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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Seroprevalence and factors affecting canine monocytic ehrlichiosis and canine brucellosis in Tanzania

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Abstract

A cross-sectional study was undertaken to determine the seroprevalence of *Ehrlichia canis* and *Brucella canis* in dogs in Morogoro Tanzania. The study was conducted between June and September 2010. A total of 100 randomly selected dogs were tested for the presence of *Ehrlichia canis* and *Brucella canis* antibodies using the Immunocomb® dot-ELISA tests (Biogal, Israel). Epidemiological factors such as age, sex, breed, health status, body condition and tick infestation were studied. *E. canis* antibodies were detected in 25% (n=100) of the dogs. *B. canis* antibodies were not detected in any of the study dogs. The difference in seroprevalence between old and adult dogs was statistically significant ($P<0.05$). There was also a significant difference in seroprevalence between dogs in good and those in fair body conditions ($P<0.05$). Seropositivity to *E. canis* was not associated with the other epidemiological factors. This study provides the first serological evidence of *E. canis* infection but found no evidence of antibodies to *B. canis* in dogs in Morogoro. Canine ehrlichiosis was found to be a prevalent disease in Morogoro and calls for regular testing and treatment of clinical cases and tick control measures to protect dogs from *E. canis* infection. The study also points out the need for further investigation on the presence of canine brucellosis.

Key words: Canine Ehrlichiosis, Canine Brucellosis, Seroprevalence, Morogoro

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Introduction

Ehrlichia canis which causes Canine Monocytic Ehrlichiosis (CME) is endemic in tropical and subtropical areas although it has also been found in temperate zones (Sáinz, et al., 1996). In Africa, seroprevalence of CME has been reported in Egypt, Ivory Coast, Gabon (Davoust et al., 2006), and Cameroon (Ndip et al., 2005). There is also serologic or molecular evidence of *E. canis* infection in South Africa, Tunisia, Senegal, Chad and Zimbabwe (Ndip et al., 2005). Canine brucellosis (CB) has been widely reported in most countries in the world, especially those with many stray dogs (Radojičić et al., 2001). However, there are no official reports of CME and CB in Tanzania. Recently cases of dogs with clinical signs resembling CME or CB have been reported in Tanzania but no laboratory confirmation was performed (SUA teaching animal hospital records, unpublished data). Clinical signs included depression, anorexia, fever, vomiting dyspnoea, lymphadenomegaly, deep yellow urine, dark tarry faeces, weight loss, abortion and infertility. Most of these have been reported to be presenting signs in CME (Waner and Harrus, 2000) whereas CB presents with abortion in female dogs and infertility in males (Oncel, 2005).

High mortalities and losses to dog owners associated with CME and CB like illness press for the need to have confirmatory diagnosis of these conditions, in addition to the need to establish baseline

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data for these diseases. The present study was therefore designed to determine the serological prevalence of *Ehrlichia canis* and *Brucella canis* infections and their associated factors in dogs in Morogoro municipality.

Specific diagnostic tests for CME include demonstration of *E. canis* morulae in monocytes, culturing the rickettsia, serology and polymerase chain reaction (PCR) (Nakaghi et al., 2008). Serological detection of *E. canis* antibodies can be done through indirect immunofluorescence antibody (IFA) test, which is considered the serological ''gold standard'', or using commercial serological tests for *E. canis* immunoglobulin-G (IgG) antibodies (Waner et al., 2000). These include the dot-ELISA tests, among them being the Immunocomb® (Biogal, Israel).

Bacteriological isolation for the diagnosis of CB is difficult, time consuming and poses health risk to personnel (Kim et al., 2006). The microbiological methods depend on the bacterial viability and consequently, may pose a threat to laboratory personnel because of zoonotic nature of *Brucella canis* (Keid et al., 2007). Serology, therefore, remains to be the most reliable method in the diagnosis and in the establishment of baseline data for these diseases.

