV-14 was on the dorsal midline, just cranial to the front edges of the scapulae in a depression cranial to the dorsal spinous process of the first thoracic vertebrae, and the needle was inserted to the hub at a 90° angle to the skin subcutaneously. In both groups inoculation was implemented using a 3 cc syringe with a 25 gauge, 5/8″ hypodermic needle for dogs that weighed less than 13.62 kg (30 lbs.). Inoculation was implemented using a 3 cc syringe with a 25 gauge, 1.5″ hypodermic needle for dogs that weighed more than 13.62 kg. A new needle and syringe were used on each patient. Both groups were inoculated with the same vaccine used at the previous wellness visit.

Blood samples were taken on Day 0 (just prior to vaccination) and on Day 14 post-vaccination using 22ga or 25ga hypodermic needles with a 3 cc syringe from the cephalic or lateral saphenous vein. The blood was immediately transferred to a serum separator tube and allowed to clot at room temperature (approx. 23C) for 15–20 min after collection. Centrifugation for 10 min at 3500 X g was performed on the blood samples, and the serum was transferred (to a 3 ml plastic tube), labeled, dated, and stored in a frost-free freezer in Styrofoam containers containing ice-packs to assure a stable constant temperature (−20 °C). Sera were stored at the Sanctuary Animal Clinic until all samples had been acquired, after which the frozen serum samples were transported in Styrofoam containers with ice-packs, to the laboratory at the Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine (Ithaca, NY, USA), for analysis. The laboratory personnel were blinded to the group assignment of each paired sample, as they were given 100 paired samples sequentially numbered with no other data provided.

1.3. Outcome measurement

The Serum Neutralization (SN) assay for CDV was used to measure serum antibody concentration in each collected sample. [38] The SN assay was run in batches of 25 paired samples (Day 0 and Day 14) to minimize any day-to-day assay variation between the paired samples. The SN test for CDV was done with 4 replicate dilutions/serum sample to increase the accuracy of the results. For

[1] Novibac: Canine 1-DAPPV, Lot #'s 02121715B, 02121716B Intervet Inc., Merck Animal Health, Omaha, Nebraska. A Canine Distemper-Adenovirus Type 2-Parainfluenza-Parvovirus MLV Vaccine.

the assay 50 μl of serum was added to the first well of a 96-well plate in the dilution series (wells contain 50 μl of medium as a diluent). Twofold dilutions were made carrying forward a 50 μl volume. After dilution, 50 μl of test virus was added to each well (for CDV the target challenge virus was 10–30 $TCID_{50}$/well). Following a 1–1.5 hr. incubation, a 100 μl volume of cells (Vero) was added to each well. Plates were incubated for 5 days at ~37C in 5% CO_2. Wells were scored for the presence of virus growth by individual well microscopic examination for CDV cytopathology (syncytia formation). Titers were reported as the inverse of 50% end-point serum dilutions according to Reed-Muench [39].

1.4. Data processing and statistical analysis

Due to the wide range of SN titer measurements, most studies have used geometric mean (instead of arithmetic mean) when conducting statistical inference on SN titer responses to mitigate the impact of extreme values (outliers) on the overall means. Therefore, in one aspect of the statistical analysis, each SN titer measurement was first converted to its log-transformed value (natural logarithm was applied) before conducting statistical analyses. The ultimate primary outcome measurement from each subject was the change of the log-transformed CDV SN titer value from Day 0 to Day 14. In addition, as a secondary outcome variable, the study calculated percentage of change (PoC), i.e., (Day 14 – Day 0)/Day 0, from raw SN titer values as another measurement of change in each subject. The reason for using the raw titer values for PoC analysis was that, as the change was normalized to a percentage, the effect from those extreme titer values was mitigated.

For both measurements of change, the study tested the null hypothesis (H_0): the distribution of change in the acupuncture group is the same as that in the control group, versus the alternative hypothesis (H_A): the distribution of change in the acupuncture group is different from that in the control group. Due to the non-normal distributions of these changes, non-parametric Wilcoxon Rank Sum test (2-sided) was used to test the hypotheses.

The proportion of subjects with increased CDV SN titers can be counted as the number of subjects whose PoCs are greater than 0, or greater than or equal to 0.5 for those with at least 50% increase in CDV SN titers. For each of these two conditions ($PoC > 0$ or $PoC \geq 0.5$), the proportions in Control and Acupuncture groups were compared (H_0: The proportions are equal between the two groups, vs. H_A: The proportion in the acupuncture group is different from that in the control group) using two-sample test for equality of proportions (2-sided; Chi-square distribution, degrees of freedom = 1).

For all data presentations and statistical analyses, statistical software R[2] was used. A null hypothesis was rejected when the resulting p-value was ≤ 0.05.

1.5. Sample size and power analysis

A sample size of 50 subjects in each group ensured that the applied Wilcoxon Rank Sum test would have approximately 95% power to reject the null hypothesis when the true difference between the control and acupuncture groups (effective size) was at least 1.0 × standard deviation (pooled from the two groups) with a significance level of 0.05.

[2] R version 3.4.1 (2017–06-30) -- "Single Candle" Copyright (C) 2017 The R Foundation for Statistical Computing; Platform: x86_64-w64-mingw32/x64 (64-bit) Venables WN, Smith DM, and the R Core Team. An Introduction to R. Notes on R: A Programming Environment for Data Analysis and Graphics; Version 3.4.3, 2017.

