Brucella melitensis Infection in Sheep-dogs in IRAN*

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Summary

The results of a survey on natural Brucella melitensis *infection in sheep-dogs in Iran is presented. Serum sample from one hundred and twelve dogs belonging to eleven sheep flocks with* Brucella *infection were prepared and tested. Samples were tested by RBPT, SAT and 2-MET. Four samples (3.57%) showed laboratory evidence of* Brucella *infection.*

At autopsy, B. melitensis *biovar 1 was isolated from the dogs that had shown positive serological responses.* Brucella *was isolated from retropharyngeal and mesenteric lymph nodes as well as the spleen and the liver. This is the first time that* B. melitensis *infection in dogs is reported in Iran. The possible role of dogs in maintaining foci of* Brucella *infection in sheep flocks is speculated.*

Introduction

Canine brucellosis, that is caused by *Brucella* other than *B. canis,* is increasingly gaining importance as a significant source of disease for other animals and human beings. Infection of dogs with these organisms usually occurs through contact with or ingestion of infected foetal membranes or aborted foetuses.

Brucella melitensis infection in the dog has been recorded, relatively, infrequently. Dogs seem to be resistant to brucellosis, as the infection is not persistent and rarely culminates in clinical signs. Nonetheless, there are

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reports from many countries that pregnant bitches have aborted their foetuses due to infection by *B. melitensis* as well as *B. abortus* or *B. suis* (Morse *et al.*, 1953; Schwarz, 1954; Ehrlein *et al.*, 1963; Phillipon *et al.*, 1969; Taylor *et al.*, 1975; Bicknel *et al.*, 1976; Srinlvasan *et al.*, 1992). Furthermore, polyarthritis, undulant fever and muscle stiffness with isolation of the microorganism from the blood and urine have been described in male dogs (McErlean, 1966; Cleg and Rorrison, 1968; Hall, 1974). Also, symptomless infections in male farm dogs with isolation of the organism from the spleen, after death, have been recorded (Prior, 1976; Bicknell and Bell, 1979). Therefore, canine infection can potentially be a source of disease for animals and persons.

The possibilities of brucellosis being transmitted, from dogs to other animals and human beings, has been considered by many investigators; a few cases can be found in the literature. Roux (1991) assumed that sheep-dogs with urinary excretion of *B. melitensis* biovar 2 were responsible for the infection of many sheep flocks in north of France and Switzerland. A bitch that had aborted was responsible for brucellosis in a man who previously assisted cleaning the uterus of the dog (Nenzani, 1935). Ostertag *et al.* (1958) reported brucellosis, in 2 human beings in North Wurtemberg, that was traced to a dog that had been kept in a *Brucella* infected sheep flock. Numerous isolations of *B. melitensis* were made from dogs that had shown positive reaction to one or more serological tests (Ostertaga and Mayer, 1958). Nicoletti *et al.* (1967) suspected the source of a *B. suis* infection, in a housewife in Massachusetts, to be a dog that had aborted. Dargein and Plazy (1922) concluded that brucellosis was contracted by 7 naval officers who in their quarters commonly owned a dog that had aborted her foetuses. Also, according to McCullough (1964) a man became infected by a dog which was ill with fever and orchitis.

The present communication reports on serological and bacteriological results in sheep-dogs tested for *Brucella* infection.

Materials and methods

Serum samples: A total of 112 samples were collected from dogs of 11 farms with the history of *B. melitensis* infection. The serum samples were tested on the same day of collection or stored at 4°C and tested within 2-3 days from the collection day.

Serological tests: 1)Rose Bengal plate test (RBPT). The antigen was prepared and standardised at Razi Vaccine and Serum Research Institute (RVSRI) according to the method recommended by Alton *et al.* (1988). The test was carried out by mixing 0.03 ml serum with 0.03 ml antigen on a plastic tray, reading the results after shaking on a shaking machine for 4 min at room

temperature. 2) Serum agglutination test (SAT). The antigen for the SAT was prepared at RVSRI according to the international standards. Serial serum dilutions, from 1:10 to 1:640, were prepared using 5% saline to minimise the prozone phenomenon. A 50% or more agglutination in the 1:40 (100 i.u.) or higher serum dilution was considered a positive reaction. 3) 2-Mercaptoethanol test (2-MET). This test was performed according to the standard procedures described by Alton *et al.* (1975) and Brinley *et al.* (1978). A 50% or more agglutination in the 1:20 or higher serum dilution was considered positive.

