An alternative combination therapy with metronidazole, clindamycin and doxycycline for *Babesia gibsoni* (Asian genotype) in dogs in Hong Kong

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ABSTRACT. *Babesia* spp. are globally distributed hemoparasites that cause disease in many mammalian species. The species *Babesia gibsoni* (Asian genotype) is prevalent and endemic in many Asian countries but has also been reported in growing numbers in countries outside of Asia. Standard therapies for the treatment of *B. gibsoni* often fail to result in consistent and successful clearance of the organism. This study evaluated the use of a combination of three antibiotics: metronidazole, clindamycin and doxycycline after atovaquone and azithromycin failed to eliminate the infection on a polymerase chain reaction (PCR) test. The aim of this study was to determine whether the triple antibiotic combination was an appropriate alternative or additional treatment for the elimination of *B. gibsoni*. The medical records of 24 patients treated from December 2012 to July 2015 were retrospectively analyzed. The diagnosis of *B. gibsoni* was confirmed with a PCR test that was also used to assess treatment response. All patients were initially treated with the standard therapy, atovaquone and azithromycin with a 25% success rate clearing *B. gibsoni*. Dogs that remained positive on PCR using the standard therapy were then treated with the triple antibiotic protocol achieving an 87% success rate. The inclusion of an alternative and potentially effective protocol for the treatment of *B. gibsoni* would increase the options for the current therapeutic options, could aid in clearance of the organism and offer a more affordable option for clients.

KEY WORDS: babesia, infectious diseases, protozoan infection, therapy, tick-borne disease

Canine babesiosis is a ubiquitous and widespread infectious hemoparasitosis. The disease is now endemic to North America, North Africa, East Africa, the Middle East and Asia [3, 7, 8, 24] and has been recently reported in Australia and in some European countries, including Hungary and Italy [12, 15]. *Babesia* species are intra-erythrocytic parasites transmitted by ixodid vectors or by direct transmission through transfusions and bite wounds with a geographic and vector dependent prevalence in the canine population [3, 25].

*Babesia gibsoni* was first described in 1910 [26] and is most commonly found in Asia (Asian genotype), with high serologic prevalence in Malaysia, Taiwan, Korea and Japan, with up to 30% of Tosa dogs reported to be infected [6, 18, 22, 27, 30]. Subclinical disease is common and infected dogs typically show mild hematological abnormalities, posing a risk as potential carriers. Severely affected dogs present with signs such as fever, hemolytic anemia and thrombocytopenia, potentially leading to euthanasia and death in hyper-acute cases or if untreated [4, 21]. Immunosuppressed states can lead to clinical disease in asymptomatic and chronically infected dogs posing an epidemiological risk for imports [4, 21].

Standard and classic treatment options for *B. gibsoni* fail to consistently eliminate the organism from the bloodstream, usually only achieving a reduction in parasitemia. Some of these protocols traditionally used for the treatment of *B. canis* include chemotherapeutic agents such as imidocarb dipropionate, diminazene aceturate, pentamidine isethionate and phenamidine isethionate [9–11]. Other protocols using a mono-therapy approach including drugs such as clindamycin or atovaquone have also shown limited success due to drug resistance [23, 33]. The addition and combination of drugs has positively decreased infection recurrence and increased clearance of parasitemia as it has been reported with clindamycin, diminazene and imidocarb, diminazene and clyndamcin, atovaquone and azithromycin, metronidazole, doxycycline and enrofloxacin, and metronidazole, clindamycin and...
doxycycline [2, 19, 20, 29, 31].

Atovaquone and azithromycin (AA) is currently considered the first-line therapy for *B. gibsoni* infection in most countries worldwide. Although AA appears to effectively ameliorate clinical signs, it has been associated with high relapse rates presumably due to atovaquone resistance associated with *B. gibsoni* cytb gene mutations [5, 13, 28]. The need to explore alternative therapies has therefore become necessary. The objective of this research was to evaluate the success rate of metronidazole, clindamycin and doxycycline (MCD), a protocol first reported in 2007 [31], as an alternative or additional treatment protocol for *B. gibsoni* (Asian genotype) infection in dogs where AA had failed to eliminate the hematozoa by a polymerase chain reaction (PCR).