2. Results

2.1. Dogs

All 100 enrolled dogs completed the study. By design, the random assignment procedure resulted in 50 dogs inoculated at the acupoint GV-14 (acupuncture group) and the other 50 dogs inoculated conventionally in the non-acupuncture site (control group). Statistical comparisons on four important characteristic variables, age, weight, sex, and time since last CDV vaccination between Acupuncture and Control groups did not suggest significant difference (with 0.05 significance level), and therefore, these variables were not likely to confound the study outcome (Table 1). There were no adverse consequences reported on any of the subjects.

2.2. Raw data of CDV SN titers

Visual examination on the raw data of CDV SN titers in the Control and Acupuncture groups suggested that there were outliers in both groups (Fig. 1). As expected, none of this data followed a normal distribution, nor were the distributions of the change from Day 0 to Day 14 (based on Shapiro-Wilk normality test, all p-values < 0.05). Due to the significant effect of outliers on mean values, the change in the Control group (mean = 182.2; SD = 630.3) was larger than that in the Acupuncture group (mean = 134.1; SD = 199.4). This outlier effect can be noticed by the observation that the median change was 20 in the Control group compared to 65.5 in the Acupuncture group. Overall, 37 out of 50 subjects in the Control group (74%) had increased CDV SN titers from Day 0 to Day 14, whereas in the Acupuncture group, 43 out of 50 subjects (86%) had increased CDV SN titers.

2.3. Log-transformed data of CDV SN titers

After applying (natural) Log-transformation on raw data of CDV SN titers, compared with the raw data (Fig. 1), the outlier effects are greatly reduced in both groups (Fig. 2). Furthermore, none of the distributions of Log-transformed data (Control and Acupuncture subjects on Day 0 and Day 14) can be rejected for being normally distributed, based on Shapiro-Wilk normality test (all $p > 0.1$).

Before comparing treatment effects, it is important to examine whether the baseline (Day 0) outcome values are different between the compared groups, which could confound the outcome of the treatment effect comparison. The main summary statistics (mean, median, SD) in the Control group are (4.73, 5.03, 1.71), and in the Acupuncture group are (4.39, 4.60, 1.41). Based on the Wilcoxon Rank Sum test, the null hypothesis that the two treatment groups had the same distribution of Day 0 Log-transformed CDV SN titer value cannot be rejected (p = 0.208) under a 0.05 significance level.

The changes of Log-transformed SN titers from Day 0 to Day 14 in Control and Acupuncture subjects were assessed (Fig. 3). The mean change in the Control group was 0.36 (median = 0.35, SD = 0.67) and was 0.72 (median = 0.52, SD = 0.79) in the Acupuncture group. For both groups, the change of Log-transformed SN titers remained far away from any normal distributions ($p = 1.0 \times 10^{-4}$ and 3.86×10^{-6} for Control and Acupuncture subjects, respectively). The statistical comparison of the distribution of changes between the two groups suggested significant group difference (Wilcoxon Rank Sum test, p = 0.0193). Since both mean and median in the Acupuncture group were greater than those in the Control group, it can be concluded that, in term of their distributions, the change of Log-transformed SN titers in the Acupuncture group was greater than that in the Control group.

Table 1
Summary statistics of subject characteristic data.

	Acupuncture group	Control group	p-value
Age (years)	Mean = 5.64 Median = 5.85 SD = 2.72 Range: 1.20–10.60	Mean = 5.52 Median = 5.50 SD = 2.68 Range: 1.20–10.60	0.8013
Weight (lbs)	Mean = 48.44 Median = 42.35 SD = 31.97 Range: 8.00–129.20	Mean = 41.41 Median = 32.45 SD = 28.87 Range: 4.75–117.00	0.2915
Sex (%)	44% – Fs 56% – M/Mc	44% – Fs 56% – M/Mc	1.000
Time duration since last CDV vaccine (months)	Mean = 13.88 Median = 12.50 SD = 3.73 Range: 9–30 15.92 ± 6.71	Mean = 15.92 Median = 13.00 SD = 6.71 Range: 11–37	0.411

2.4. Percentage of change (PoC) in CDV SN titers

While Log-transformation is commonly used for data with extreme values, the nonlinear transformation makes interpretation of the conclusions from statistical analysis not as straightforward as that when using raw data or more intuitive conversions such as Percentage of Change (PoC). Therefore, to examine the data more thoroughly, the study further compared the difference between Control and Acupuncture groups with respect to the PoC data (Fig. 4).

Wilcoxon Rank Sum test was applied to test the statistical significance of the difference of the PoC distribution in Control (83%) and Acupuncture (242.3%) groups (Fig. 5). The null hypothesis (H_0) is that the PoC distribution in Acupuncture subjects is the same as that in Controls. The test concluded a rejection of the null hypothesis ($p = 0.0196$), which supported the evidence that the PoC in the Acupuncture group is significantly larger (difference in distribution with a shift to the right) than the PoC in the Control group.

With respect to the proportion whose PoCs were greater than 0 (any increase), the test concluded that the difference between the two groups (Control group = 0.74 vs. Acupuncture group = 0.86) was not statistically significant ($p = 0.211$; left panel in Fig. 6). However, with respect to the proportion whose PoCs were greater than or equal to 0.5 (at least 50% increase), the test concluded that the difference between the two groups (Control group = 0.40 vs. Acupuncture group = 0.62) was statistically significant ($p = 0.045$; right panel in Fig. 6).

3. Discussion

The main findings from this study are: (1) using Log-transformed CDV SN titer data, both Control and Acupuncture groups had significantly increased CDV SN titers 14 days after receiving the vaccination, and the Acupuncture group has a significantly greater increase than the Control group; (2) the Acupuncture group had higher Percentage of Change (Day 0 to Day 14) in CDV SN titers, and also had much higher proportion of subjects with significant increase (≥50%) of CDV SN titers.