Bacteriology: Four dogs, which showed positive reaction to serological tests, were killed by euthanasia. Both pairs of the parotid, submaxillary, retropharyngeal, prescapular, iliac, mediastinal, inguinal, and mesenteric lymph nodes as well as specimens from the liver, lungs, kidneys, spleen and testicles were sent to the Brucellosis Department of RVSRI. Each lymph node and other specimens were cultured on 3-4 plates of serum dextrose agar with antibiotics, and all plates were incubated at 37°C in an ordinary incubator. These were checked, after 4 to 7 days of incubation, for *Brucella* colonies. Subcultures of *Brucella* isolates were biotyped, using techniques described by Corbel *et al.* (1978).

Typing of biovars: Biovar typing was carried out by tests of sensitivity to dyes, failure to produce H_2S, monospecific serum agglutination and lack of Tb phage sensitivity (Corbel *et al.*, 1978).

Results

The results of serological tests in 112 sheep-dogs from 11 *Brucella* infected sheep flocks are summarised in Table 1. The proportion of positive reactions to RBPT (20.53%) was higher than to SAT (16.96%) and 2-MET (6.25%). Only four dogs (3.57%) positively responded to serological tests for brucellosis. The end point titres, using SAT and 2-MET, are shown in Table 2.

Brucella was isolated from lymph nodes and other organs of the dogs with positive serum (Table 2). *Brucella* was most frequently isolated from retropharyngeal lymph nodes and, in decreasing order, from mesenteric lymph nodes, spleen and liver. The microorganisms isolated by culture were determined to be *B. melitensis* biovar 1 (the endemic biovar of Iran).

Discussion

The first report on isolation of *B. melitensis,* the causative agent of abortion in sheep and goats in Iran, dates back to many years ago (Kaveh, 1950).

Since then many review articles on brucellosis due to *B. melitensis* in sheep and goats, cattle, camels, and human beings have been published (Keyhani and Entessar, 1969; Sabaghian and Nadim, 1974; Zowghi and Ebadi, 1982; Zowghi *et al.*, 1984; Zowghi and Ebadi, 1985; Zowghi and Ebadi 1986; Zowghi and Ebadi, 1988). In the present investigation, the first report of the occurrence of *B. melitensis* Biovar 1 in sheep-dogs in Iran is given.

Brucellosis has been diagnosed in dogs in many countries and there are many reports on natural and experimental *B. abortus* infection in this animal (Forbes, 1990; Scalan *et al.*, 1989), but infection of dogs with *B. melitensis* is rare (Islamov, 1973; Galiro *et al.*, 1991). In Iran, sheep and goats are the principal farm animals, and *B. melitensis* is common in many areas of the country. Hence, the occurrence of *B. melitensis* in sheep-dogs is not surprising.

Table 1-*The results of serological tests in 112 sheep-dogs*

Test	RBPT		SAT			2-MET		
Titres	-	+	-	<1:40	>1:40	-	< i:20	>1:20
Total sera tested	89	23	93	15	4	105	3	4
Positive sera	23		19			7		
%	20.53		16.96			6.25		

Table 2 *The end point of sera of four dogs tested for brucellosis*

No.	RBPT	SAT	2-MET	Culture
1	+	1:40	1:20	+
2	+	1:40	1:20	+
3	+	1:40	1:40	+
4	+	1:40	1:40	+

The circumstantial evidence suggested that the dogs had become infected by ingesting aborted foetuses or placentas from *Brucella*-infected sheep. The possibilities of transmission of the disease from dogs to farm animals as well as the risk to human health have been considered by many workers. Direct contact with infective material is well recognised to be a source of infection (Henderson and Hill, 1972) and children, in particular, could be in danger when handling an infected dog, especially one that appeared healthy. Therefore, to eliminate this risk and for better fulfilment of eradication programmes it is proposed that: Dogs in *Brucella*-infected sheep and goat flocks be tested by serological tests, at least once a year, and the reactors be destroyed. The movement of animals should be controlled by appropriate regulations. Infected animals, including dogs, must be killed and other animals be vaccinated.

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