### MATERIALS AND METHODS

#### Data collection and population

Electronic medical records were retrospectively evaluated in dogs that tested positive by PCR for *Babesia gibsoni* at a referral veterinary center in Hong Kong between December 2012 and June 2015. Dogs with relevant clinical signs, hematological abnormalities, diagnosed by PCR and treated with AA or MCD were identified. Dogs that did not have PCR results following treatment were excluded from the study. A total of 24 dogs met the inclusion criteria (Table 1). The population consisted of client-owned dogs living in Hong Kong of various ages and breeds. Data collected included age, breed, sex, clinical signs, diagnostic testing and therapeutic protocols.

#### Diagnostic testing

Diagnostic testing on dogs selected for the study included physical examination, hematological exam, microscopy and PCR testing. Blood samples were collected and approximately 1 ml from every patient was placed in EDTA to be analyzed. A laser counter (ProCyte Dx Hematology Analyzer, Idexx, Wetherby, UK) was used for complete blood count and a light microscope (Leica DM 750, Leica Microsystems, Wetzlar, Germany) was used for blood smear assessment after staining with a Wright-Giemsa stain.

DNA identification by PCR was used to diagnose *B. gibsoni* infection and/or clearance of infection following treatment. The samples were sent within 2–12 hr to the Department of Microbiology, Veterinary Division, Hong Kong University (HKU), where they were processed. For the extraction of DNA from the EDTA blood samples, a commercial kit (EZ1 mini kit, QIAGEN GmbH, Hilden, Germany) was used according to the manufacturer instructions, adjusted in 200 µl of TE buffer and stored at −20°C until further processed. The amplification process was performed for each PCR test from a 25 µl reaction mixture that contained

| Patient | Breed | Gender | Age Years | HCT % | PLT K/µl | Treatment overview | Micro | Outcome |
|---------|-------|--------|-----------|-------|----------|--------------------|-------|---------|
| 1       | Pomeranian | M (N) | 7         | 13.1  | 131      | 2AA + BT           | N–    | F-AA    |
| 2       | Pomeranian | F (N) | 8         | 14.2  | 66       | 1AA + BT           | NP    | CR-AA   |
| 3       | Schnauzer | M (N) | 2         | 20    | 43       | 2AA                | P+    | F-AA    |
| 4       | Mongrel   | M (N) | 6         | 52    | 19       | 1AA/1MCD           | F–    | CR-MCD  |
| 5       | Collie    | F (N) | 4         | 28.1  | 55       | 1AA/1MCD           | F–    | CR-MCD  |
| 6       | Pekingese | M (N) | 12        | 20.4  | 19       | 2AA + BT           | NP    | F-AA    |
| 7       | G.Retriever | M (N) | 10        | 22    | 16       | 1AA                | NP    | CR-AA   |
| 8       | Shih Tzu  | M (N) | 6         | 40.4  | 6        | 2AA                | N–    | CR-AA   |
| 9       | Shih Tzu  | F (E) | 7         | 32    | 2        | 2AA                | N–    | CR-AA   |
| 10      | Akita     | F (E) | 8         | 23.8  | 0        | 1AA                | P+    | CR-AA   |
| 11      | Labrador  | F (N) | 4         | 9     | 35       | 2AA + BT/1MCD      | NP    | F-AA/CR-MCD |
| 12      | Maltese   | M (E) | 7         | 15    | 8        | 4AA + BT/1MCD      | NP    | F-AA/CR-MCD |
| 13      | Corgi     | M (N) | 7         | 16.8  | 55       | 3AA + BT           | P+    | F-AA, NF-AA |
| 14      | Mongrel   | F (E) | 13        | 14.5  | 1        | 2AA + BT           | NP    | F-AA, NF-AA |
| 15      | Husky X   | M (E) | 6         | 16    | 27       | 2AA                | N–    | F-AA, NF-AA |
| 16      | Poodle    | F (N) | 7         | 32    | 8        | 2AA/1MCD           | NP    | F-AA/ NF-MCD |
| 17      | Schnauzer | F (N) | 9         | 17.4  | 41       | 6AA                | N–    | F-AA    |
| 18      | Cocker    | M (E) | 9         | 6.3   | 62       | 2AA + BT/1MCD      | N–    | F-AA/CR-MCD |
| 19      | G.Retriever | F (N) | 3        | 21    | 10       | 3AA/1CDM           | NP    | F-AA/CR-MCD |
| 20      | Samoyed   | M (E) | 1         | 12    | 46       | 1AA/1MCD           | P+    | F-AA/ NF-MCD |
| 21      | Poodle    | M (N) | 7         | 25.6  | 6        | 2AA/1MCD           | N–    | F-AA/CR-MCD |
| 22      | G.Retriever | M (N) | 14        | 11.2  | 92       | 1AA + BT           | NP    | F-AA, NF-AA |
| 23      | Schnauzer | F (N) | 6         | 19.5  | 824      | 3AA/1MCD           | NP    | F-AA/F-MCD |
| 24      | Maltese   | M (N) | 5         | 29.3  | 49       | 1AA                | NP    | CR-AA   |