It was not surprising that both the Control and Acupuncture groups had significant increases in SN titer over the 14-day test period, as this only confirmed that the vaccine effectively induced a humoral immune response regardless of the site of inoculation in most subjects. That a MLV CDV vaccine would induce an anamnestic response in previously vaccinated healthy adult dogs is consistent with the literature [7]. The lack of a measurable anamnestic response in individuals from both groups is not surprising given

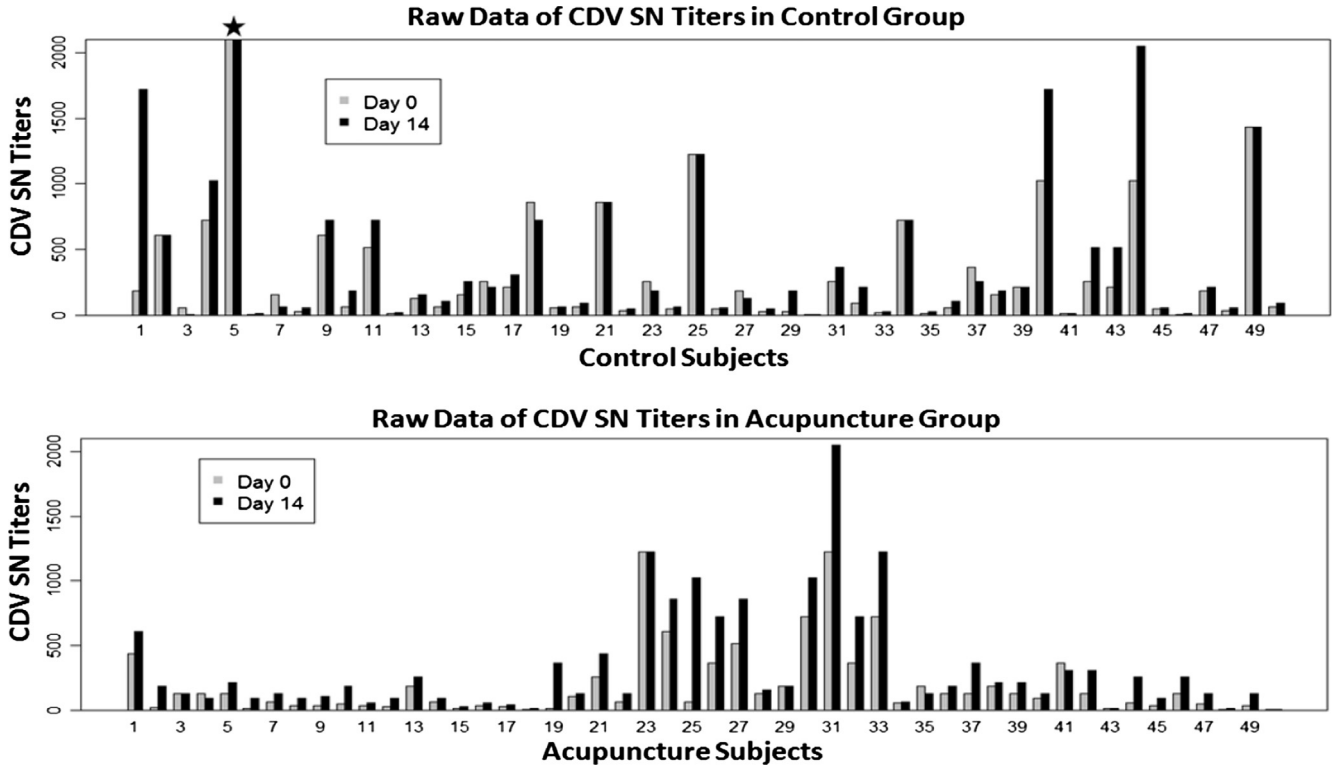

Fig. 1. Raw data of CDV SN titers in Control and Acupuncture groups. Note that the 5th Control subject (marked with a star on the top) had extremely large values of SN titers on both Day 0 (5734) and Day 14 (9830).

