First Diagnosis of *Brucella canis* Infection in Dogs in Israel

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ABSTRACT

Brucella canis is a worldwide infectious disease of dogs and a zoonotic infection. Until now the disease has not been diagnosed or documented in Israel. Recently, an outbreak of canine brucellosis in Northern Israel has been documented and confirmed for the first time in Israel using bacteriological, serological and molecular methods. The exact source of the infection was not determined but was possibly associated with a single female dog which was imported into Israel from abroad or with more than one imported dog. It was expected that the extent of the outbreak would be limited by taking the necessary measures described in this document and by renewing the vigilance and awareness of Israeli veterinarians.

Keywords: *Brucella canis*; Israel; Bacteriology; Serology; PCR; Zoonosis

INTRODUCTION

Brucellosis is a chronic infectious disease in dogs caused by gram-negative coccobacilli of the genus Brucella. There are four species known to cause disease in dogs: *Brucella melitensis, Brucella abortus, Brucella suis,* and *Brucella canis*. The most dangerous and significant infectious species for dogs is *B. canis* (1).

Israel is endemic to *B.melitensis* in small ruminants, cattle and camels (2). However Israel has been free of *B. abortus* since 1985 (2). Until 2019, no serologic and bacteriologic evidence of infection of dogs with *B. canis* in Israel has been found.

Brucellae enter the host by penetrating mucosal epithelium usually of the reproductive tract, after sexual or reproductive transmission, or through oral transmission, and are transported as free bacteria, or within phagocytic cells to regional lymph nodes (4). *B. canis,* like other *Brucella spp*., has a strong tropism for the reproductive and lymphoendoreticular systems. Colonization of reproductive tissue, mammary glands, and the spleen, as defined by positivity on culture, is common (4). Continued proliferation of *B. canis* within the uterine wall, testis, and mammary tissue, as well as lymph nodes and spleen, leads to reproductive failure and nonspecific, cyclic, febrile disease (4).

In the majority of reported human cases, *B. canis* infection is the direct result of exposure to whelping or aborting females, since high concentrations of *Brucella* organisms occur in the birth fluids and vaginal discharges (5). Other human cases have been reported as a result of laboratory infections or contact with infected dogs or their excrements.

Reported clinical signs associated with human brucellosis include fever (often periodic and nocturnal), fatigue, headache, weakness, malaise, chills, sweats, weight loss, hepatomegaly, splenomegaly, and lymphadenopathy. Serious complications from *B. canis* infection in humans include septic arthritis, aortic valve vegetations, calvarial osteomyelitis, epidural abscess, pleural effusion, oral lesions, lower extremity aneurysms, and culture negative endocarditis. Deaths are rare except with serious underlying sites of infection or delayed treatment. Unlike in dogs, treatment of human *B. canis* infections has been associated with elimination of the organism (6).

Infected dogs pose a threat to other dogs for an average of 12 months. Males, even after treatment and recovery, can often remain carriers of the pathogen. Females, after spontaneous termination of pregnancy or abortion, continue to spread bacterial secretions for months (1).

The prevalence of canine brucellosis varies according to the animal's age, housing conditions, breed and geographic location. Pet dogs in suburban environments have a lower prevalence compared with stray dogs. However, infection with *B. canis* poses a serious threat to the breeding of dogs which are producing pups for the pet trade. Additionally, the disease can be spread to other areas due to the transport of infected dogs to new locations. It is generally assumed that countries with a large stray dog population have a higher prevalence of infection, since stray dogs can contribute to distribution and retention of this organism in dog populations (7, 8, 9, 10).

Brucella canis infection was first reported from the United State in 1966-1969 (6). *B. canis* appears to be widely distributed, and has been reported in North, Central and South America, Canada and parts of Asia, Africa and Europe. (1). The disease is considered endemic in Southern USA, in Central and South America and in Mexico (11, 12, 13). Numerous reports of infections with *B. canis* also originate from Asia (China, Japan, India) and Africa (South Africa, Nigeria, Zimbabwe) (14, 15, 16). It is rare in Australia and does not occur in New Zealand (17, 18, 19). In Germany, *B. canis* was reported in 1976 in a colony of Beagle dogs (20). Rare cases originate also from other European countries, such as Sweden (21, 22), the United Kingdom (23, 24), Austria (25), Italy (26), and Hungary (27).

This article documents the first serological, bacteriological and molecular demonstration of *Brucella canis* in dogs Israel.