Neutered male (M (N)), entire male (M (E)), neutered female (F (N)), entire female (F (E)), lowest hematoctrit (HCT) pre-treatment, platelets (PLT) pre-treatment, atovaquone and azithromycin (AA) number of courses, blood transfusion (BT), metronidazole, clindamycin and doxycycline (MCD) number of courses, microscopic blood smear assessment (Micro), negative (N–), positive (P+), not performed (NP), failed to clear *B. gibsoni* after AA or MCD (F-AA, F-MCD), not followed up after AA or MCD (NF-AA, NF-MCD), clinical recovery with clearance of *B. gibsoni* with AA or MCD (CR-AA, CR-MCD). When two protocols where used (/) was used to separate protocols and outcomes respectively.
0.75 U of Taq DNA polymerase, 20 mM concentrations of each deoxynucleoside triphosphate, 10 mM Tris-HCl, 50 mM KCl, 1.6 mM MgCl$_2$ and 5 µl of DNA template with specific primer sets for Babesia spp. and B. gibsoni as shown in Table 2 [1, 14]. Using the standard PCR conditions, the amplification was done with an annealing temperature at 55°C and 35 cycles. Distilled water and DNA of each agent were included as negative and positive controls in each PCR reaction. DNA sequencing was done using a BigDye Terminator v1.1 Cycle Sequencing Kit and analyzed on an Applied Biosystems 3500 geneEc sequencer (Applied Biosystems, Foster City, CA, USA). The sequences of the PCR products were compared with known sequences by BLAST analysis against the NCBI database.

Quantitative PCR was performed on the PCR-positive samples using a LightCycler Taqman Master kit (Roche Molecular Systems, Inc., Pleasanton, CA, USA). A ready-to-use hot start reaction mix for PCR using the LightCycler Carousel-Based System Instruments with hydrolysis probes, containing LightCycler FastStart Enzyme, LightCycler FastStart TaqMan Reaction Mix and Water, PCR Grade. The cDNA was amplified in a 2.0 LightCycler (Roche Molecular Systems, Inc.). The primers and probes sequences used for quantitative PCR of Babesia spp. are shown in Table 3. The limit of detection is 10 plasmid copies per reaction. Positive controls of various dilutions were run along with the test samples to calculate the limit of detection. The cut off for detection on PCR was 1.2 copies per microliter. Animals with higher copies were deemed as PCR positive and animals below that limit were considered as PCR negative and therefore free or clear of babesiosis.