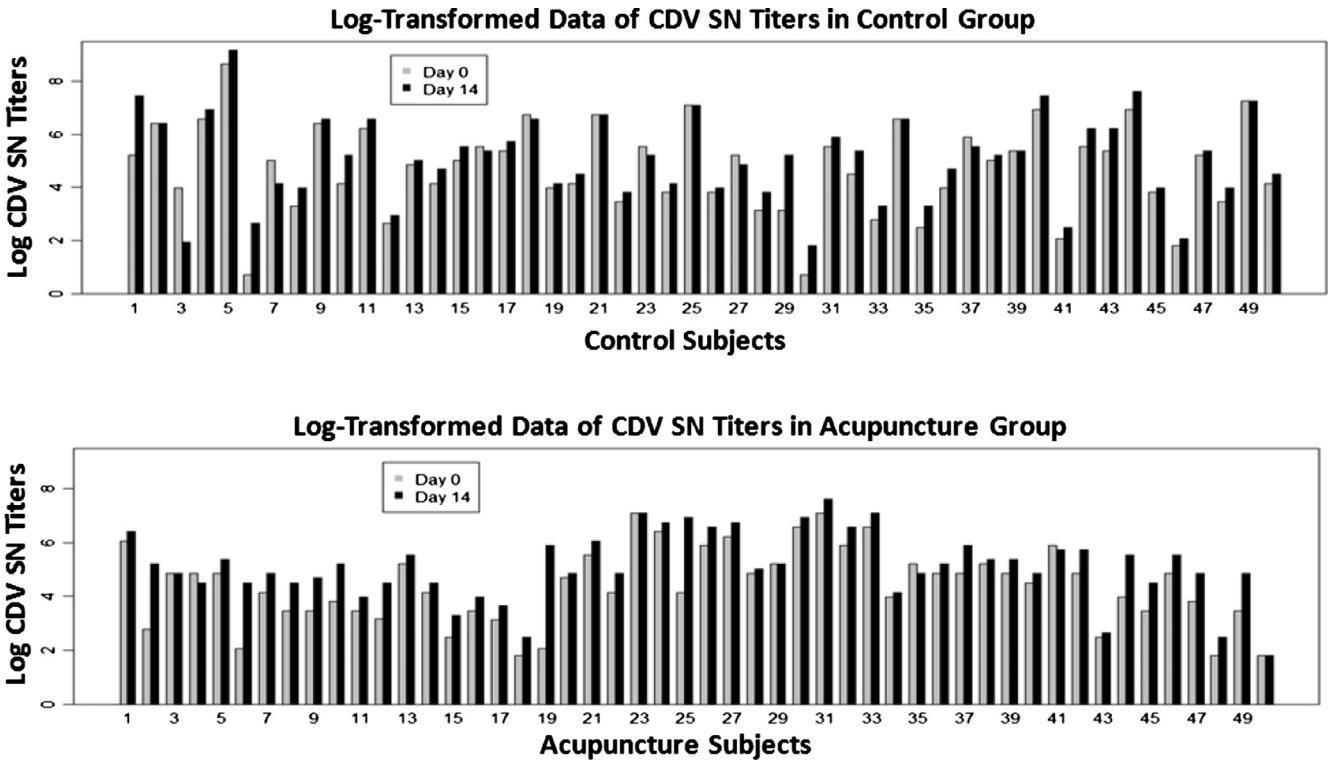

Fig. 2. Log-transformed data of CDV SN titers in Control and Acupuncture groups.

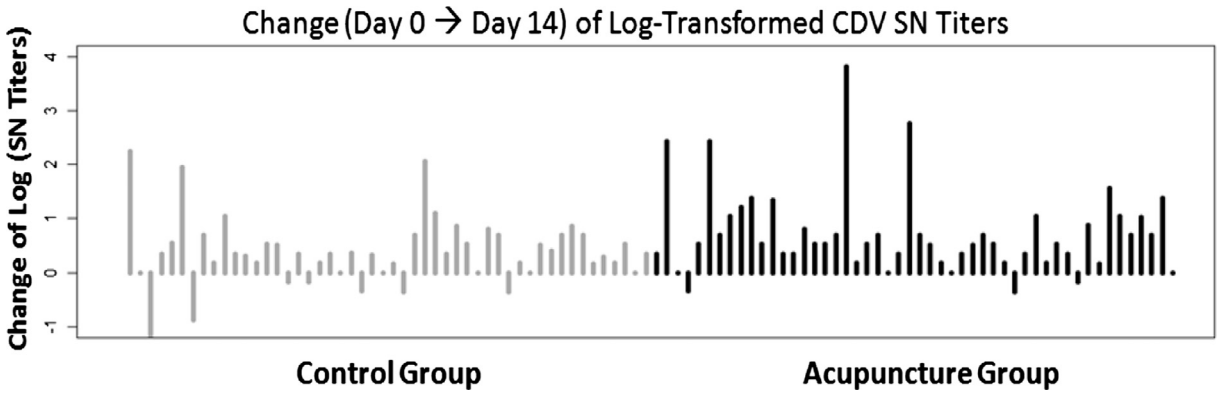

Fig. 3. Change of Log-transformed CDV SN titers from Day 0 to Day 14 in Control and Acupuncture subjects.

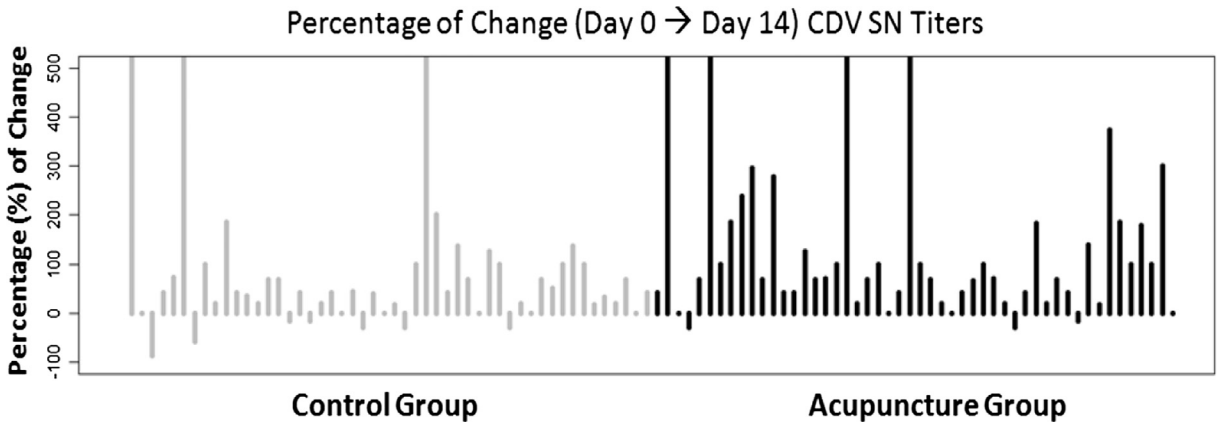

Fig. 4. Percentage of Change (PoC) in CDV SN titers from Day 0 to Day 14 in Control and Acupuncture subjects.