Serological tests for *B. canis* include rapid slide agglutination tests (RSAT), tube agglutination test, an indirect fluorescent antibody (IFA) test, agar gel immunodiffusion and enzyme-linked immunosorbent assays (Wanke, 2004). Other tests such as complement fixation and counter-immunoelectrophoresis are used mainly in research (Wanke, 2004). Titres vary between individuals and with the detection method. Cross-reactions between *B. canis* and other Gram-negative bacteria can occur in some tests, particularly agglutination tests. Non-specific agglutination reactions also occur in some dogs (Kim et al., 2006). To eliminate this problem, researchers have developed ELISA techniques using purified specie-specific antigens and/or monoclonal antibodies (Radojičić et al., 2001). The dot-ELISA technique has been found to be reliable and highly specific for rapid diagnosis of brucellosis in dogs (Radojičić et al., 2001). For clinical cases repeated blood cultures and serologic monitoring are required before a dog can be declared negative (Oncel, 2005). For epidemiological studies, serological procedures devised for serodiagnosis of *B. canis* infection in dogs have been applied successfully without the bacteriological methods (Flores-Castro et al., 1977).

Materials and Methods

Study area and animals

This study was conducted in the Morogoro municipality in Morogoro region, Tanzania. Morogoro is situated 190 km west of the commercial capital, Dar es Salaam. The dog population was estimated at 3135 (Morogoro municipal livestock office, 2010, Internal Report). The sample size was calculated using the formula described by Stevenson (2008). Using the expected prevalence of 3% (Davoust et al., 2006), with 95% certainty of including at least one positive animal, and maximum acceptable error of 5%, a sample size of 93 was calculated. The sample size was rounded to 100 dogs which included 57 males and 43 females.

Study design

A cross-sectional study was employed where blood samples were taken from the animals once between June and September, 2010.

Selection of study animals

The Municipality of Morogoro was divided into five areas namely Kilakala, Forest Hill, Chamwino, Tungi and Town centre, based on the administrative locations of the area (Appendix 1). Dogs from these areas were randomly sampled for inclusion into the study. Dogs from the police force, a security company and missionary unit formed one group called a working dogs group. Another group called a suspect group comprised of dogs suspected to be suffering from CME at SUA hospital or recovering from such illness, or coming from a pack which experienced recent mortalities. In total there were seven groups which formed seven sampling clusters. The dogs were categorised into three age groups which were: the juvenile (0-6 years), the adult (7-8 years) and the old (>8 years). The distribution and composition of the different dog groups are summarised in Table 1. All dogs in the sampling cluster were tested for antibodies against *E. canis* and *B. canis*. The owners also provided information on sex and breed as well as dog's contact with other dogs. On breed statistical analysis based on whether or not the dog was a German Shepherd Dog (GSD). Health status was assessed on three levels. Healthy dogs were not sick, recovering dogs had been sick but were recovering and sick dogs were sick at the time of data collection. The dog's body condition was recorded as good, fair or poor. Presence and history of tick infestation was noted.

Data collection

The 100 study dogs were obtained by visits to selected homes in the five areas of the Municipality. Dogs were restrained manually and whole blood was collected from the cephalic vein in 5 ml plain and ethylenediamine tetra-acetic acid (EDTA) tubes. The blood in plain tubes was centrifuged at 2500 rpm for 10 minutes using a Sigma 3E-1 centrifuge (Sigma Harz, Germany). Serum obtained was stored at -20^{o}C prior to use for serological tests.

