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Brucellosis outbreak in an organized dairy farm involving cows and in contact human beings, in Himachal Pradesh, India

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ABSTRACT

Brucellosis is an important disease of livestock species and wild animals, as well as a significant health hazard in contact human beings, causing a variety of reproductive disorders in cattle under intensive farming. This report is about an outbreak of brucellosis in an organized dairy farm, leading to abortions, retained placenta and stillbirths in cows. The *Brucella abortus* biotype-I was isolated from placentas, uterine discharges, vaginal swabs and foetal stomach contents collected from infected animals. The serological study, employing rose bengal plate test (RBPT) and serum agglutination test (SAT), revealed involvement of both *B. abortus* and *B. melitensis* in all affected cows. Seropositive cases for *B. melitensis* were also found among 10% of contact animal handlers, having a history of human brucellosis-like symptoms. The isolates were found sensitive to streptomycin, chlortetracycline, ciprofloxacin, ampicillin, tetracycline and gentamicin. The haematological study revealed severe monocytosis averaging 33% and lymphocytosis averaging 32.8% in all infected animals.

Key words: abortion, *Brucella abortus* biotype-I, *Brucella melitensis,* cows, human beings

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Introduction

Brucellosis is caused by different species of *Brucella* and is an important disease of livestock species and wild animals, as well as a significant health hazard when they are in contact with human beings. In organized dairy farming, reproductive disorders, viz. infertility, retained placenta, abortions and endometritis, etc. are the main impediments to profitability. Various infectious and non-infectious etiological agents are the cause of such maladies. A careful scrutiny of literature revealed that brucellosis has been recognized, both by isolation and serological studies, as one of the most serious diseases among different livestock species as well as animal handlers in the past few decades, in Himachal Pradesh in particular and in India in general (KATARIA and VERMA, 1969; KULSHERSHTHA et. al. 1978; SHARMA et al., 1979; CHAKRABORTY and KWATRA, 1980; MANICHAM and MOHAN, 1987; KATOCH et al., 1996; VERMA et al., 2000). This paper deals with a severe outbreak of brucellosis in an organized dairy farm as well as its transmission to in-contact animal handlers.

Materials and methods

There was sudden outbreak of abortions followed by retained placenta among cows, aged from 5 to 9 years, between 2 to 5 lactations, at the last trimester of gestation, as well as an increase in cases of retained placenta with apparently normal calvings, in a dairy herd of about 290 animals (Jersey and Jersindh i.e. Jersey × Redsindhi). This outbreak lasted for four weeks and a total of 24 pregnant cows in the farm at the time of outbreak were affected. The in-contact animal handlers also reported that they had suffered symptoms resembling human brucellosis/Malta fever. Samples were collected from five aborted cows and from other cows with retained placenta. The samples included items of placenta, vaginal swabs, samples from aborted foetus (abomasal contents, heart blood, peritoneal cavity fluid) as well as paired serum samples from affected animals. Serum samples were also collected from 20 persons who were in contact with the affected animals. Isolation was attempted by inoculation of morbid materials/swabs on 8% sheep blood agar and enriched Brucella agar plates. These plates were incubated both aerobically and micro-aerobically in a candle jar at 37 °C for 4 to 7 days. The isolates were initially identified on

the basis of cultural, morphological characteristics, along with biochemical features (BUCHANON and GIBBONS, 1974). The serum samples were screened by rose bengal plate test (RBPT) and serum agglutination test (SAT) employing standard procedures. The isolates were biotyped at the Division of Public Health, Indian Veterinary Research Institute (IVRI), lzatnagar, India. Antimicrobial drug susceptibility profile was conducted employing disc diffusion test. Blood samples of the affected animals were also taken for haematological studies.

Results

In this outbreak, of 24 affected pregnant cows only 6 (25%) cows aborted, 17 (53.13%) cows suffered from retained placenta with normal calves, and one (4.17%) cow delivered a dead calf. After processing the samples *Brucella abortus* biotype-I was isolated from the morbid materials from all cows. The isolation results are shown in Table 1.