### Treatment protocols

All dogs were initially treated with the standard therapy, atovaquone and azithromycin. Atovaquone (Mepron 750 mg/5 ml, GlaxoSmithKline, Triangle Park, NC, USA) was prescribed at 13.3 mg/kg PO thrice daily and azithromycin (Zithromax 250 mg, Pfizer, Tadworth, UK) at 10 mg/kg PO once daily in all cases for 10–17 days. Ten out of the 24 dogs were further treated with metronidazole (Metodan 250 mg, Winsor, Hong Kong) 15 mg/kg PO twice daily, clindamycin phosphate (Dalacin 150 mg, Pfizer) 25 mg/kg PO twice daily and doxycycline hydrochloride (Vibramycin 50 & 100 mg, Pfizer) 5 mg/kg PO once daily for 30–90 days following dosages of previous reports [31]. The MCD protocol was used in a number of patients only after PCR results for B. gibsoni had shown copies above the detection limits following treatment with the AA protocol. The variability in the duration of treatment was determined by clinician discretion and client compliance.

### Statistical analysis

Descriptive analyses were performed using Microsoft Excel for data collection, frequency and graphing. The online open-source OpenEpi version 3 statistical program was used to compare observed versus expected counts in some of the data. Comparison between observed counts and expected frequencies per null hypothesis were obtained and documented in a two by two table. A Fisher exact test for comparison with 95% confidence intervals was used and P-values <0.05 were considered significant.

### RESULTS

Amongst the population of dogs in this study shown in Table 1, there were more males (14/24; 58%) than females (10/24; 42%). The number of neutered males (10/24; 42%) and females (7/24; 29%) were higher than the number of entire males (4/24; 17%) and females (3/24; 13%). The age of the animals ranged from 1 to 14 years. Most dogs (18/24; 75%) were less than 9 years old with a median age of 7 years. Breed types included poodle (2), mongrel (2), miniature schnauzer (3), golden retriever (3), shih tzu (2), miniature collie (1), Pomeranian (2), Maltese (2), cocker spaniel (1), Welsh corgi (1), Labrador retriever (1), Akita (1), Pekingese (1), husky cross (1) and Samoyed (1).

All 24 selected dogs were symptomatic and presented with a variety of clinical signs including lethargy, splenomegaly, pallor of mucous membranes, icterus, heart murmur and tachycardia. All had hematologic abnormalities (24/24; 100%) including anemia and thrombocytopenia. Anemia was severe (HCT <20%) in 12 out of 24 animals (50%), moderate (HCT 20–30%) in 8 out of 24 dogs, while the rest were mild (HCT 30–40%). There was no significant correlation between the severity of anemia and the duration of treatment.

### Table 2. Sequences of primers for PCR of Babesia spp. and B. gibsoni used in the study

| Pathogen     | Gene    | Sequence                  | Amplicon size (bp) |
|--------------|---------|---------------------------|--------------------|
| Babesia spp. | 18S rRNA| Forward 5’ CTCTTGTAATTGGGAATGATGG | 560                |
|              |         | Reverse 5’ CCAAAGACTTTGATTTCTCTC |                    |
| Babesia gibsoni | 18S rRNA | Forward 5’ CTCGGCTACTTGCCTTGTC | 650                |
|              |         | Reverse 5’ GCCGAAACTGAAATAACGGC |                    |

### Table 3. Primers and probes for quantitative PCR of Babesia spp. positive samples

| Pathogen     | Gene    | Sequence                  | Product length (bp) |
|--------------|---------|---------------------------|---------------------|
| Babesia spp. | 18S rRNA| Forward 5’GACTAGDGATTGGAGGTCGTCTRT | 79                  |
|              |         | Reverse 5’TCCCCCAGAACCCAAAAG |                    |
|              |         | Probe 5’[FAM] CCTTCAGSACCTTGGAGA[MGB] |                    |
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animals (33%) and mild to normal (HCT >30%) in 3 out of 24 dogs (12%). Blood transfusions were given to 9 out of 24 (38%) dogs as a consequence of the severe anemia. Thrombocytopenia was severe (PLT <50 × 10⁹/l) in 17 out of 24 dogs (71%) and mild to moderate (PLT >50 × 10⁹/l) in 6 out of 24 dogs (25%). Only one dog (4%) presented without thrombocytopenia but had a thrombocytosis of 824 × 10⁹/l.