Table 1: Cluster composition and seroprevalence of CME

Cluster	Composition	No. of dogs	Seroprevalence (%)	CI
Working	Police dogs K.K security Salvatorian Missionaries	18	5/18 (27.8)	9.7-53.5
Kilakala	Kilakala Kigurunyembe Kola Bigwa	10	2/10 (20)	2.5-55.6
Forest Hill	Forest Hill LITI SUA Vibandani	9	3/9 (33.3)	7.5-70.1
Mazimbu	Mazimbu Kihonda Tungi/Nanenane	15	5/15 (33.3)	11.8-61.6
Tungi	Mafisa Mji mpya	11	1/11 (9.1)	0.2-41.3
Town centre	Town centre Misufini Chamwino	13	7/13 (53.8)	25.1-80.8
Suspects	Clinical cases	24	2/24 (8.3)	1.0-27.0

Sample laboratory analysis

Dogs' sera collected were used for detection of specific anti-*Ehrlichia canis* IgG antibodies by Immunocomb® dot-ELISA test (Biogal, Israel) as previously described (Waner et al., 2000). The results were expressed in ''S'' units on a scale of 0–6 (S0-S6) on a color-coded Combscale provided in the Immunocomb1 kit. Three ''S'' (S3) units were calibrated by the manufacturer to a titre of 1:80. A result of greater than or equal to three ''S'' units (≥ S3) in the Immunocomb1 test was considered positive. A positive control spot developing colour changes equal to three ''S'' units was present on each test.

Similarly, sera were used for detection of canine brucellosis by using *Brucella canis* Immunocomb® Antibody Test Kit (Biogal, Israel). The procedure is similar to the test for CME except that for this a purified *Brucella canis* antigen is attached to the Comb. The results were also expressed in ''S'' units on a scale of 0–6 (S0-S6). Manufacturer's calibration was for Score 1 (S1) to read a titre ≤ 1:50, Score 2 (S2) a titre 1:50-1:200, and Score 3-6 (S3 – 6), titre ≥ 1:200. Tests with levels 3-6 (S3-S6) were considered positive. Level 1 (S1) of antibody was considered a negative result (no infection) and level 2 (S2) indicated suspicion of infection.

Statistical analysis

Data were recorded in Microsoft Excel® version 4.0. The stored data were then transferred to Epinfo® Version 3.5, 2008 for Windows (CDC, 2009) for descriptive statistical analysis. Seroprevalence was calculated for each risk factor studied, as the number of dogs with titre greater than 1:80 divided by the total number of dogs analysed with the 95% confidence interval (CI). Logistic regression was used to evaluate risk factors. The difference was considered significant when $P<0.05$.

Results

Study dogs

A total of 57 male and 43 female dogs were used in the study. Thirty two of the dogs were pure breed dogs, twenty three were cross breeds, twenty four local and twenty one dogs were of mixed breeds. Cross breeds in this case refer to dogs with mixed blood of two breeds. Dogs of mixed breeds refer to dogs which have mixed blood of more than two breeds. The distribution and number of dog breeds sampled is shown in Table 2.

Table 2: Distribution of dog breeds in the study

Breed	Frequency	Percent
Boerboel	2	2.0%
Doberman/GSD cross	1	1.0%
Doberman	2	2.0%
Doberman cross	1	1.0%
GSD	26	26.0%
GSD cross	19	19.0%
Mixed	21	21.0%
Mongrel	24	24.0%
Pomeranian	1	1.0%
Ridgeback cross	2	2.0%
Rottweiler	1	1.0%
Total	100	100.0%

The age of the study animals ranged from 3 months to 15 years. The mean age was five years and the mode was seven years. Thirty three dogs were in the juvenile group, fifty six in the adult group and eleven were in the old age group. Tick infestation was noted in five

dogs and eighty three dogs had no ticks at the time of data collection. No information was available for twelve dogs. Dog owners admitted to have noticed ticks infestation at one or more times in the life of all the dogs. Ticks found on the dogs were not identified. Forty five dogs had had contact with other dogs while thirty one dogs had no contact. Information was lacking for twenty four dogs.

Seroprevalence of Canine Monocytic Ehrlichiosis

Out of the 100 serum samples, a total of 25 (25%) had IgG antibodies reactive to *E. canis*. Titres for positive reactors were 1:80 (n=8), 1:160 (n=9) and 1:320 (n=8).