Table 1. Animal and sample-wise isolation findings

Cow N^{o}	Breed	Gestation lenght*	Sample processed	Isolation
1119	Jersey	7 month	Placenta Uterine discharge	*B. abortus* biotype-I Nil
1191	Jersindh	8 month	Uterine discharge	*B. abortus* biotype-I
3037	Jersindh	7 month	Placenta Uterine discharge Foetal lung, liver, spleen Foetal stomach contents Foetal peritoneal cavity fluid	*B. abortus*** *B. abortus* biotype-I Nil *B. abortus* biotype-I Nil
3178	Jersindh	7 month	Uterine discharge Placenta	*B. abortus***
3228	Jersey	8 month	Uterine discharge	*B. abortus***

* At the time of abortion
** Could not be biotyped

The animal and human sera samples were screened by SAT and RBPT for the presence of antibodies against *B. abortus* and *B. melitensis* using the respective antigens procedure at the Central Research Institute, Kasauli, Himachal Pradesh, India. The results of these tests are shown in Tables 2 and 3.

Table 2. Serological evidence of brucellosis among aborted cows

Cow N^o	Seropositive by RBPT		Seropositive by SAT			
			B. abortus titre		*B. melitensis* titre	
	I	II	I	II	I	II
1119	+	+	1:80	>1:5120	Nil	1:160
1136	-	+	1:20	1:640	Nil	1:160
1191	-	+	1:40	>1:5120	Nil	1:40
3037	+	+	1:80	>1:5248	1:10	1:160
3178	-	+	1:20	1:2560	1:40	1:40
3228	+	+	1:40	1:640	1:10	1:20

I - Serum samples collected at the time of abortion
II - Serum samples collected after 21 days of abortion

Table 3. Serological evidence of brucellosis among animal handlers

N^o of sera samples screened		RBPT	N^o of sera samples showing SAT titres						% age positive
			Nil	1:10	1:20	1: 40	1:80	1:160	
B. abortus	20	One ± (doubtful)	19	1	-	-	-	-	nil
B. melitensis	20	-	15	1	2	1	1	-	10*

* Titre of 1:40 and above was taken as positive

The drug sensitivity profile of the isolates revealed their sensitivity to streptomycin, chlortetracycline, ciprofloxacin, ampicillin, tetracycline and gentamicin. The haematological study revealed severe monocytosis averaging 33% (ranging from 16 to 46%) and lymphocytosis averaging 32.8% (ranging from 18 to 43%) in all animals. However, neutrophils were 26.6%, ranging from 18 to 36% in the infected animals.

Discussion

In reproduction-related problems, the significance of specific infectious agents such as *Brucella* spp., *Leptospira* spp., *Listeria monocytogenes, Campylobacter* spp., *Chlamydia psittaci, Mycoplasma* spp., and *Salmonella* spp. has been established beyond doubt. However, the impact of non-specific infectious causes is influenced by several perpetuating factors

(RADOSTITS el al., 1994). Brucellosis is a disease of economic importance as it adversely affects the reproductive and productive potential of the affected animals in terms of loss of calves, infertility, as well as reduction or complete loss of milk yield after the abortion. However, AKHTAR and MIRZA (1995) reported that the progeny of suffering animals might catch the infection from affected parents, although the rate of seroconversion among such progenies is not significant among cattle. In cattle, the involvement of different biotypes of *B. melitensis* and *B. abortus* has been reported, as the host specificity in different *Brucella* species is not very strict (MUSTAFA and CORBEL, 1988; ERDOGAN et at., 1993).