Microscopic examination of whole blood for identification of intra-erythrocytic parasites was performed in 16 out of 24 dogs (67%) before the PCR results were received and hemoparasites were observed in 7 out of the 16 dogs (44%) (Fig. 1).

The first line of treatment (AA) was initially used in all 24 (100%) PCR positive dogs, where 6 (25%) cleared *B. gibsoni* and 18 (75%) failed to clear the infection on PCR (Fig. 2). Eight (45%) out of the 18 dogs where AA failed to clear *B. gibsoni* had additional AA courses but never had a conclusive PCR post-treatment. Two (11%) dogs were treated with MCD but were lost to follow up and did not have a PCR test after treatment either, and 8 (45%) dogs were treated with MCD and had an outcome (Fig. 2).

Four dogs (4/24, 17%) cleared *B. gibsoni* after 1 course of AA and 20 dogs (20/24, 83%) failed to clear *B. gibsoni* at day 31 to 38. Two additional dogs cleared *B. gibsoni* after the second AA treatment at day 62 to 76. The dogs that failed to clear *B. gibsoni* after the second treatment (n=5) did not clear *B. gibsoni* regardless of the number of courses of AA they had. One dog that was treated with 6 courses of the AA protocol had 7 PCR tests that were all positive.

The MCD protocol was used in 42% (10/24) of the dogs included in the study or in 56% (10/18) of the dogs that had not cleared *B. gibsoni* using the AA protocol. Three dogs (3/10, 30%) were treated with the MCD protocol after one course of AA. Four dogs (4/10, 40%) had been treated with 2 courses of AA before they were changed to the MCD protocol. Two dogs (2/10, 20%) changed to MCD after 3 courses of the AA protocol and 1 dog (1/10, 10%) was treated with MCD following 4 courses of the AA protocol. At the end of the treatment 1 dog treated with MCD had not cleared *B. gibsoni* on PCR and 2 dogs were lost to follow-up (Fig. 2).

Fig. 1. Intraerythrocytic *Babesia gibsoni* (asterisk) in canine red blood cells; light microscopy Wright-Giemsa stain, 100×. Images from Daniela Muguiro, VDL, City University of Hong Kong.

Fig. 2. Diagram of results by polymerase chain reaction (PCR) after treatment with atovaquone and azithromycin (AA) and metronidazole, clindamycin and doxycycline (MCD). (*) Percentages adding >100% due to rounding.
To further assess the true elimination of *B. gibsoni*, the rate of infection recurrence was retrospectively evaluated in the limited number of dogs (n=5) that had successive follow-up visits and additional data. Recurrences in dogs that had cleared infection were observed in 1 dog treated with MCD and in 1 dog treated with AA, however, it was noted that molecular resolution occurred again in the MCD treated dog and in the AA treated dog following the use of the same respective protocols. The former showed a sustained clearance on PCR in successive tests carried out 4, 11 and 20 months post-treatment. Additional information from 2 dogs that had not followed up after AA treatment included positive PCR tests 2 and 3 years later respectively, suggesting a chronic infection and resistance to atovaquone or a potential new infection remaining therefore inconclusive. One MCD success dog had several PCR follow up tests over 3 years confirming a sustained clearance on PCR. No further data was available for these or the rest of the dogs included in the study.