Statistical association of potential risk factors such as age, body condition, breed, health status, tick infestation, and sex is summarised in Table 3. None of the above mentioned factors was associated with *E. canis* seropositivity (P>0.05). However, when seroprevalence of the old and adult age groups were compared the difference was statistically significant (P<0.05) with the disease more prevalent in the old dogs. The difference between dogs in good and fair body condition was also statistically significant (P<0.05). The difference in seroprevalence between dogs in fair and poor body condition and that between dogs in good and poor body conditions were not statistically significant (P□0.05). The seroprevalence in dogs in poor body condition is highest among the three levels.

Table 3: Logistic regression to detect factors associated to antibody response to *E. canis*

Variable	No. of positive samples (%)	P-value
Age		
Juvenile	16/56 (28.6)	
Adults	4/33 (12.1)	
Old	5/11 (45.5)	0.0563
Old/adult 0.0258*		
Body condition		
Fair	10/22 (45.5)	
Good	10/49 (20.4)	
Poor	2/4 (50)	0.065
Good/Fair 0.034*		
Breed-GSD		
Yes	4/26 (15.4)	
No	21/74 (28.4)	0.1881
Health status		
Healthy	20/73 (27.4)	0.6269
Recovering	¼ (25)	
Sick	4/23 (17.4)	
Presence of ticks		
Yes	3/5 (60)	
No	22/83 (26.5)	0.1068
Sex		
Male	15/57 (26.3)	
Female	10/43 (23.3)	0.7264

* Statistically significant (P < 0.05)

Only one dog had a level of 0 (S0) on the *E. canis* Immunocomb dot ELISA scale (Fig. 1). Among the seven clusters, the highest prevalence was recorded from the Town centre cluster (53.8%). The lowest prevalence came from the suspect cluster with a prevalence of (8.3%) (Table 1). Seventy six dogs had records on whether or not they had been in contact with other dogs. Contact with other dogs did not significantly influence seroprevalence. With respect to breed, there was no significant difference (P□0.05) between GSDs and other breeds on the seroprevalence of *E. canis* antibodies.

