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EFFECT OF DOXYCYCLINE, AZITHROMYCIN AND IMIDOCARB ON HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS AND HEALTH STATUS OF *Ehrlichia canis* INFECTED DOGS

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Abstract. The objective of this study was to evaluate the treatment effect of doxycycline, azithromycin and imidocarb on dogs with natural *E. canis* infection. 98 dogs that tested positive for *E. canis* were randomly divided into three regimens: regimen 1, 38 dogs, treated with doxycycline; regimen 2, 33 dogs, azithromycin, and regimen 3, 33 dogs, imidocarb. Hematological parameters: white blood cells, red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), platelet (PLT); blood biochemical parameters: amylase, lipase, aspartate transaminase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), urea and creatinine, blood smear and clinical health status were analyzed within and among regimen(s) on day 0 and day 7, 15, 21, 28, and 60 post-treatment. The results show that RBC, HGB, HCT, and PLT increase gradually through treatment. The biochemical parameters decrease gradually from day 0 to day 28. In regimen 2 and on day 60, amylase, urea, AST, ALT, and GGT rise again. By day 60 post-treatment, 6/38 (15.8%) dogs in regimen 1, 12/27 (44.4%) dogs in regimen 2, and 8/33 (24.2%) dogs in regimen 3 show signs of disease recurrence. The blood smear exam on day 60 shows that 7/38 (18.4%) dogs in regimen 1, 14/27 (51.8%) dogs in regimen 2, and 11/33 (33.3%) dogs in regimen 3 still have *E. canis* morulae in monocytes and/or neutrophils. In conclusion, doxycycline shows the best effect on treating dogs with natural *E. canis* infection compared with azithromycin and imidocarb at mentioned dosages and therapeutic duration.

Keywords: *Ehrlichia canis*, doxycycline, imidocarb, azithromycin

1 Introduction

Ehrlichia canis (*E. canis*) is one of the bacteria causing Ehrlichiosis in dogs (Canine Ehrlichiosis, a tick-borne disease). The disease seriously affects the health of infected dogs [1]. *E. canis* infections in humans have also been reported [2–5], raising concerns about the detrimental effects of such disease on human health, especially for dog owners or people having contact with infected dogs and/or dog ticks. *Rhipicephalus sanguineus* (*R. sanguineus*), also known as brown ticks, is a vector-borne that transmits *E. canis* and other rickettsia pathogens to dogs [1] and sometimes humans as well [6]. This tick can be found on dogs living in urban and rural areas. They are highly adaptable

to live in human houses and are active throughout the year, not only in tropical and subtropical regions but also in some temperate areas. Depending on factors such as climate and host availability, *R. sanguineus* can reproduce up to four generations per year. Recent studies have demonstrated that ticks exposed to high temperatures attach and feed on humans and rabbits more rapidly. This observation suggests that the risk of transmission of zoonotic agents (including *E. canis*) to humans could increase in warm and long summer regions [1]. Currently, there is no effective vaccine to prevent diseases caused by *E. canis*. Preventive measures are primarily to protect the host from tick bites by using periodic tick killers [7], while the use of preventive antibiotics is not recommended.

According to Vu et al. [8], 37.9% of dogs in Thua Thien Hue brought to Okada Pet Hospital by residents have tested positive for *E. canis*, with various symptoms, including fever, nasal hemorrhage, weakness, pale mucous membranes, dermatitis, anorexia, vomiting, ascites and/or depression. In bitches, there may be metritis and/or stillbirth [8]. Vu et al. [8] also reported that *E. canis* morulae are present in monocytes and/or neutrophils but not in other types of white blood cells.

There is currently no effective vaccine to prevent *E. canis* disease. The main measures for disease control include prevention (mainly the protection of the host from the attack of ticks by the use of periodic tick killers [7]) and the treatment of the disease with various antibiotics to eliminate the pathogens in the body. Tetracycline has been an antibiotic of choice for the treatment of many bacterial diseases, including those related to rickettsia pathogens. Doxycycline (an antibiotic of the tetracycline group) is a treatment option for Ehrlichiosis in dogs, and it is often used to treat *E. canis* orally. However, the effectiveness of doxycycline in eliminating *E. canis* infection remains controversial. McClure et al. [9] reported that *E. canis* remained in peripheral dog blood and in *R. sanguineus* ticks that sucked blood from infected dogs after a 14-day doxycycline therapy course. Conversely, when intravenously infecting dogs with *E. canis*, a 28-day doxycycline therapy course can completely remove *E. canis* from infected dogs [9]. Fourie et al. [10] reported that most dogs infected with *E. canis* were cured by giving doxycycline tablets. However, other reports have shown the possibility of persistent infection after a doxycycline treatment regimen of 7 to 85 days in dogs with natural and experimental infections [1, 11]. Despite the controversy over the effectiveness of treatment, a 28-day course of oral doxycycline is the currently recommended drug issued by a consensus statement by the American College of Veterinary Medicine to treat *E. canis* infection in dogs [9]. Using doxycycline for a too long time (four weeks continuously) causes fatigue for infected animals, doctors, as well as owners. Therefore, we tested whether azithromycin and imidocarb with shorter treatment periods were effective in *E. canis* elimination.