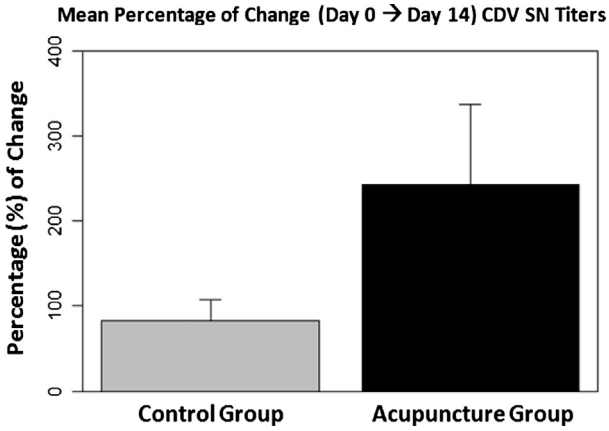

Fig. 5. Mean percentage of change in CDV SN titers, Control and Acupuncture groups. The length of each error bar stands for the standard error of the corresponding estimated mean value. Control Group: Maximum = 845.1% Minimum = -87.0% Median = 41.6% Acupuncture Group: Maximum = 437.5 Minimum = -29.7% Median = 68.8%.

the short duration since their last vaccination. The fact that there was no statistically significant difference between groups in regard to duration since last vaccination, supports the conclusion that this did not affect the outcome measurements between groups. Future studies would be required on dogs with longer duration since last vaccination, and ideally a study utilizing naïve unvaccinated dogs to better assess the difference in the immune response to CDV vaccination. Additionally, it would be logical to choose other 'non-acupuncture' sites for inoculation of the control group to test for titer response variation. This would lend further support to the acupoint hypothesis should the acupoint group remain consistently higher. The authors chose the particular site (for the control group) described in the Materials and Methods section based on a commonly used area for vaccination by veterinarians in practice.

The most important outcome of this study was a significantly higher humoral antibody response (SN titers) in dogs inoculated at GV-14 compared to subjects inoculated at a non-acupuncture site. The question of what titer affords adequate protection with regards to CDV SN titers is elusive, as the interplay of cell mediated immunity, host stressors, and challenge dose all affect clinical outcomes. The desired effect of vaccination is to induce a protective antibody response, and higher titers can be assumed to offer better protection, or protection of longer duration. [40] CDV SN titers have been considered protective against exposure to CDV in dogs when the SN titer is equal to or greater than 1:32. [41] The duration of protection is typically for 3–5 years, based on challenge studies. [7,42] The present study investigated whether the site of vaccination has an impact on the ability to boost an existing titer. To answer the question as to whether acupuncture vaccination is more protective than conventional methods would require challenge studies in the future.

The randomization and controlled nature of this study helped mitigate the likelihood that the outcomes of group comparison were influenced by internal factors. This is supported by the observations that the signalment (Table 1) showed no significant difference between Acupuncture subjects and Controls. The importance of this lack of difference between groups is twofold: first, since the Acupuncture subjects and Controls were similar in measurable biological variables, under the null hypothesis, it can be inferred that they should have equal chance to respond to the treatment (CDV vaccination) with no reason to believe they are biased populations; and second, that it is reasonable to extrapolate the findings to the general dog population at large. Further evidence of this generalization was that 39 breeds were represented, and these are among the most popular breeds seen in clinical practice. The breed diversity in both groups makes extrapolation of the study's findings to the dog population at large more robust.

There are limitations to randomized, controlled clinical trials such as ours which need to be addressed. Ideally blinding of the participants decreases inadvertent bias, however the authors did not believe that blinding of the animal's owner was necessary, and that study participation was positively affected with full disclosure. Performance bias on the part of the clinician administering the vaccine was anticipated and reduced by having a clearly defined inoculation protocol, which did not vary between subjects. The possibility of detection bias was obviated by blinding the laboratory personnel from any individual sample's treatment group designation. All samples were numbered in chronological sequence, and treatment group was not available during analysis. It is possible for sampling bias to occur, given the need for client