Earlier, in Himachal Pradesh VERMA et al. (2000) reported the involvement of *B. abortus* biotype-III based on isolation and monitoring serological response in abortions of cows in Himachal Pradesh. NAGAL et al. (1994) ascribed the association of *B. melitensis* biotype-III in abortion among cows, in the same dairy herd both by isolation and serological titres. However, on this occasion, by isolation studies *B. abortus* biotype-I was established as the etiologic agent for the current outbreak. Surprisingly, the serological studies evidenced *B. melitensis* infection along with *B. abortus.* However, isolation of *B. melitensis* was not successful. This can be attributed to the fastidious nature of the organism, and the isolation of *Brucella* is influenced by several factors, such as a lesser number of viable organisms, putrefaction, overgrowth of contaminants, fastidious nature of organism, etc. Similarly to this investigation, in the southern states of India, RAMANATHA and GOPAL (1992) have also reported abortion in a cow due to *B. abortus* biotype-I.

Indeed, the different species of *Brucellae* are not strictly host specific, but *Brucellae* are potentially highly pathogenic and insidious in nature and a human health hazard, causing a variety of disease syndromes varying from symptomless carrier stage to undulant fever manifesting symptoms, viz. anorexia, nocturnal perspiration, malaise, depression, fatigue, loss of body weight and muscle aches etc. The bacteria persist in individuals, affecting heart and brain tissues. In this investigation, of 20 in-contact dairy handlers having a history of brucellosis-like symptoms, only two samples were positive for antibodies against *B. melitensis.* However, the number of seroreactors for *B. abortus* and *B. melitensis* were higher, i.e.

5% (1/20) and 25% (5/20), respectively. The major route of transmission in human beings is ingestion of contaminated, raw, unpasteurised milk from victim cows, as well as contact with infected aerosols or aborted materials from *Brucella*-infected cows (SUDERSHAN et al., 1996; ACEDO et al., 1997). There are reports of brucellosis among in-contact humans both from India and abroad (MRUNALINI and RAMASASTRY, 1999; EL-ANSARI et al., 2001) and seropositivity was adjudged to the extent of 15.86% and 1.0%, respectively. The prevention of human infection is mainly dependent upon control and elimination of animal infection.

In the haematological study, the increase in number of monocytes may be attributed to the presence of tissue debris in the uterus, as natural uterine cleaning is hampered owing to retention of placenta in brucellosis, the monocytes acting as scavengers in such cases (BENJAMIN, 1995). The isolates were sensitive to certain antibiotics but it is emphasized that brucellosis can be controlled only by efficient management practices. Treatment of affected animals is not cost effective and is usually not recommended unless the animal has a high pedigree. From this investigation it was concluded that the main etiology behind the reported outbreak of abortions and other reproductive disorders among cows was *B. abortus* biotype-I. The seroevidence of association of *B. melitensis* among affected animals and in-contact human beings was revealed but could not be positively established due to the previous history of a *B. melitensis* outbreak at the same farms, as well as other factors related to the isolation of this fastidious organism.

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SAŽETAK

Bruceloza je važna bolest domaćih i divljih životinja, koja predstavlja prijetnju za zdravlje ljudi u dodiru s inficiranim životinjama. Očituje se različitim reprodukcijskim poremećajima. Opisana je pojava bruceloze na farmi mliječnih krava, gdje je uzrokovala pobačaje, zaostajanje posteljice i mrtvorođenja. *Brucella abortus* biotip I bila je izdvojena iz posteljica, maternicnih iscjedaka i vaginalnih obrisaka te sadržaja sirišta pobačenih plodova. Serološkim pretragama pomoću aglutinacijskog i serum aglutinacijskog testa u zaraženih životinja bile su dokazane vrste *B. abortus* i *B. melitensis*. Serološki pozitivni nalazi za *B. melitensis* dokazani su u 10% osoba koje su bile u dodiru sa životinjama, a u anamnezi je bilo ustanovljeno da su imale kliničke znakove slične brucelozi. Izdvojene brucele bile su osjetljive na streptomicin, klortetraciklin, ciprofloksacin, ampicilin, tetraciklin i gentamicin. Hematološkom pretragom dokazana je monocitoza u 33% te limfocitozu u 32,8% svih inficiranih životinja.

Ključne riječi: pobačaj, *Brucella abortus* biotip I, *Brucella melitensis*, krave, ljudi