Furthermore, when pregnant bitches suffer from the disease, the use of doxycycline is thought to bring numerous side effects, especially to the fetus [12]. The use of azithromycin has therefore been taken into account to replace and reduce the side effects of doxycycline on pregnant animals.

Azithromycin has been tested for treating most diseases caused by rickettsia [13–15]. The antibiotic has the potential to treat intracellular parasitic pathogens because of its ability to concentrate inside neutrophils and monocytes [15]. However, the therapeutic effect of azithromycin on the elimination of *E. canis* in dogs has not been clarified.

Imidocarb, a diamidine of the series of carbanalide compounds with antibiotic activity, is also used independently or in combination with doxycycline to treat *E. canis* infection in dogs. However, its therapeutic effect is also inconsistent. Matthewman et al. [16] used imidocarb dipropionate for dogs that are purposely infected with *E. canis* and concluded that the drug is effective in treating Ehrlichiosis in dogs. In contrast, Eddlestone et al. [17] indicated that imidocarb dipropionate was incapable of completely removing *E. canis* from infected dogs.

Conflicting reports of the therapeutic effect of doxycycline, azithromycin, and imidocarb on Ehrlichiosis diseases suggest that some factors (such as dosage, duration of treatment, or virulence of the strain of pathogenic bacteria, health condition of infected dogs, and /or living condition) can affect the outcome of these treatments. The existence of *E. canis* post-treatment in dogs remains a challenging problem for veterinarians in field practice. With an aim to save infected dogs, we evaluated the effectiveness of doxycycline, azithromycin, and imidocarb on the animals infected with *E. canis* in Thua Thien Hue province.

2 Material and methods

2.1 Experimental design

Ninety-eight dogs tested positive for *E. canis* at OKADA PET HOSPITAL in Hue City through clinical examination, hematology tests, Elisa tests, and blood smears were randomly divided into three regimens (38, 27, and 33 dogs in regimens 1, 2, and 3, respectively). Dogs tested positive for *E. canis* are described by Vu et al. [8]. The drugs used to treat *E. canis* infection in this study include regimen 1: doxycycline capsule 10 mg/kg, once a day, continuously for 28 days [18]. Regimen 2: azithromycin capsule 10 mg/kg, once a day, continuously for ten days [14, 19]. Regimen 3: imidocarb injection solution, 6.6 mg/kg, subcutaneous injection, two injections separated by two weeks.

2.2 Measurements

About 4 mL of blood samples were collected from the cephalic veins of sick dogs, of which 2 mL of blood was stored in a sterile anticoagulant blood sample vial containing ethylene diamine tetra acetic acid (EDTA, used for hematology test and blood smear), the remaining 2 mL was transferred to a test tube containing heparin (used for biochemical analysis). Blood samples were collected on days 0 (pre-treatment), 7, 15, 21, 28, and 60 (post-treatment) from each infected dog and analyzed within 10 minutes after collection.

Hematological parameters, including red blood cell count (RBC, 10^6/uL), hemoglobin (Hb, g/dL), hematocrit (HCT, %), platelet (PLT, 10^3/uL), total white blood cell count (WBC, 10^3/uL), lymphocytes (LYM, 10^3/uL), granulocytes (Gran, 10^3/uL), and other white blood cells (Mid, 10^3/uL), were analyzed by using an automatic hematology analyzer (Dymind Hematology Analyzer-DH36).

Some blood biochemical parameters (amylase, lipase, AST, ALT, GGT, urea, creatinine) were analyzed by using an automatic dry biochemical analyzer (Vetube 30, Mindray). The blood smear with Diff-Quick staining was also performed for microscopic examination to detect *E. canis* morulae on days 0 and 60 of the course of treatment. The blood smear preparation was carried out according to Vu et al. [8]. All dogs involved in this study received Fiprofort as a tick killer and were administered according to the manufacturer's instructions [20].