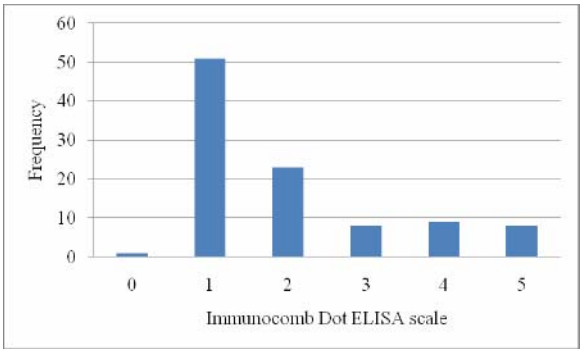


Fig 1: Frequency of Immunocomb dot ELISA scale levels for CME

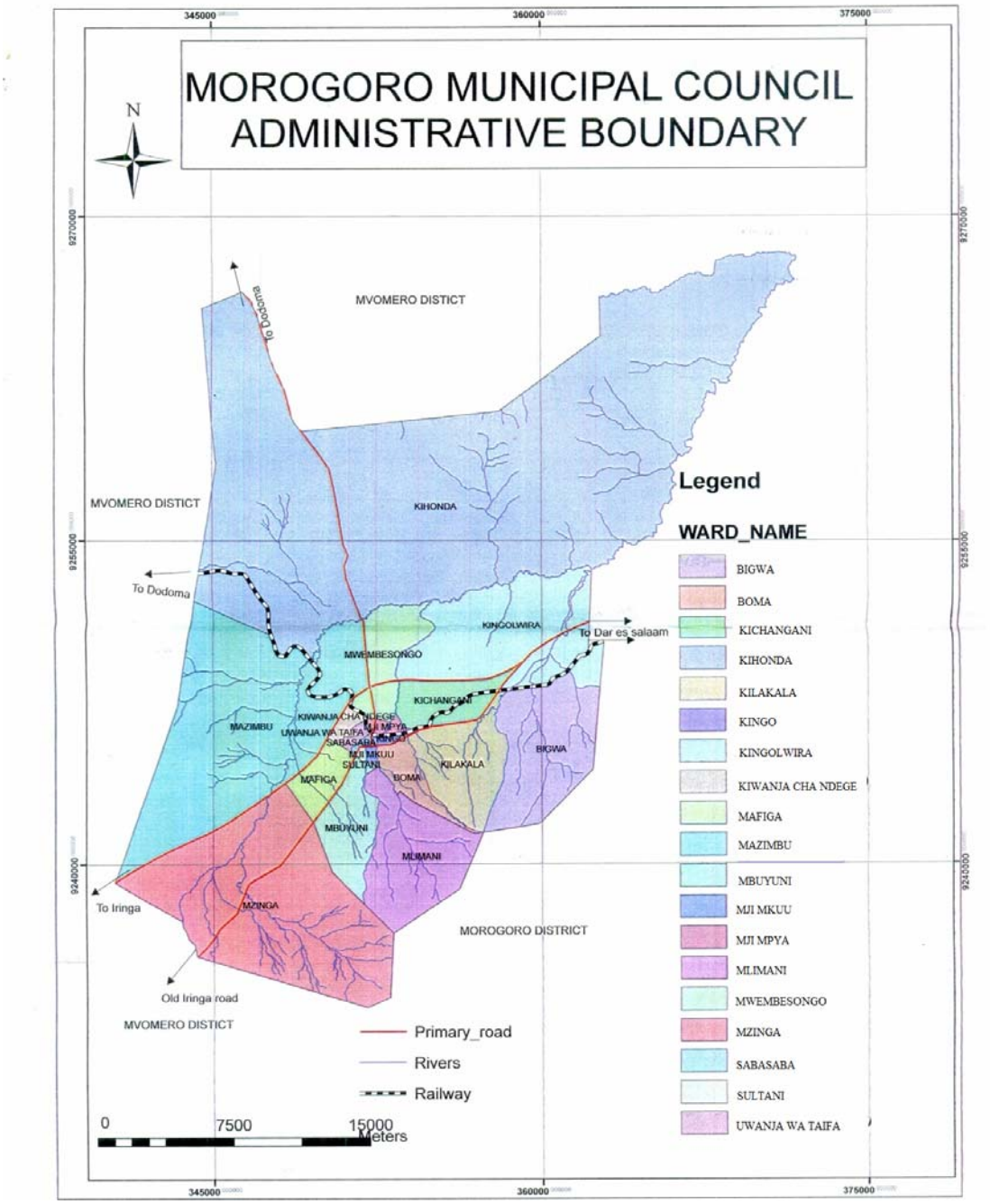
Seroprevalence of Canine brucellosis

There were no positive *Brucella canis* Immunocomb test results from all the 100 study dogs, implying a seroprevalence of 0% for Canine brucellosis in Morogoro. However, eight dogs had titres of ≤ 1:50 (S1) and five dogs had titres of 1:50-1:200 (S2). Only three dogs had signs of reproductive disorders. One of these three dogs had had pregnancy which ended up with no puppies. The *B. canis* antibody titre for this dog was of the level S2. The other two had no antibody titres (S0). These thirteen dogs with low levels of antibodies to *B. canis* belonged to the following breed categories: GSDs (n=3), GSD crosses (n=2), Mixed (n=3), mongrels 4) and ridgeback cross (n=1).

