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Seroprevalence of *Brucella canis* in dogs and at-risk humans in Jordan

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Abstract: *Brucella canis* infection is a neglected zoonotic disease and its seroprevalence in dogs and at-risk humans has not been previously studied in several countries including Jordan. The main aim of this study was to determine the seroprevalence and identify risk factors of *B. canis* infection in police, breeding and stray dogs and in at-risk humans in Jordan. A total of 169 sera samples from apparently healthy dogs and 185 samples from apparently healthy people (85 from dog handlers and 100 from the general population) were tested in the study. Antibodies against *B. canis* were tested using the canine D-Tec® CB Rapid Slide Agglutination Test (RSAT) kit with secondary 2-mercaptoethanol (ME-RSAT). Overall, 8.3% of the dog sera samples tested positive to antibodies against *B. canis*, and 37.8% of stray dogs tested positive. Seroprevalence was higher in male dogs than in females. Furthermore, none of the tested human samples was positive to antibodies against *B. canis*. There was a significant association between seropositivity and the type of dog. The study reports preliminary findings that suggest the presence of *B. canis* among stray dogs in Jordan. Thus, preventive measures should be taken to control the transmission of this pathogen from stray dogs to other dogs and humans as well.

Keywords: *Brucella*; dogs; stray; breeding

Canine brucellosis, caused by *Brucella canis*, is considered to be an under-recognized and neglected disease in both humans and animals in many parts of the world. This disease causes reproductive failure in dogs and is considered one of the most economically significant diseases of breeding dogs (Henderson et al. 1974; Gyuranecz et al. 2011). It can also lead to ocular signs and discospondylitis in spayed or neutered dogs (Henderson et al. 1974; Gyuranecz et al. 2011). Many infected dogs appear to be clinically healthy and therefore infected dogs can go unnoticed thus creating diagnostic challenges to health care providers in veterinary settings (Marzetti et al. 2013). However, history of abortion or signs of poor reproductive performance in dogs may increase the index of suspicion for veterinarians. On the other hand, there have been reports that dogs infected with *B. canis* may remain asymptomatically bacteraemic for prolonged periods of time (Lucero et al. 2002). This may suggest that contact with apparently healthy and asymptomatic dogs might increase the chances of occupational transmission to pet owners, veterinary students, veterinarians, laboratory technicians and dog handlers (Krueger et al. 2014).

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The most commonly reported routes of transmission of canine brucellosis to humans are through contact with infected dogs, contact with their secretions or occupationally through exposure to clinical cases, during assisting parturient or aborting dogs, handling stillbirths, or contact with canine biological samples including blood, urine, placenta or aborted foetuses (Krueger et al. 2014). Until today, in many parts of the world, routine testing for *B. canis* is not performed in either humans or dogs even in suspected cases. In humans, *B. canis* causes mild and non-specific clinical signs that usually do not increase the index of suspicion for physicians, meaning that this zoonotic disease is potentially underdiagnosed (Krueger et al. 2014). The zoonotic risk of *B. canis* has been considered fairly high for persons who handle breeding dogs in kennels or who are exposed to infected animals (Lucero et al. 2010).

B. canis appears to be widely distributed and has been reported in the USA, Canada, India, South and Central America and some European and African countries (Poester et al. 2002; Cadmus et al. 2011; Kaden et al. 2014). Yet, there is a scarcity of reports on the prevalence and risk factors of *B. canis* infection in human and dogs worldwide. In the Middle East, most of the research was conducted to estimate prevalence rates of *Brucella* species in food animals (Musallam et al. 2015). Therefore, the main aim of this study was to estimate the prevalence of *B. canis* in three classes of apparently healthy dogs: specifically, police, breeding and stray dogs. The study also aimed to estimate the prevalence of *B. canis* in apparently healthy at-risk professionals including veterinary students, veterinarians, laboratory technicians, police dog handlers and kennel workers. Furthermore, in this study we also explored potential risk factors that might be associated with *B. canis* infection in dogs and humans in Jordan.