2.3 Data analysis

The data collected were stored in Microsoft Excel worksheets, and the statistical analysis was conducted with the Minitab and SPSS software. A p-value of 0.05 or lower is considered statistically significant.

3 Results

3.1 Hematological parameters

Changes in total white blood cell count (WBC), lymphocyte (Lym), granulocyte (Gran), other types of white blood cells (Mid), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT) and platelets (PLT) pre- and post-treatment

Hematological parameters are widely used in clinical practice to assess health and disease conditions. The reference ranges are also important tools as biomarkers to assess disease progression or response to therapy. Prior to treatment (day 0), the WBC (Fig. 1A) in regimens 1 and 3 was within the reference interval but higher in regimen 2. Lym (Fig. 1B) in all three regimens was higher than the reference threshold; Gran (Fig. 1C) in all three regimens was in the

reference range. Mid (Fig. 1D) in regimens 1 and 3 was in the normal threshold but higher in regimen 2. There was no statistically significant difference in white blood cell types between regimens ($p > 0.05$). After treatment, WBC, Lym, and Gran in regimens 1 and regimen 3 tended to increase but decreased in regimen 2 on the 60th day after treatment. However, statistically significant changes ($p < 0.05$) were observed in Gran and Mid of regimen 3 between day 0 and day 60 within regimens.

Prior to treatment, RBC (Fig. 1 E) in regimens 1 and 2 was lower than the reference threshold but higher in regimen 3 (4–5.2 × 10^6/uL). In contrast, HGB (Fig. 1F) and HCT (Fig. 1G) were all lower than the reference threshold (12–15 g/dL, 35–40%). PLT (Fig. 1H) in all three regimens was very low compared with the reference threshold. After treatment, RBC, HGB, HCT, and PLT in all three regimens tended to increase throughout treatment and were statistically higher on day 60 than on day 0 within regimens ($p < 0.05$).