Discussion

The present study is the first serological investigation of canine monocytic ehrlichiosis in Tanzania. It provides the first serological evidence of *E. canis* infection in dogs in Morogoro. The investigation found that 25% of the study dogs had antibodies reactive with *E. canis* by Immunocomb Dot-ELISA test (Biogal, Israel), demonstrating an overall seroprevalence of 25%. As for canine brucellosis the seroprevalence of canine brucellosis in Morogoro municipality was 0%.

Appendix 1: Map of the Morogoro Municipal



Source: Morogoro Municipal Director's Office, 2010

Canine Monocytic Ehrlichiosis

The seroprevalence of CME in Morogoro municipality was lower than the seroprevalence reported in other tropical countries such as South Africa (42%) (Jiménez-Coello et al., 2009), Egypt (33%), Ivory Coast 67.8 %, (Davoust et al., 2006), and Cameroon 32% (Ndip et al., 2005). However, it was higher than the seroprevalence of 3.1% reported in Gabon (Davoust et al., 2006) and that of 8.1% among stray dogs in Yucatan Mexico (Jiménez-Coello et al., 2009). The variation in seroprevalence among different studies can be attributed to the diversity of experimental designs and diagnostic protocols used, environmental factors involved in the epidemiology of ehrlichiosis in the studied regions and the cut-off values utilized in the IFAT for each study (Souza et al., 2010). In a study

done in Brazil by Oliveira et al. (2000), seroprevalence was higher (92.3%) than all the reports mentioned above. The study by Oliveira et al. (2000) involved only dogs with clinical signs of CME and resulted in higher prevalence of CME compared to the other studies. Jiménez-Coello et al. (2009) mentioned inclusion of a low number of dogs, dogs with different health status, origins and sanitary conditions as the possible reasons for variations in reported prevalence. Serological screenings in animals with clinical signs of CME results in a high number of positive cases (Jiménez-Coello et al., 2009). The present study included animals with different health status and sanitary conditions.

In the present study eighty three dogs (94.3%) had no tick attached out of the eighty eight dogs which had information on whether they had ticks attached or not. However, all the study dogs had a history of tick attachment. Although tick identification was not done, these findings indicate that the *E. canis* vector tick, *R. sanguineus,* is endemic in Morogoro. Previous studies such as that by Fyumagwa et al. (2008) have indicated presence of the tick *R. sanguineus* in Tanzania.

With regard to health status twenty out of seventy three healthy (27.4%) dogs were seropositive for CME, and five out of twenty seven unhealthy (sick or recovering) dogs (18.5%) were seropositive. However, the difference was not statistically significant. There are only a few studies in which seroprevalence were compared between healthy and unhealthy dogs. In one of the studies, by Ndip et al. (2005) unhealthy dogs had a higher seroprevalence (20/39) than healthy dogs (13/65). Macieira et al. (2005) used PCR and established a prevalence of 30/112 (26.8%) in thrombocytopenic dogs and 4/114 (3.5%) in non-thrombocytopenic dogs. Thrombocytopenia is considered the most common and consistent haematological finding in acute CME (Waner and Harrus, 2000). Davoust et al. (2006) used dogs without any clinical signs of illness and the seroprevalence of CME was 67.8% and 3.1% in Ivory Coast and Gabon respectively. The small sample size of unhealthy dogs (27) in the present study is a possible reason for variation of these results from previous studies. Another possible explanation is that some of these dogs probably suffered a clinical CME, recovered from the disease, and had *E. canis* antibodies at the time of testing, while others were probably subclinical carriers of *E. canis*. In addition, clinical signs in the sick dogs were not specific for CME and some had signs unrelated to CME, or had other diseases such mange infestation, transmissible venereal tumours, and flea allergy dermatitis.