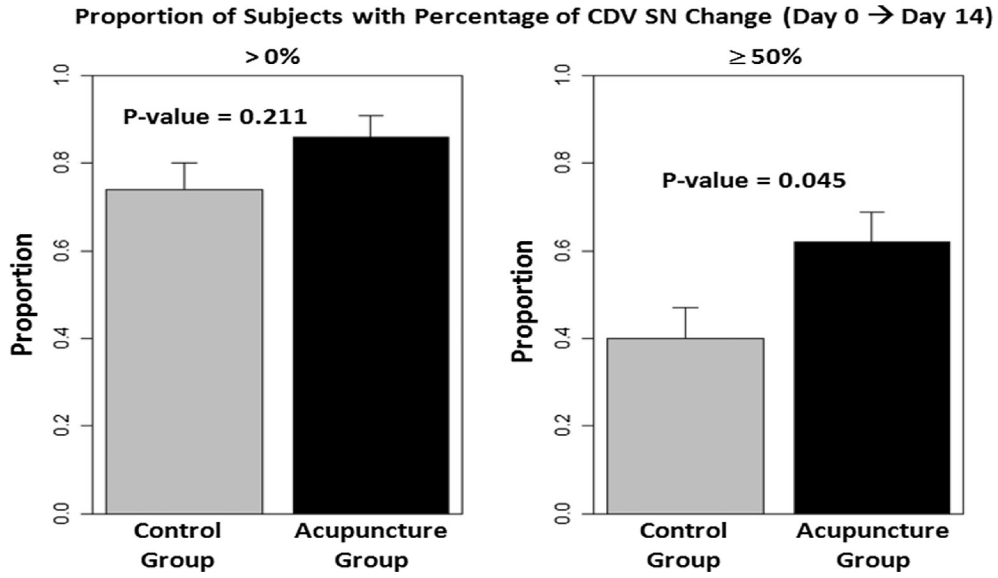

Fig. 6. Proportion of subjects with increased CDV SN titers (Left: any increase; Right: increased at least 50%). The length of each error bar stands for the standard error of the corresponding estimated proportion.

consent. However, records were kept on clients who refused participation, and the signalment of their dogs did not vary significantly from the enrolled dogs (data not shown).

A search of the literature showed only one study in which CDV vaccination was used in combination with acupuncture [43]. In that study, using Artic Fox pups, the authors did not inoculate the pups in an acupoint and did not evaluate the serological response as an outcome measurement. The vaccine was given subcutaneously in an undocumented anatomical location, simultaneously with dry-needle acupuncture. The authors reported enhanced cellular immunity based on a significantly prolonged lymphocytosis in the vaccinated group compared to the control group. Another study, in which 10 Rottweiler dogs were vaccinated at three immunomodulating acupoints (including GV-14) with a multivalent MLV vaccine (containing CDV) and then followed serologically for 6 weeks, showed a higher titer than baseline values. No controls were included in the study and the low number of subjects (4 in one group, 3 in each of 2 separate groups) was inadequate for statistical analysis [44].

Acupuncture has been used in veterinary medicine for thousands of years, whereas it has only been 30 years since acupuncture vaccination was introduced in China [45]. The method is different from Western medicine in the route of intramuscular or subcutaneous injection and is also different from traditional acupuncture therapy. Immunological/biological agents (antigens, antibodies, etc.) are injected into specific acupoints thought to enhance immunity. The idea is that stimulation of the acupoints can amplify the effect of the vaccine and thus more efficiently enhance the immune response. A summary of the technique with respect to the choice of acupoints, types of vaccines, species applied, and some results from clinical studies can be found in a review article [45].

Studies that reported results in acupoint vaccination were in farm animals. Wang and colleagues reported a study on the immunity of chicken infectious bursal disease, in which results of vaccination by means of acupoint injection at GV-1, oral administration, and intramuscular injection were compared [46]. The authors found that the serum of the acupoint injection group had significantly higher immunoglobulin (IgG) than the other two groups but had lower immunoglobulin (IgA) than the oral administration group. Furthermore, based on the ratio of bursa weight to body weight, the acupoint injection group showed better immune function than the other two groups. Similar results on GV-1 acupoint inoculation were reported by Guo with a Newcastle disease oil emulsion vaccine for enhancing immune function in chickens [47]. A review article on porcine epidemic diarrhea virus (PEDV) reviewed studies of vaccination through GV-1 acupoint injection [48]. One study showed the active immunization protection rate in 3-day-old piglets was 77% and the passive immunization protection rate was 97% [49]; Another study demonstrated 85% for both active and passive immunization protection rate [50]; and another group observed 95.5% and 96.2% active and passive immunization protection rate, respectively, in 3- to 6-day-old piglets. [51] A study was conducted in dairy cattle to compare the result of foot-and-mouth disease (FMD) vaccination with GV-1 acupoint inoculation versus conventional intramuscular inoculation. [52] The proportion of cattle achieving the desired antibody titer was 79% in the acupoint inoculation group, and only 35% in the conventional intramuscular injection group, 21 days after vaccination. Furthermore, the authors found that acupoint vaccination led to lower stress levels than conventional intramuscular inoculation, based on the average milk production lost in the time period after inoculation (1.7 kg/day for acupoint vaccinated cattle vs. 5 kg/day for conventionally vaccinated cattle). In preventing abortion in sheep, vaccination with GV-1 acupoint injection required a significantly lower vaccine dosage to attain similar immune effects. Only 1/3 of the normally prescribed vaccine dosage was needed for the GV-1 vaccinates compared to conventional intramuscular inoculation. [53] All these studies suggested that vaccination in a specific acupoint could be an effective and efficient method of immunization.

The mechanisms by which acupuncture, and by extension acupoint vaccination, can affect the immune response is still in its formative stages. [26,54,55]. The nociceptive effect of manual acupuncture incites a local inflammatory reaction that activates the neuro-endocrine-immune network. [56,57]. Systemic effects include alterations in peripheral blood leukocyte populations (including lymphocyte subpopulations) and increases in cytokine concentrations (IL-6 and IL-10). A recent study demonstrated that acupuncture at specific acupuncture points, including GV-14, increased IL-4 and IL-10. [58] A review supports the hypothesis that acupuncture has positive effects on the activities of helper T cells including Th1 and Th2 cells with consequent stimulation of B-cell antibody production. [59] These immunological responses may be involved in the mechanism(s) by which vaccine inoculation at the acupuncture point GV-14 can enhance humoral antibody production.