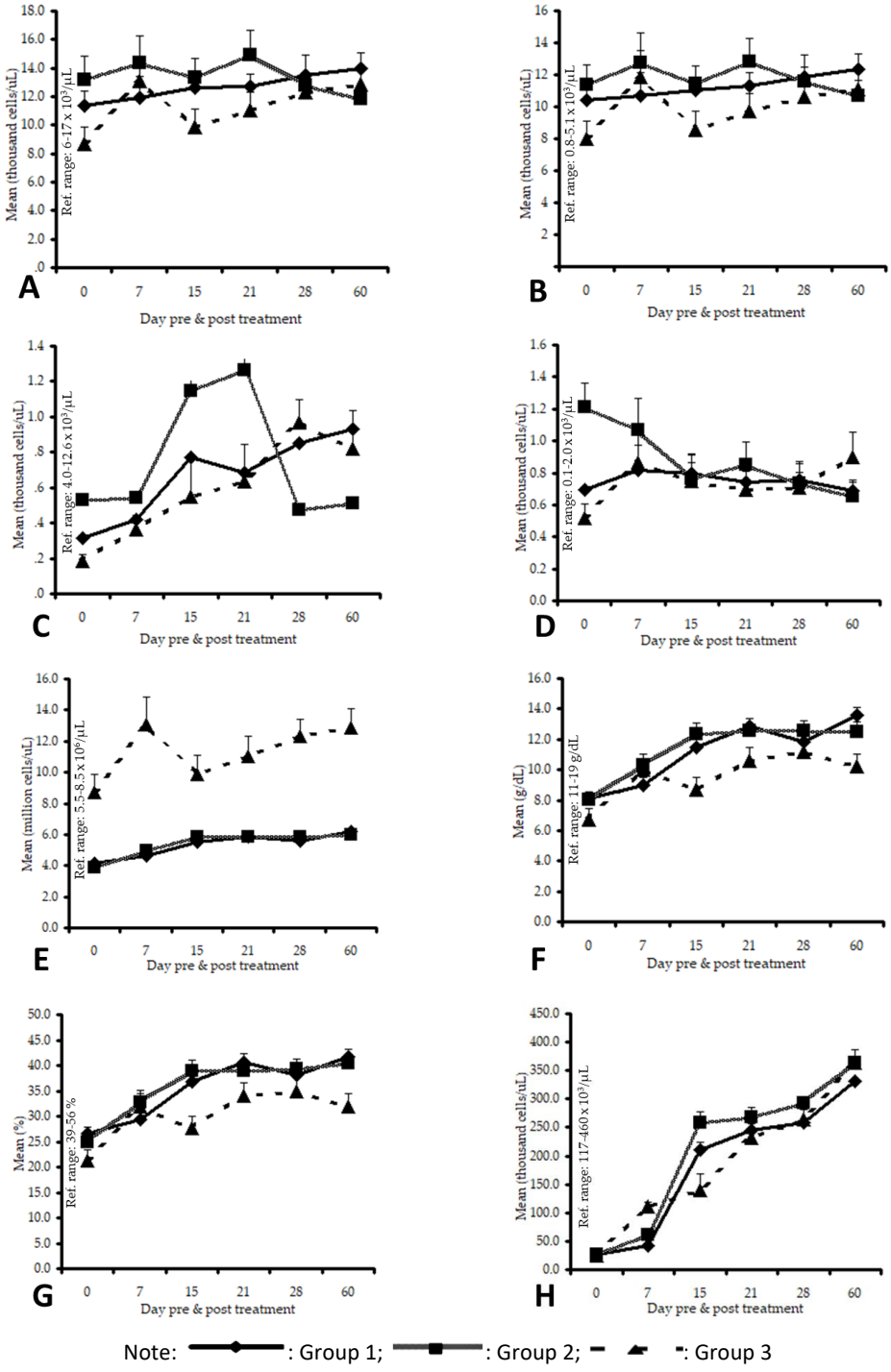

Fig. 1. Changes of WBC (A), Lymphocytes (B), Granulocyte (C), Mid cells (D), RBC (E), HGB (F), HCT (G) and PLT (H) pre-and post-treatment

3.2 Biochemical parameters

Changes of amylase, lipase, AST, ALT, GGT, urea and creatinine pre- and post-treatment

Before treatment, amylase (Fig. 2A), lipase (Fig. 2B), AST (Fig. 2C), ALT (Fig. 2D), GGT (Fig. 2E), urea (Fig. 2F), and creatinine (Fig. 2G) were higher than reference ranges. After treatment, in all three regimens, these biochemical parameters tended to decrease gradually from day 0 (pre-treatment) to 28 (post-treatment). In regimens 1 and 3, the biochemical parameters were lower on day 60 than on day 0 ($p < 0.05$). However, in regimen 2, amylase, urea, AST, ALT, and GGT were higher on day 60 than on day 0, but the changes were statistically insignificant.

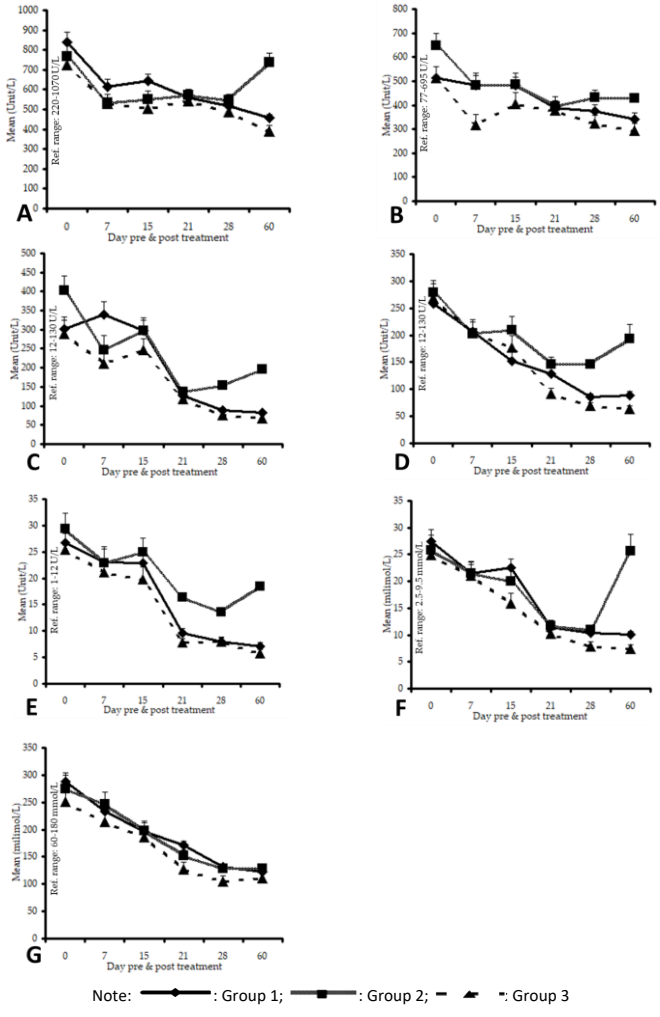

Fig. 2. Changes of amylase (A), lipase (B), AST (C), ALT (D), GGT (E), urea (F) and creatinine (G) pre-and post-treatment