Hematologic abnormalities improved gradually taking an average of 25 days to show normalizing values. All negative PCR dogs treated either with AA (n=6) or AA followed by MCD (n=7) had normal hematological findings. Sixty percent of the dogs that failed to clear *B. gibsoni* or did not have a follow-up PCR still showed clinical improvement after treatment, with HCT and PLT counts normalized or only mildly decreased (HCT >30%; PLT >100 × 10^9/l), on the other hand, the remaining 40% of that group of dogs did not show a sustained improvement and HCT and PLT counts were significantly lower. No dogs were known to have died from babesiosis in this retrospective evaluation.

Overall a high success rate was observed after the additional treatment with MCD with 87% (7/8) clearance rate on PCR. There was a statistically significant difference amongst those considered treatment successes (PCR negative) between the AA and MCD protocols with significantly more dogs resulting PCR negative after the addition of the MCD protocol compared to the AA protocol alone (P-value 0.0064).

The observed PCR positives and negatives treated with AA and MCD respectively were significantly higher than the expected frequencies per null hypothesis (Table 4). There was therefore a significant difference between the observed results and the expected frequencies, with inadequate goodness of fit.

### DISCUSSION

This study suggested that the MCD protocol was an effective alternative for the treatment of *B. gibsoni* in dogs with persistently detectable parasitemia in Hong Kong after limited response to the AA protocol according to PCR. Although clinical resolution after treatment was observed in most cases, the criterion used for determining a successful result was based solely on molecular resolution. Whilst it could be argued that persistent PCR positive parasitemia may be of no adverse clinical consequence, these dogs may serve as a reservoir for infection. In addition, a recrudescence of persistent parasitemia might occur particularly in the face of immunosuppression such as chemotherapy. Lastly, given the increased movement of people and their pets, it is also possible that asymptomatic dogs could serve as a Trojan horse of infection to areas where *B. gibsoni* is not endemic.

Asymptomatic dogs are not routinely tested for babesiosis even though the prevalence in Hong Kong has been reported to be as high as 44% in stray dogs and 31% in pet dogs [32]. False negatives could have occurred in cases where hematological abnormalities were present but PCR did not detect DNA copies above the detection limits that could be explained by intermittent presence of parasites in venous blood during chronic infection or by low levels of infection. Reports of intermittent positive PCR results during chronic infections in dogs that had been experimentally infected with *B. gibsoni*, further support the possibility of false negatives post-treatment [16]. False positives or chronic infections were suspected in this study where cases showed clinical improvement but never eliminated *B. gibsoni* on PCR. Most of these cases were not followed up and they were not retested.

Treatments classically used for *B. gibsoni* infections such as diminazene aceturate, pentamidine isethionate and phenamidine isethionate are not commonly used in Hong Kong due to concerns regarding their potential side effects, their limited availability and their reported lack of success. Other treatments like imidocarb dipropionate are readily available, but despite its convenient 2-injection dosage administration its efficacy for *B. gibsoni* is considered to be low. The use of imidocarb in Hong Kong is generally limited for the treatment of *B. canis* infections. The lack of success of previous treatments has encouraged research and the use of novel protocols such as atovaquone and azithromycin, metronidazole, enrofloxacin and doxycyclin, and metronidazole, clindamycin and doxycycline, all showing good response rates for the treatment of babesiosis [2, 20, 31].

The standard protocol (AA) was initially used in all 24 dogs of the present study, however, the success rate was significantly lower than in previous studies [2]. Possible reasons for this discrepancy include the development of drug resistance associated to the mutation in cytochrome b genes, false positives, a difference in parasite load due to a higher prevalence of *B. gibsoni* in Hong Kong compared to the US, or the failure of drug dosing compliance by clients. Atovaquone resistance due to an M121I variant

| PCR Positive | PCR negative | Marginal treated totals |
|--------------|--------------|-------------------------|
| Treated with AA | 18 (14.25) | 6 (9.75) | 24 |
| Treated with MCD | 1 (4.75) | 7 (3.25) | 8 |
| Marginal PCR totals | 19 | 13 | 32 (Grand total) |

**Table 4.** Single table analysis for statistical associations for PCR and treatment protocols
population has been reported as a potential cause of treatment failure \[5, 13, 17, 28\]. Quantification of an M121I variant population by allele-specific real-time PCR was not performed in the present study, therefore the authors were unable to determine how many MCD success cases might have been resistant M121I strains. The possibility that the MCD protocol was more effective against atovaquone resistant (M121I) \( B. \) \( \text{gibsoni} \) should be investigated further to assess and compare its efficacy against wild-type \( B. \) \( \text{gibsoni} \).