The present study has shown that there is no significant difference in seroprevalence of CME between sex and seroprevalence was significantly different between dogs in the old and adult groups ($P<0.05$). Significant difference in seroprevalence was also found when dogs in good and those in fair body conditions were compared. The seroprevalence of 27.8% in the working dogs group is very close to seroprevalence of CME among military dogs in Egypt (29%) established in a study by Botros et al. (1995). CME is generally considered to have no age or sex nor predilection (Waner and Harrus, 2000). Other studies have indicated an increase in seroprevalence with age. Rodriguez-Vivas et al. (2005) found that dogs older than two years had higher association with a seropositive result, in a study conducted in Yucatan, Mexico. Rahman et al. (2010) found that, older dogs (10-16 years) had highest percentage of infection. This association is attributed to the increased probability of a dog being exposed to *E. canis* as it gets older, rather than to a higher susceptibility among older dogs (Costa et al., 2007). The difference between dogs in good and fair body conditions is possibly associated with the nutritional status which may influence the immunologic status of the dogs.

The suspect cluster had the lowest seroprevalence among the clusters. This implies that most of the dogs in the suspect group were not in any of the three stages of the disease. It has been documented that high levels of IgG develop during the acute stage of CME (Guimarães et al., 2009). Dogs that fail to eliminate the infection during the acute stage usually become carriers (Guimarães et al., 2009). The findings are contrary to other studies in which dogs suspected to have E. *canis* infection were used. In a study by Pusterla et al. (1998) in Switzerland, dogs suspected of having ehrlichiosis had the highest prevalence of antibodies to *E. canis* compared to healthy dogs and dogs with other diseases, and the differences were statistically significant. Thrombocytopenic dogs had a higher prevalence than non-thrombocytopenic dogs in a study by Macieira et al. (2005). This disagreement of results could be attributed to the fact that the present study criteria for selection of animals to this group were not specific to the suspicion of the actual study subjects to have signs of CME. Some of the study animals in this group were even healthy.

Brucellosis

Five dogs had *B. canis* titres of 1:50-1:200 (S2) which were categorised as suspicious results. Since clinical signs related to the disease have been observed in Morogoro, it is suggested that further studies involving larger sample size of dogs should be conducted. This is because while some previous studies had high prevalence of CB, some had as low as 1% (Brown et al., 1976). In another serological survey for canine brucellosis that had been conducted on 341 dogs from different regions of the province of Quebec, a

significant titre was found in six sera (1.6%) (Higgins et al., 1979). In a study by Bosu and Prescott (1980) sera from 2000 dogs were tested for antibodies to *Brucella canis* by a rapid slide agglutination test and seroprevalence was 0.3%. Thirty-one sera gave suspicious titres. Other reports have reported canine brucellosis prevalence of 4.9% in Ahvaz Iran (Mosallanejad et al., 2009), 21.5% in Konya region, Turkey (Uçan et al., 2010) and 7.3% in the city of Buenos Aires, Argentina (Boeri et al., 2008). Among the study dogs in the present study, only three dogs had signs of reproductive disorders which are major clinical signs leading to suspicion of canine brucellosis. They were all seronegative for *B. canis*. This number of animals with suggestive clinical signs for canine brucellosis is too low to be meaningful in a study involving such a sample size. Moreover, the exact time of occurrence of the clinical signs in these dogs was not investigated. It is possible that they were previously seropositive but the antibodies had waned at the time of this investigation, or the reproductive disorders were caused by different infections. It is also important to understand that not all cases that had related signs with brucellosis are caused by *B. canis* (Mosallanejad et al., 2009).

Conclusions and recommendations

From this study it can be concluded that CME is common in Morogoro and therefore should be considered in the differential diagnosis of canine diagnosis presenting with a complex of clinical signs. More emphasis should be given to testing and treatment of clinical cases as well as tick control measures to protect dogs from *E. canis* infection. As for CB findings of this investigation points out to the need for further studies on the presence of the disease. Since this study took only a short period of time, it is recommended that a longer study with a larger sample size be conducted to be able to gather more information on the diseases. Such a study will be able to purposively involve clinical cases which can be monitored over a period of time.

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