3.3 Clinical health monitoring and blood smear

From the 7th to the 28th after treatment, all infected dogs in all three regimens showed good progress in clinical health. However, by day 60 post-treatment, 6/38 (15.8%) dogs in regimen 1, 12/27 (44.4%) dogs in regimen 2, and 8/33 (24.2%) dogs in regimen 3 had shown signs of *E. canis* infection, including anorexia, coughing, vomiting, and dermatitis. The blood smear results on day 60 showed that 7/38 (18.4%) dogs in regimen 1, 14/27 (51.8%) dogs in regimen 2, and 11/33 (33.3%) dogs in regimen 3 still had the sign of *E. canis* morulae in monocytes and/or neutrophils.

Table 1. Clinical health monitoring of dogs pre- and post-treatment

Regimen	No. of infected dogs at day 0	Day 7–28				Day 60			
		No. of dogs cured	%	No. of dogs remained infected	%	No. of dogs cured	%	No. of dogs remained infected	%
1	38	38	100	0	0.0	32	84.2	6	15.8
2	27	27	100	0	0.0	15	55.6	12	44.4
3	33	33	100	0	0.0	25	75.8	8	24.2
Sum	98	98	100	0	0.0	72	73.5	26	26.5

Table 2. Sign of *E. canis* morulae in the canine peripheral blood post-treatment

	Day 0			Day 60	
Regimen	No. of dogs positive	No. of dogs negative	%	No. of dogs positive	%
1	38	31	81.58	7	18.42
2	27	13	48.15	14	51.85
3	33	22	66.67	11	33.33
Total	98	66	67.35	32	32.65

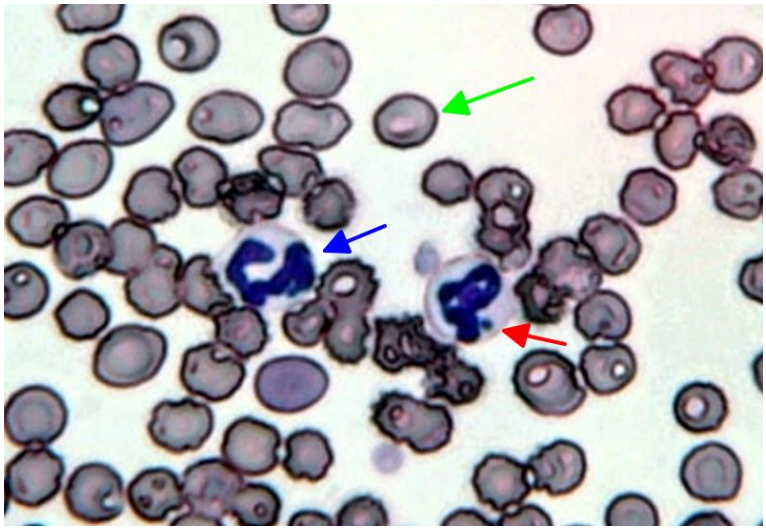

Fig. 3. Representative blood film under microscope with 100× oil lens showing neutrophils with (red arrow) and without (blue arrow) signs of *E. canis* morulae. The green arrow indicates a red blood cell.

Photo: Vu Van Hai

4 Discussion

Tick-borne diseases caused by the family *Anaplasmataceae* are increasingly critical, and *E. canis* is the most common. Some pathogens of this regimen have infected dogs naturally and have increased in recent decades [5, 21]. The disease can appear acute, sub-acute or chronic and is mainly identified through experimental injecting *E. canis*-infected blood into the host [22–26]. The acute stage of the disease begins about 10 days after the infusion of the pathogen, with changes including leukopenia, thrombocytopenia, fever, depression, and anorexia. The clinical signs diminish gradually about 20 to 30 days after injection, usually followed by a sub-acute period lasting several months to years. Although clinical signs in the sub-acute phase may disappear, hematological changes, such as leukopenia, anemia, and thrombocytopenia, still remain, of which mild thrombocytopenia is the most common under experimental conditions [25]. The chronic stage is the third one, which can range from mild to severe, with clinical signs and recurrent hematology, including thrombocytopenia, hemorrhage, mononucleosis, lymphocytic leukocytosis, and weight loss [25, 27]. However, in this study, the positive dogs are all naturally infected, and we could not determine when the dogs have been infected and at what stage, but our findings of pre-treatment thrombocytopenia (day 0) in infected dogs in all three treatment regimens are consistent with results from previous studies [22–27]. The clinical signs described above are also similar to those reported in our previous study [8]. Furthermore, we found that