4. Conclusion

The results reported from this randomized controlled study support the hypothesis of enhanced humoral immune stimulation when vaccinating at acupoint GV-14 [30] and highlight the potential value of the Traditional Chinese Veterinary Medicine (TCVM) Meridian System and at least one specific acupoint (GV-14) to the field of vaccinology. Further studies on different vaccines and vaccination protocols will be required before the veterinary community can be confident in the use of acupoint vaccination. Reproduction of our results by other independent researchers is encouraged, as well as permutations involving other vaccines, and other acupoints thought to be immunopotentiating.

Acknowledgements

The authors thank Edward J Dubovi, PhD. Director of Virology at the Animal Health Diagnostic Center and Professor in the Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY for his generous support and expertise in the implementation of the CDV serological testing.

Conflict of interest

The authors declare that there were no conflicts of interest in this study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

[1] Martella V, Elia G, Buonavoglia C. Canine distemper virus. Vet Clin North Am Small Anim Prac 2008;38:787–97.
[2] Deem SL, Spelman LH, Yates RA, et al. Canine distemper in terrestrial carnivores: a review. J Zoo Wildl Med 2000;31:441–51.
[3] Green EC, Appel MJ. Canine distemper virus. In: Greene C, editor. Infectious Diseases of the Dog and Cat. Philadelphia: WB Saunders Co; 1990. p. 226–41.
[4] Davis-Wurzler GM. 2013 update on current vaccination strategies. Vet Clin North Am Small Anim Prac 2014;44:235–63.
[5] Galan A, Gamito A, Martin EM, et al. Uncommon acute neurologic presentation of canine distemper in 4 adult dogs. Can Vet J 2014;55:373–8.