MATERIAL AND METHODS

This study was approved by Institutional Animal Care and Use Committee as well as the Institutional Research Board (approval No. 7601). Blood samples from human participants were obtained after the signing of informed consent forms. All participants were briefed about the objectives and the voluntary nature of this study. All human participants enrolled in the study were over 18 years of age, apparently healthy, and had no history or clinical signs suggestive of immunodeficiency. A self-administered questionnaire was filled out by the participants which documented the following information: age, sex, health status of participants (presence of active or chronic infection), duration of contact with dogs, history of handling or assisting parturient or handling aborted materials, history of being sick after exposure to sick dog and educational level. At-risk participants were enrolled in the study if they were exposed to dogs on a regular basis as a part of work, training or ownership and included kennel workers (police dogs), breeders, veterinary students, veterinarians, police dog handlers, veterinary assistants and laboratory technicians. The canine non-exposed group included participants who presented themselves at the governmental health centre for routine health exam and who had no clinical signs and no exposure to dogs in the past year as part of their daily life.

Blood samples of dogs were obtained from densely populated areas in Jordan including Amman, Jarash, Irbid, Ajloun, Al-Shouneh, Al-Ramtha and Jawa. Human blood samples were obtained from the same areas. A form was filled in for each dog that documented the age, sex, breed, category (stray, police or breeding dogs), vaccination status, abortion history and history of sickness. In stray dogs, vaccination status or abortion history could not be obtained or verified. History of sickness was determined based on general health examination and the apparent body condition scores. Blood samples were obtained from the cephalic vein of dogs. Upon collection, the samples were shipped on the same day under cold conditions to the Zoonotic Diseases Laboratory, Jordan University of Science and Technology. Upon arrival at the laboratory, samples were registered in the sample logs with unique identifications. Sera were harvested from the samples by centrifugation at 1200 *g* for 10 minutes. The sera were divided into two aliquots, pipetted into Eppendorf tubes and stored at –20 °C.

Antibodies against *B. canis* were tested in the study population using the canine D-Tec® CB Rapid Slide Agglutination Test (Synbiotics Corporation, Kansas City, MO, USA) kit with secondary 2-mercaptoethanol (ME-RSAT). To decrease the occurrence of false positives, the kit also included a 2-mercaptoethanol (ME-RSAT) confirmatory procedure that eliminates IgM antibodies which

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are usually responsible for this unspecific agglutination. The test was performed after 60 µl of serum and prepared antigen were pipetted and mixed with a stir stick within an oval circle as specified on the provided test card according to the manufacturer's instructions. The card was gently rocked to swirl the mixture for 15 seconds and then allowed to rest for two minutes. The mixture was then observed for agglutination. If a serum sample produced a positive agglutination reaction, the ME-RSAT procedure was then completed. The test kit included a positive control. Results were interpreted blindly by three readers as strongly positive (extensive agglutination), weakly positive (visible agglutination) and negative (absence of agglutination).

The overall seroprevalence of the *B. canis* antibodies among participant subjects was calculated from the total number of samples tested and expressed as a percentage. The measured dependent outcome was serological reactivity evaluated using the rapid slide agglutination test (RSAT). The obtained data were used to identify potential risk associations between the outcome variable (seropositivity to *B. canis)* and the independent risk factors using logistic regression. Statistical results were considered significant if the P value was ≤ 0.05. All statistical analyses were performed using SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA).

RESULTS

In this study, 85 at-risk human subjects known to be exposed to dogs were enrolled and submitted serum sample for analysis, of which there were 65 males with a mean age of 24.7 ± 5.2 years and 20 females with a mean age of 21 ± 1.6 years. Meanwhile, 100 non-exposed human subjects were enrolled, with 74 males with a mean age of 27 ± 3.1 and 26 females with a mean age of 23 ± 2.9. None of the sera samples obtained from exposed or non-exposed groups tested positive to antibodies against *B. canis.* These samples included one veterinarian, one laboratory technician, 34 police dog handlers, 47 veterinary students and two canine kennel workers. Among the students, there were 36 students who reported a history of having been accidently stuck by a needle while training and 12 students reported handling non-canine placenta. None of the exposed group reported being sick after handling dogs in the years prior to sample collection.