WBC, Lym, Gran, and Mid pre-treatment (day 0) did not decrease compared with the reference range. This finding is in contrast to that of Waner et al. [25]. It is possible that the sick dogs in these studies are in different stages of disease. Therefore, hematology tests reveal that the signs of decreases regarding RBC, HCT, and PLT are probably more specific and valuable than signs of leukopenia.

By contrast, before pre-treatment (day 0), WBC and RBC in regimen 3 did not decrease but were higher than the reference interval. In fact, we have seen a lot of cases in which dogs are seriously ill and anemic, but the CBC results show that the values of blood parameters increased rather than decreased. The reason might be that the sick animals were vomiting, suffering from anorexia or diarrhea that caused the body to become severely dehydrated, and thickens the blood, finally leading to the increase in the values of hematology parameters in the CBC report. Therefore, when performing a diagnosis, it is necessary to be cautious. Normal or increased hematology parameters' values do not mean that the blood is in good condition. The doctor needs to connect different clinical data to thoroughly and accurately conclude the problem.

The hematologic and biochemical test results on days 7, 15, 21, 28, and 60 post-treatment show that WBC, Lym, and Gran in regimen 1 and regimen 3 tended to increase, while they tended to decrease on day 60 in regimen 2. Statistically significant changes ($p < 0.05$) were observed in Gran and Mid of regimen 3 between days 0 and 60. RBC, HGB, HCT, and PLT in all three regimens tended to increase throughout treatment, with statistical significance between day 0 and day 60 in all three regimens ($p < 0.05$). On day 60 in regimens 1 and 3, the biochemical parameters' values continued declining compared with those of day 0 ($p < 0.05$). However, on day 60, amylase, urea, AST, ALT, and GGT tended to rise again compared with day 28 in regimen 2, but there was no statistical significance ($p > 0.05$). This result shows that all three drugs seem to increase the hematology parameters' values and reduce biochemical parameters to the reference threshold. This means that the drugs destroy or inhibit the causative agent (*E. canis*), thereby improving or restoring liver, kidney and pancreas function. However, in regimen 2 (using azithromycin) on day 60 post-treatment, amylase, urea, AST, ALT, and GGT tended to increase again, suggesting the signs of disease recurrence. This is in line with our clinical health monitoring results where, on days 7–28 post-treatment, all infected dogs in three tested regimens showed good progresses in clinical health, while by day 60 post-treatment, more dogs in regimen 2 had exhibited the recurrence of the disease than in regimens 1 and 3. Specifically, 6 out of 38 (15.8%) dogs in regimen 1, 12 out of 27 (44.4%) dogs in regimen 2, and 8 out of 33 (24.2%) dogs in regimen 3 showed the signs of recurrence of the disease, including poor appetite, coughing, and ulcers on the skin. The blood smear results on day 60 showed that 7 out of 38 (18.4%) dogs in regimen 1, 14 out of 27 (51.8%) dogs in regimen 2, and 11 out of 33 (33.3%) dogs in regimen 3 still had *E. canis* morulae in monocytes and/or neutrophils. Our findings are consistent with those of

previous reports [9, 11, 17] indicating that removing *E. canis* from the host body is not easy. Failure to completely remove Ehrlichia pathogens from the hosts upon treatment can result in asymptomatic carriers that become the source of infection for intermediate hosts (vector ticks) and vertebrate hosts [24] (dogs, cats or humans). Further alternative medications/treatment regimens need to be continuously evaluated.

5 Conclusion

Based on the results of hematology and biochemistry changes and clinical health monitoring, as well as the presence of *E. canis* on the blood film, we conclude that doxycycline (dose of 10 mg/kg, given for 28 consecutive days) shows the best therapeutic effect on the *E. canis* treatment in dogs. Azithromycin has the least treatment effect. Although there were positive improvements in clinical health after seven days of treatment, several sick dogs were not entirely removed from *E. canis*, posing the risk of disease recurrence even in humans. Therefore, it is necessary to perform other therapies to improve the treatment effectiveness and successfully cure dogs that unfortunately get *E. canis* infection.

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