MATERIALS AND METHODS

Epidemiological investigation

Visits to private breeding dogs' holdings and kennels and examinations of suspected dogs were carried out by the staff members of the Kimron Veterinary Institute (KIV) and the Israeli Veterinary Services.

Serological testing

Blood was collected from the cephalic vein and after clotting was separated to obtain serum, which was held at 4°C before assay.

Screening test for antibodies to *B. canis* were carried out using the *Brucella canis* Immunocomb Antibody test kit (Biogal, Kibbutz Galed, Israel). Confirmatory tests were performed with the Canine Brucella antibody test kit (D-TEC, CB, 2ME-RSAT, Zoetis).

The rapid slide agglutination test (RSAT) for the diagnosis of *Brucella canis* 2ME-RSAT was performed on all samples as follows: 25 µL of serum were mixed with 25 µL 2-mercaptoethanol followed by 50 µL *B. canis* total antigen produced from the M-strain (a less mucoid *B. canis* strain used as antigen for serological diagnosis of canine brucellosis), mixed and kept in an orbital shaker for two minutes. An agglutination viewer was used to determine presence of agglutination. The test was positive when agglutination was observed.

Furthermore, positive and suspected samples were sent to the USDA reference laboratory for confirmation. For this purpose, the agar gel immunodiffusion test (AGID) was used. AGID is considered gold standard serologic test currently available (28). This test identifies antibodies against cytoplasmic antigens of *B. canis*, which are highly specific to the *Brucella spp*.

Furthermore, dogs' serum samples were also tested at the KVI using Rose Bengal Test (RBT), Complement-Fixation (CFT) and Agglutination (SAT) Tests for *B. melitensis/ abortus* antigens**.**

Bacterial culture

Smears were taken from the vaginal discharges, aborted material and stained with Stamp's modified Ziehl–Neelsen (28).

Samples were collected from blood, aborted tissues, vaginal discharges and from male semen. The samples were collected in TSA using aseptic technique and shipped at 4°C to the laboratory (KVI).

Traditional gold standard diagnostic test for B. canis were carried out after culture in TSA medium with and without antibiotic. After the isolation of bacteria, definitive tests were carried out and included TSA with fuchsin, TSA with thionine and chemical assays (28).

The isolates were identified at the genus level by conventional microbiological methods and bio-typed, as previously described based on requirement of CO_2 for growth, urease activity, production of H_2S, sensitivity to the penicillin, streptomycin, growth on fuchsin and thionin dyes, Tb and

Iz phage typing, agglutination pattern with mono-specific anti-A and anti-M sera (28).

Molecular testing

DNA isolation was prepared from canine blood samples vaginal swabs and abortion material using the DNeasy blood and tissue kit (QIAGEN Inc., Valencia, CA, USA), following the manufacturer's instructions. PCR confirmation of *B. canis* was performed as described previously (29).

CASE REPORTS

First case

During 2019, three reports concerning aborting bitches were reported to the Israeli Veterinary Services, suspicious of canine Brucellosis. All cases were reported from northern Israel.

The first report was received from a dog breeding facility in February 2019 concerning an aborting Yorkshire terrier bitch during her third trimester of pregnancy. Blood samples were collected from the bitch and sent to the KVI. The sample were found to be positive for antibodies to *B. canis* using Biogal ImmunoComb, RSAT 2ME and AGID serological tests.

No abortion material or placentas were sent for further testing. The Yorkshire terrier female died a few days after the abortion. The female was not sent to the KVI for post mortem examination.

During the epidemiological investigation, it was found that the female Yorkshire terrier had been mated with a Yorkshire terrier from an individual living in the neighborhood of the breeding facility.

In April 2019, a King Charles spaniel female, which was also kept in the same breeding facility, miscarried during her third trimester of pregnancy. All the puppies were born dead. The King Charles spaniel male which had mated her was held at the facility. At the time of mating he was one year and four months old. He was brought to the breeding farm as a puppy. It was not known whether he was born in Israel or was imported from abroad.

Until these abortions of the Yorkshire Terrier and the King Charles Spaniel at the breeding facility, no other miscarriages or other abortions were reported. Serum was collected from nine dogs at the facility and all serological results were negative.

Later a reminder was sent to the kennel for follow-up investigation. At this time the dogs had disappeared from the facility without any further explanations and therefore no additional examinations were carried out.

Second case

Brucella canis in a multi-dog household in Alma village

In September 2019, a client with a number of dogs from Alma village, situated in the Upper Galilee in Northern Israel, visited a private veterinary clinic with a Pomeranian bitch following an abortion. At that time, the veterinarian aware of the recent abortions reported in bitches, caused by *Brucella canis*, advised the owner to send samples to the governmental laboratory, but the owner refused.