[6] Blixenkrone-Moller M, Svansson V, Have P, et al. Studies on manifestations of CDV in an urban dog population. Vet Microbiol 1993;37:163–73.
[7] Abdelmagid OY, Larson L, Payne L, et al. Evaluation of the efficacy and duration of immunity of a canine combination vaccine against virulent parvovirus, infectious canine hepatitis virus, and distemper virus experimental challenges. Vet Ther 2004;5:173–86.
[8] Citron ML, Moss BM. Pneumococcal-vaccine-induced thrombocytopenia. J Am Med Assoc 1982;248:1178.
[9] Neiderud J. Thrombocytopenic purpura after a combined vaccine against morbilli parotitis and rubella. Acta Paediatr Scand 1983;72:613–4.
[10] Beeler J, Varricchio F, Wise R. Thrombocytopenia after immunization with measles vaccines: review of the vaccine adverse events reporting system (1990 to 1994). Pediatr Infect Dis J 1996;15:88–90.
[11] Souayah N, Nasar A, Suri MF, et al. Guillain-Barré syndrome after vaccination in United States: data from the centers for disease control and prevention/food and drug administration vaccine adverse event reporting system (1990–2005). J Clin Neuromuscul Dis 2009;11:1–6.
[12] Duval D, Giger U. Vaccine-associated immune-mediated hemolytic anemia in the dog. J Vet Intern Med 1996;10:290–5.
[13] McAnulty JF, Rudd RG. Thrombocytopenia associated with vaccination of a dog with a modified-live paramyxovirus vaccine. J Am Vet Med Assoc 1985;186:1217–9.
[14] Straw B. Decrease in platelet count after vaccination with distemper-hepatitis (DH) vaccine. Vet Med Small Anim Clin 1978;73:725–6.
[15] Axthelm MK, Krakowka S. Canine distemper virus-induced thrombocytopenia. Am J Vet Res 1987;48:1269–75.
[16] Moore GE, Guptill LF, Ward MP, et al. Adverse events diagnosed within three days of vaccine administration in dogs. J Am Vet Med Assoc 2005;227:1102–8.
[17] Meyer KE. Vaccine-associated adverse events. Vet Clin North Am Small Anim Prac 2001;31:493–514.
[18] Karafillakis E, Larson HJ. The benefits of the doubt or doubts over benefits? A systematic literature review of perceived risks of vaccines in European populations. Vaccine 2017;35:4840–50.
[19] Plotkin SA. Vaccines, vaccination, and vaccinology. J Infect Dis 2003;187:1349–59.
[20] Shams H. Recent developments in veterinary vaccinology. Vet J 2005;170:289–99.
[21] Singh M, O'Hagan D. Advances in vaccine adjuvants. Nat Biotechnol 1999;17:1075–81.
[22] Leroux-Roels G. Unmet needs in modern vaccinology: adjuvants to improve the immune response. Vaccine 2010;28:C25–36.
[23] Pulendran B, Li S, Nakaya HI. Systems vaccinology. Immunity 2010;33:516–29.
[24] Xie H, Preast V, Atria S. Etiology and Pathology. In: Xie H, Preast V, editors. Traditional Chinese Veterinary Medicine: Fundamental Principles. Reddick: Chi Institute Press; 2013. p. 157–200.
[25] Kim SK, Bae H. Acupuncture and immune modulation. Auton Neurosci 2010;157:38–41.
[26] Cui M. Present status of research abroad concerning the effect of acupuncture and moxibustion on immunologic functions. J Tradit Chin Med 1992;12:211–9.
[27] Habacher G, Pittler MH, Ernst A. Effectiveness of acupuncture in veterinary medicine: systematic review. J Vet Intern Med 2006;20:480–8.
[28] Xiao L, Wang H. Acupuncture: another new adjuvant. J Tradit Chin Med 2012;32:1124–6.
[29] Kou W, Bell JD, Gareus I, et al. Repeated acupuncture treatment affects leukocyte circulation in healthy young male subjects: a randomized single-blind two-period crossover study. Brain Behav Immun 2005;19:318–24.
[30] Fujiwara R, Tong ZG, Matsuoka H, et al. Effects of acupuncture on immune response in mice. Int J Neurosci 1991;57:141–50.
[31] Li H, Li XH, Zhang LF. Influence of electroacupuncture of "Dazhui" (GV-14) "Mingmen" (GV-4) and non-acupoint on the inflammation and immune reactions in adjuvant arthritis rats. Zhen Ci Yen Jiu 2009;34:225–9.
[32] Xiao L, Jiang GL, Zhao JG, et al. Clinical observation on effects of acupuncture at Dazhui (GV-14) for abating fever of common cold. J Tradit Chin Med 2007;27:169–72.
[33] Rogers PA, Schoen AM, Limehouse J. Acupuncture for immune-mediated disorders. Literature review and clinical applications. Probl Vet Med 1992;4:162–219.
[34] Joos S, Schott C, Zou H, et al. Immunomodulatory effects of acupuncture in the treatment of allergic asthma: a randomized controlled study. J Altern Complement Med 2007;6:519–25.
[35] Wang Y, Zhu Z, Yang W. Acupuncture at Dazhui (GV 14) and Geshu (BL 17) for 39 cases of leucopenia. J Tradit Chin Med 2016;36:6.
[36] Ling JY, Shen L, Liu Q, et al. Impacts on chronic fatigue syndrome and Qi deficiency syndrome and T cell subgroups in patients treated with acupuncture at selective time. J Tradit Chin Med 2013;33:1061–4.
[37] Ye F, Lui D, Wang S, et al. Effects of electro-acupuncture on T cell subpopulations, NK activity, humoral immunity, and leukocyte count on patients undergoing chemotherapy. J Tradit Chin Med 2007;27:19–21.
[38] Appel M, Robson DS. A microneutralization test for canine distemper virus. Am J Vet Res 1973;34:1459–63.
[39] Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. Am J Hyg 1938;27:493–7.
[40] Tizard I, Ni Y. Use of serologic testing to assess immune status of companion animals. J Am Vet Med Assoc 1998;213:54–60.
[41] Mozin DE, Lorenzen MJ, Haworth JD, et al. Duration of serologic response to five viral antigens in dogs. J Am Vet Med Assoc 2004;224:55–60.
[42] Twark L, Dodds J. Clinical use of serum parvovirus and distemper virus antibody titers for determining revaccination strategies in healthy dogs. J Am Vet Med Assoc 2000;217:1021–4.
[43] Sciesinski K. Cellular immunity after acupuncture stimulation at the points Dazhui, Hegu and Zusanli together with prophylactic vaccinations in young polar foxes. Scientifur 1990;14:274–8.
[44] Hu S, Xu Y. The immune responses induced by injection of vaccines at different regions of the body surface. In: Proceedings of the 18th Annual International TCVM Conference, Beijing, China. p. 86–92.
[45] Diao X. The research progress of acupoint vaccination in animals. Chin J Vet Med 2002;38:38–9.
[46] Wang XM. Comparative test of effects of immunization by Hou-hai point and other ways in chicken. Chin J Vet Sci Tech 1995;2:21–2.
[47] Guo Y. Chicken newcastle disease oil emulsion inactivated seedlings of astragalus polysaccharides at GV-1 injection on the function in AA+ chickens. Inner Mongolia University Master's Thesis; 2011.
[48] Sun D, Wang X, Wei S, et al. Epidemiology and vaccine of porcine epidemic diarrhea virus in china: a mini-review. J Vet Med Sci 2016;78:355–63.
[49] Wang M, Ma SQ, Zhou JF, et al. Development of the inactivated vaccine of porcine epidemic diarrhea virus. Chin Anim Infect Dis 1993;5:17–9.
[50] Ma SQ, Wang M, Zhou JF, et al. Adaptation of porcine epidemic diarrhea virus to vero cells and evaluation of the inactivated vaccine against porcine epidemic diarrhea virus. Chin Anim Infect Dis 1994;2:15–8.
[51] Tong YE, Feng L, Li WJ. Development of attenuated vaccine strain of porcine epidemic diarrhea virus. Chin Anim Infect Dis 1998;20:329–32.
[52] Tan Y. Study on synchronized immunization method with foot-and-mouth disease vaccines of dairy cattle. Huazhong Agricultural University Master's Thesis; 2007.
[53] Gao SD. Experimental studies on inoculation of inactivated vaccine against chlamydial abortion in ewes on Hou-hai point. Chin J Vet Sci Tech 1995;1:7–8.
[54] del Ray A. Immune-neuro-endocrine reflexes, circuits, and networks: physiologic and evolutionary implications. Endocrine Immunol 2017;48:1–18.
[55] Cabioğlu MT, Eren Cetin B. Acupuncture and Immunomodulation. Am J Chin Med 2008;36:25–36.
[56] Zhou D, Pan P, Guo Y, et al. Inflammatory reaction caused by acupuncture is one of the initial factors of acupuncture effect. Zongguo Zhen Jiu 2009;29:32–4.
[57] Ding SS, Hong C, Wang Y, et al. Acupuncture modulates the neuro-endocrine-immune network. QJM Int J Med 2014;107:341–5.
[58] Jiang W, Wang LL, Wu S, et al. Effects of mild moxibustion intervention on serum and liver amyloid-a protein, serum IL-6, TNF-alpha and IL-10 contents in atherosclerosis rabbits. Zhen Ci Yan Jiu 2014;39:63–7.
[59] Takahashi T, Sumino H, Kanda T, et al. Acupuncture modifies immune cells. J Exp Clin Med 2009;1:17–22.