A total of 169 dogs were enrolled in this study and these belonged to three categories: 37 stray dogs, 55 police dogs and 77 breeding dogs housed at kennels (small farms made from brick). In total, there were 27 spayed females, 69 intact females and 73 males. Represented dog breeds included 92 Mongrel dogs known locally as Canaan breed dogs, 19 Huskies, 18 Labrador Retrievers, 17 German Shepherds, 15 Belgium Shepherds, five Springer Spaniels, one Pitbull, one Rottweiler and one Terrier. Police dogs were all reported to be current on all vaccinations and deworming medicine, while vaccination status for most of the breeding dogs was unknown. All stray dogs were reported to have active ticks. Exposure to stray dogs was reported in about 59 dogs. None of the police dogs was reported to be exposed to any stray dogs. None of the serum samples obtained from police or breeding dogs tested positive against *B. canis,* while 14 (8.3%) of the dogs tested positive against *B. canis.* The seroprevalence was higher in male

Table 1. Factors associated with seroprevalence of *Brucella canis* among the tested dogs

Variable	Category	Negative	Positive	*P*-value
Dog group	kennel dogs	77	0	< 0.05
	police dogs	55	0	
	stray dogs	23	14	
Sex	male	64	9	
	female (intact)	64	5	
	female (spayed)	27	0	
Breed	Mongrel	78	14	
	Belgium Shepherd	15	0	
	German Shepherd	17	0	
	Husky	19	0	
	Labrador Retriever	18	0	
	Pitbull	1	0	
	Rottweiler	1	0	
	Springer Spaniel	5	0	
	Terrier	1	0	
Exposure status	confined	110	0	
	exposed/ commingling	45	14	
Abortion	yes	12	0	
	no	143	14	
Pregnancy	yes	8	0	
	no	147	14	

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stray dogs (nine males positive) than in females (five females positive). From these samples, there were 12 (7.10 %) reported cases of abortion in the year prior to blood collection. The abortion cases were all reported at the breeding kennels and the aetiology of abortion was never investigated by the owners. There was a significant association between the seropositivity and group of dogs (stray, police and breeding dogs). The data are summarised in Table 1.

DISCUSSION

In this study, we determined the seroprevalence of antibodies against *B. canis* in the sera of three categories of the dog population as well in the sera of two categories of the human population. The study showed that none of the human or dog samples were seropositive against *B. canis* except for samples from stray dogs. The overall seroprevalence was 8.3% for all tested dogs and 37.8 % in the tested stray dogs. The seroprevalence of canine brucellosis appears to differ by location and ranges from 0% to % 15% (Brower et al. 2007; Cadmus et al. 2011; Behzadi and Mogheiseh 2012; Castrillon-Salazar et al. 2013; Ayoola et al. 2016). However, it appears to be sporadic in other parts of the world including Europe and the Middle East (Sayan et al. 2011). No dogs seropositive against *B. canis* have been reported in Australia and New Zealand. The differences in seroprevalence by region might be explained by the testing method. Specifically, the high prevalence reported in our study might be attributed to the fact that RSAT and 2ME-RSAT have been reported to yield false-positive reactions (Carmichael et al. 1984). On the other hand, these agglutination methods have been shown to have lower sensitivity than other serological tests, mainly ELISA (Wanke et al. 2002; Wanke et al. 2006), thus opening the possibility that some dogs that tested negative by agglutination in this study were currently or previously infected by *B. canis*.