The veterinarian reported the case to the regional veterinary officer. An agriculture police team together with a government veterinarian visited the owner in his home. There they found six Pomeranian bitches; one of which had a microchip from abroad. Another two had Israeli microchips and the remaining three had not been microchipped at all. The dog which had been presented to the clinic was absent. Furthermore, there were no male at the owner's home.

Samples (serum and vaginal swabs) were taken from all six bitches. The imported bitch was positive and the other two microchipped bitches were found to be suspicious. The remaining bitches were negative to *B. canis*. Serological tests on the three female dogs found one to be positive and two were suspect. No Brucellae bacteria were isolated from the vaginal swabs collected.

The microchipped bitches were all euthanized. The three negative bitches were sterilized, administered antibiotic treatment and adopted by new owners. One of them was tested three months later and found to be negative to *B. canis.*

Third case

In November 2019, a third report was received from the city of Carmiel in Northern Israel from a multi-dog household, where five dogs (one male and four females) were kept. The KVI received two aborted pups due to suspicion that brucellosis might have been the cause of the abortion. The female was 2 year old and had aborted during her 6th week of pregnancy.

The epidemiological investigation revealed that about 6 months previously, one of their bitches had aborted. The pups were taken to a local clinic, however due to the state of their decomposition, the pups were not examined nor taken

for further investigation. After the abortion in November, the owner, who had previously worked as a veterinary assistant, decided to take all their remaining dogs to a private veterinary clinic where samples were collected from all the dogs and sent to the KVI. The following samples were collected:

Serum samples from five dogs
Sperm ejaculate from the male dog
A vaginal swab from the aborting female.
Details of the dogs were as follows:

Name	Date of Birth	Breed	Gender	Other details
Bubochika	March 2017	Mongrel	Female	Aborted in July 2019
Mai	April 2019	Chihuahua	Female	Daughter of Niki and Shoshana
Niki	Unknown	Chihuahua	Male	Mated with Shoshana
Shoshana	May 2017	Chihuahua	Female	Aborted 12 November 2019
Sherry	April 2013	Chihuahua	Female	No additional information

According to the owner of the five dogs, all were purchased together. The owners did not recall when the purchase was carried out nor any details concerning the vendor. In April 2019, the male dog mated with Sherry, which whelped two pups, one male and one female. The pups were sold to two different buyers of unknown location. To the best knowledge of the owners, Niki did not mate with any bitches outside of their home since his purchase.

RESULTS

Visit to the home where the dogs were kept in Carmiel

The flat was situated on the fourth floor of a building where the dogs were kept together. The dogs appeared to be in a good physical condition. Further blood samples were collected from all the dogs and a vaginal swab was prepared from the female which had aborted. All dogs were treated with antibiotics by protocol and were later sterilized.

Results of bacteriological tests for Brucella from aborted material

All 5 dogs from the apartment mentioned prior, were tested for serology and bacteriology. A smear taken from the aborted material and stained with Ziehl-Neelsen was found to be presumptively suspicious for *Brucella species* by microscopic examination. *Brucellae* bacteria were isolated from fetal tissues, the vaginal swab (collected two weeks after abortion) and blood from one female canine, Shoshana.

Results of the antibody tests from the sera of the five dogs for *Brucella canis* antibodies were as follow:

Name of the dog	Positivity for *B. canis* antibodies
Bubochika	Suspicious
Mai	Suspicious
Niki	Suspicious
Shoshana	Strongly positive
Sherry	Suspicious

Serologically, all dogs were negative in the rose Bengal, complement-fixation and agglutination tests with *B. melitensis/abortus* antigens.

The examination of the samples using PCR demonstrated the presence of *Brucella canis*.

DISCUSSION

This report documents the first outbreaks of canine *Brucella canis* in Israel, occurring in the north of Israel. The diagnosis was confirmed using serological, bacteriological and molecular methods. Until this time, there have been no guidelines in Israel for veterinarians concerning brucellosis in dogs. Therefore, there was considered an urgent need for improved coordination between professional bodies and document clear guidelines concerning the management of single cases or outbreaks of canine brucellosis, taking into account the current epizootic situation and urban conditions.

An effective vaccine against canine brucellosis has until now not been developed (30). Infected dogs are isolated and sterilized or euthanized. There are currently no effective treatments for brucellosis in dogs. This disease is considered incurable. Conservative treatment includes symptomatic therapy and long term strategies of antibiotics (31).