A protocol including clindamycin, diminazene and imidocarb has been reported as a potential alternative for AA resistant strains \[19\], however, this protocol is not commonly used in Hong Kong due to limited drug availability and physician or client preference for the shorter AA protocol. The MCD protocol was used instead as the alternative or additional protocol in all cases in animals that had been treated with AA but had failed to clear babesiosis. The lack of a standardized length of MCD treatment and its evaluation as a potential primary therapy was not possible due to its retrospective nature. The length of treatment with MCD has not been established in previous studies \[31\], and in the present case, it was decided by the clinician, on an individual basis, based on client compliance, clinical signs and PCR results. The encouraging results observed in this case series with the MCD protocol are supported by a previous study where clearance of \( B. \) \( \text{gibsoni} \) by PCR occurred in 75% of the dogs with the use of the MCD protocol \[31\]. The dog population in that study was lower (4 instead of 10), had been pre-treated with another protocol (diminazene instead of AA) and had been experimentally infected as opposed to the naturally occurring infection of the dogs in this report.

The typical recommendation for PCR testing post-treatment is two consecutive tests at days 60 and 90 \[2\]. This is due to the possibility for false-positive results on PCR from residual, though possibly non-viable, DNA still present that could be amplified on PCR. It is worth noting that approximately 90% of the dogs in the study from which this recommendation is derived, were already negative at day 60 and no results were available for day 30 to assess earlier conversion or potential early false negatives or positives \[2\]. Recent studies reported no significant differences in results when comparing PCR testing post-treatment at days 30 and 60, with 95% of dogs testing negative at day 30, reducing the financial burden involved in the cost of follow up testing \[17\]. In the present study dogs that cleared \( B. \) \( \text{gibsoni} \) after AA therapy had PCR testing post-treatment at days 31 to 38 (n=6) and days 62 to 76 (n=2), whereas dogs that cleared \( B. \) \( \text{gibsoni} \) after MCD had at least 2 PCR tests post-treatment, one on day 31 to 38 (n=7) and an another on day 61 to 121 (n=7).

A more accurate way of assessing the true elimination of babesiosis could have included assessing the rate of infection recurrence, comparing both AA and MCD success groups. Additional PCR data was collected before the final submission of this article but the interpretation of that data was limited due to the low number of dogs that had followed up. This reinforces the need for prospective studies that include recurrence rates to assess more objective clearance of babesiosis.

The economic impact of the treatment protocols is an important consideration. The cost of the standard protocol (AA) in Hong Kong could be a financial limitation for the pet owners, costing approximately 400 USD for a 10-day course for a 10 kg dog, especially when several courses of this protocol had to be repeated for refractory or resistant cases. Despite the longer duration of treatment for the MCD protocol, it is half the cost of the standard protocol in Hong Kong. The longer length of the triple antibiotic treatment could be, however, one important reason amongst pet owners and practitioners for not choosing this protocol and therefore other shorter therapies such as AA as a first choice is preferred, despite the higher cost. The number of cases that were treated with the triple antibiotic therapy, MCD, was relatively small but considerably relevant taking into account the paucity of data in this field, with similar or smaller number of dogs observed in previous studies. Data regarding alternative treatments remain scarce. Further evaluation of this protocol is needed with a more standardized approach to diagnostic testing along with therapeutic protocols, ideally in a prospective nature.

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An alternative combination therapy with metronidazole, clindamycin and doxycycline for Babesia gibsoni (Asian genotype) in dogs in Hong Kong

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