Epidemiological studies characterizing occurrence and risk factors associated with *B. canis* in dogs or humans are scarce and have not been carried out in many parts of the world including Jordan. In dogs, only a few studies evaluated the risk factors associated with seropositivity against *B. canis*, which include a history of *B. canis* seropositivity, non-culling of seropositive dogs, a history of abortion, the presence of exotic breeds and a history of feeding foetuses from cows (Cadmus et al. 2011; Behzadi and Mogheiseh 2012; Castrillon-Salazar et al. 2013). Just recently, a study conducted in Iran compared the seroprevalence against *B. canis* between native and foreign dogs, and the authors suggested that native dogs are less likely to be seropositive when compared with exotic or imported dogs (Behzadi and Mogheiseh 2012). In humans, meanwhile, reported risk factors associated with seropositivity against *B. canis* include poor hygiene and lack of personal protection during reproductive procedures, unsafe practices during care for abortions, location, such as e.g. urban areas and immune status, particularly in children (Abarca et al. 2011; Fernandes et al. 2011; Agudelo-Florez et al. 2012; Angel et al. 2012). Previous studies have suggested that exposure to *B. canis* occurs in people in occupational contexts. In this study, we tested at-risk groups of people including veterinary students, handlers, breeders, shelter workers and veterinarians. Although all subjects appeared to be seronegative, they still should follow safety measures and proper precautions to eliminate the chance of contacting this disease. Just recently, Osoro et al. (2015) have provided evidence of a strong association between human and animal seropositivity at the household level. Overcrowded shelters or breeding kennels may potentially create a perfect environment for transmission of infectious disease. The zoonotic risk of *B. canis* has been considered fairly high for persons who handle breeding dogs in kennels or who are exposed to infected animals (Krueger et al. 2014). In the current study, all of the abortion cases appeared to have occurred at the breeding kennels where dogs tested negative against *Brucella* in the agglutination test. This also may suggest that abortion could potentially be caused by other causes which require further testing.

This is the first research to document the occurrence of *B. canis* in Jordan. The results reported here reveal a high level of seropositivity against *B. canis* in stray dogs. The extent of the stray dog crisis in many parts of developing countries including Jordan is beyond acceptable. In Jordan, the numbers of stray dogs have been on the rise recently due to many reasons, including population growth from an influx of refugees from neighbouring countries and poor waste disposal management.

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In addition, a lack of policies that encourage and enforce responsible dog ownership or adoption is another factor. Pet ownership is much less common in countries of the Middle East due in part to cultural and religious barriers. In Jordan, although there are no accurate statistics of the size of the stray dog population, estimates put the number at thousands of stray dogs who wander the streets and outskirts of communities potentially spreading diseases to other animals and humans. In the past few years, a "Capture, neuter and return" program was adopted in Jordan just in the capital city; however, this is considered insufficient to control the issue of stray dogs at the national level. The number of stray dogs is a national issue and regulatory bodies are in the process of developing guidelines, policies and strategies to better control the numbers of stray dogs. Furthermore, a significant drive by veterinarians to increase public health awareness through workshops, campaigns and the media has been initiated. In addition, adopting national strategies and policies to activate or regulate identification, registration, vaccination, sales and breeding of dogs as well the testing of foreign or exotic dogs that are imported into the country should be initiated. More importantly, there is an increasingly growing concern over the uncontrolled movement of dogs across borders secondary to wars in neighbouring countries in addition to increasing numbers of breeding farms that are not attended by state or local veterinarians. Stray dogs can be a potential source for *B. canis* for other companion animals as well as humans.

Brucellosis caused by *B. canis* is globally underestimated or underreported in human and veterinary medicine for several reasons, including non-pathognomic symptoms and a lack of familiarity on the part of medical professionals. In addition, the routine *Brucella* test used in clinical laboratories does not detect *B. canis* infection as it is performed with smooth *Brucella* antigens. Despite finding no evidence of antibodies against *B. canis* in the tested human samples, routine testing should be part of the diagnosis in suspected cases in particular in cases with compromised immunity and in children and pregnant women. In conclusion, this study suggests the presence of *B. canis* in stray dogs in Jordan. This finding highlights the need to understand the epidemiology of *B. canis* infection to establish the necessary measures for surveillance and control.

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