The lack of targeted measures to control and combat brucellosis contributes to the spread of this threatening disease among dogs of various breeds, especially in kennels, and also poses a certain zoonotic danger to humans (5).

Existing diagnostic methods allow an accurate diagnosis of brucellosis with an individual and integrated approach to the patient. However, the lack of the necessary knowledge and experience, combined with a lack of awareness of veterinarians, owners and breeders of dogs, often leads to the

failure to identify this pathogen as a cause of reproductive failure (1). Puppies from a litter of a bitch infected with brucellosis or puppies purchased from nurseries that are suspected/positive to brucellosis, should be tested for the disease, even if the puppy appear healthy, since in young animals, the manifestation of clinical signs of infection are usually noted only after puberty (4).

The outbreak described here was limited to northern Israel. The exact origin of the infection was not determined but was possibly associated with a single female which may have been imported into Israel from overseas or with multiple introductions. It was hoped that the extent of the outbreak could be limited by taking the necessary measures described in this document and by renewing the vigilance and awareness of Israeli veterinarians.

For prevention, it is necessary:

1. To diagnose brucellosis in all dogs at risk, such as dogs in group housing, with an unknown anamnesis or when genitourinary inflammatory signs and to encourage sample delivery for both serological and bacteriological diagnosis in cases of canine abortions or infertility.
2. Before mating, it is imperative to examine the male and the female for brucellosis.
3. Investigate dogs with any deviations in reproductive function.
4. Observe the basic sanitary and hygienic standards for both group and individual dogs.
5. Isolate and exclude from breeding all dogs infected with *B. canis*.
6. Brucella-positive dogs should not be mated, even artificially.

At the time of writing this article a widespread incidence of *B. canis* has been reported. In order to control this contagion, the following document has been circulated to all veterinarians.

Translation of some highlights appearing in the Circular regarding canine brucellosis, distributed to veterinarians in Israel

In the case of suspicion of a dog infected with *Brucella canis*, based on clinical signs of disease, the following samples must be sent to the Kimron Veterinary Institute (KVI):

- Serum
- The aborted fetus and where possible the placenta
- Vaginal swabs of the suspected females
- Full blood in EDTA for PCR examination

Treatment and Prevention

Sterilization of the male or female dog reduces the chances of infection by the bacteria, *B. canis*. Thus, if a dog is found positive to *B. canis*, sterilization must be carried out immediately together with treatment with antibiotics. A combination of tetracycline and a quinolone or aminoglycoside is recommended, for 6-8 weeks. As the bacteria is intracellular, treatment must include an antibiotic that is active intracellularly. Even under these circumstances it is still possible that the therapy will not eliminate the bacteria completely in all cases.

In addition, the dog must be examined serologically every three months, until two consecutive negative tests are attained. The consulting veterinarian must explain to the client the importance of personal hygiene while caring for the dog.

The consulting veterinarian must report the case to the relevant official municipal veterinarian and must follow-up the dog prior to the approaching examinations. Furthermore, the consulting veterinarian must report the case to the Head of the relevant Regional Veterinary Department.

Dogs infected with *B. canis* can be transferred to a new family. The new adopting family must be made aware that the dog has been diagnosed with canine brucellosis and the must be instructed to follow the presence of the dog's Brucella antibodies every three months, until the findings show two consecutive negative results. In the case where the owners move to another regional veterinary zone, the municipality veterinarian of the new dwelling must be informed.

Change of ownership of a dog diagnosed with *Brucella canis* must be approved by the municipal veterinarian.

Prevention of infection by *B. canis* in dog kennels

Infected dogs need to be completely separated from other healthy dogs and if possible removed from the kennel. Periodic serological examinations of all dogs need to be undertaken until negative results are obtained for two consecutive serological examinations carried out with an interval of 3 months between them. The kennels need to be disinfected often and in particular after every parturition or abortion.

Aspects of public health

Brucellosis is zoonotic. Humans can be infected with these bacteria in spite of the fact that humans are relatively re-

sistant. Especially people experiencing immunodeficiency coming into contact with vaginal discharges associated with parturition, infected semen or urine, may become infected, as these fluids contain the highest concentration of the organism.

All veterinarians are required, in the case of any suspicion of brucellosis in dogs, and especially in case of abortions to submit a report to the local Regional Veterinary Department and in addition to collect and dispatch samples to the Kimron Veterinary Institute for further testing.

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