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## Brucellosis in Organized Dairy Farm: An Investigation

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### ABSTRACT

Brucellosis is an important zoonotic disease of animals and man causing different reproductive problems like abortion, retention of placenta, repeat breeding, still birth and many more. In this report, authors described an investigation of brucellosis outbreak in an organized dairy farm, Agra, India causing abortions and other reproductive disorders. Blood and environmental samples including feed and water samples were collected in sterile containers. About 80% sera samples collected from aborted animals and 60% sera samples from pregnant and in-contact animals, maintained at the dairy farm contained antibodies against *Brucella* spp., while all the bulls were found negative for presence of anti-*Brucella* antibodies. *Escherichia coli* and *Streptococcus* spp., isolates were recovered from water samples, while *Staphylococcus*, *Streptococcus* spp. and *Pseudomonas* spp. could be isolated from feed samples. In the present investigation, high prevalence of anti-*Brucella* antibodies was found in sera samples of the animals, indicates that they all had an exposure to *Brucella* spp. which might be the probable reason of abortion storm. Thus, on the basis of our findings, it can be deduced that *Brucella* might be associated with abortion in dairy animals even without apparent septicaemia or any other manifestations. The presence of anti-*Brucella* antibodies in sera samples of animals can be used as an indicator of infection in farm animals.

**Key words:** Animal, abortion, brucellosis, outbreak

### INTRODUCTION

Reproductive disorders particularly abortion and repeat breeding in animals are caused by various etiological agents such as *Brucella* spp., Infectious Bovine Rhinotrachitis, Bovine viral diarrhoea virus, *Leptospira* and *Listeria* spp., *Campylobacter* spp. etc (Refai, 2002; Renukaradhya *et al.*, 2002; Chahota *et al.*, 2003; Smits and Kadri, 2005; Kumar *et al.*, 2009; Dhama *et al.*, 2013; Rathore *et al.*, 2013; Verma *et al.*, 2014). Besides these some other bacteria like *Staphylococcus*, *Escherichia coli* and other pathogenic bacteria and protozoa like *Neospora* may also cause abortion in the pregnant cattle either alone or in concert with other pathogens (Van Wuijckhuise and Otten, 2008; Njiro *et al.*, 2011; Blumer *et al.*, 2011). Among all these, *Brucella abortus* is considered as the most common cause of abortion and subsequently considerable economic losses to dairy industry. Brucellosis was first time reported in India in Anonymous (1918) and since then it has been reported in many states of the country and its incidence and prevalence is increasing day by day due to increase in trade and movement of livestock (Renukaradhya *et al.*, 2002; Kumar *et al.*, 2009). According to an estimate, the annual losses due to brucellosis are

Rs 350 million per year in India (Tiwari *et al.*, 2013). Contaminated feed and water, close contact, unhygienic management practices are some of the important sources of infection for terrestrial animals (PD-ADMAS, 2011). *Brucella* can survive and remain infective for several months in water, aborted foetus and foetal membranes, faeces and liquid manure, organic material, dust and soil on buildings, equipment and clothes (Alton, 1985; FAO/WHO, 1986). This article deals with an outbreak of brucellosis in an organized dairy farm, Agra, Uttar Pradesh, India as well as its transmission.

## MATERIALS AND METHODS

**Study area:** The incident occurred in an organized dairy farm at Agra, Uttar Pradesh, India. Climatic conditions of this area is humid subtropical with dry winter type, annual rainfall (650-1000 mm), relative humidity (20-50%), annual temperature (11.0 to 36.9°C).

**Animal and management:** The animal population at the farm during the incident was 806 of different age and physiological status (Table 1). The herd were kept in groups according to their physiological stage. The animals are kept on stall feeding and fed with wheat straw along with concentrate and mineral mixture. They had free access to water sources (water troughs). Animals were vaccinated against pasteurellosis with *Pasteurella multocida* biotype A vaccine (Biological Product Section, Badshabagh, Lucknow) and against foot-and-mouth disease. The hygiene and sanitation conditions at the farm were very poor or unsatisfactory (Fig. 1a-d).

**History, clinical examination and data collection:** Abortions started in the month of March, 2013 and going on with an average of 5-10 abortions per month in dairy farm. All the animals were clinically examined by the experts from the university. The health and basic record books of the herd, compiled by veterinary and animal care staff, were also examined and analysed for occurrence of the disease, morbidity and mortality etc.

**Sample collection:** Besides, blood samples from aborted, pregnant animals and from bulls were also collected aseptically and transported on ice to the laboratory. Feed and water samples were analysed for presence of contamination. Sera from all the blood samples were separated and stored at 20°C, while clots were processed for the isolation of bacteria. Environmental samples from the dairy farm including water (from water troughs) and feed samples were collected and brought in sterile containers on ice.

Table 1: Distribution of animals according to species and physiological status

Type (Physiological status)	No. of animals	
	Cattle	Buffalo
Breeding bull	7	2
Milch animals	138	16
Dry/pregnant	122	20
Heifers	67	36
Female calf (<0.5 year)	58	1
Female calf (0.5-1 year)	40	10
Female calf (1 year)	120	12
Male calf (<0.5 year)	46	4
Male calf (0.5-1 year)	12	4
Male calf (1 year)	68	11
Total	689	117



Fig. 1(a-d): Poor hygienic conditions at the farm

**Laboratory examination:** Sera samples were tested for presence of anti-*Brucella* antibodies using Rose Bengal Plate Test as per the method described by Alton *et al.* (1975). Briefly, the test sera and Rose-Bengal antigen were kept for one hour at room temperature before the beginning of the test. A result was considered positive when there was any degree of agglutination noticeable and the absence of agglutination was considered as negative. Blood clots were processed for bacteriological examination by incubating the samples at 37°C onto nutrient agar, MacConkey agar and 5% sheep blood agar plates and examined after 24-48 h. Environmental samples from the dairy farm including water and feed samples were processed for microbiological examination as per the method described by Cruickshank *et al.* (1975).

## RESULTS AND DISCUSSION

A total of 16 sera samples from aborted animals (5), pregnant animals (5) and bulls (6) were processed for diagnosis of *Brucella* infection using RBPT. Out of these, 80.00% of aborted animals (4/5) and 60% of pregnant animals (3/5) were positive for brucellosis infection, while all the bulls were found negative. *Escherichia coli* and *Streptococcus* spp. isolates were recovered from water samples, while *Staphylococcus* spp., *Streptococcus* spp. and *Pseudomonas* spp. could be isolated from feed samples.

Reproductive-related problems in animals may be due to various infectious diseases of bacterial origin (brucellosis, salmonellosis, campylobacteriosis, leptospirosis, mycoplasmosis and listeriosis etc)

and viral origin (Infectious Bovine Rhinotrachitis and *Bovine herpes virus-1* (BHV-1) leading to major threats to dairy industry (Chahota *et al.*, 2003; Kumar *et al.*, 2009; Njiro *et al.*, 2011; Murray, 2012; Watson *et al.*, 2012; Verma *et al.*, 2014). Although, a number of different bacteria have been associated with abortion in dairy animals. In the present investigation, high prevalence of anti-*Brucella* antibodies was found in sera samples of the animals, indicates that they all had an exposure to *Brucella* spp. and which might be the probable reason of abortion storm. This observation is in concurrence with earlier study (Chahota *et al.*, 2003) revealing involvement of *Brucella* spp. in all affected cows using serological tests.

In the present study, water available to animals was heavily contaminated ( $10^5$  CFU mL<sup>-1</sup>) particularly with faeces such as faecal coliforms as count was much higher (80/100 mL<sup>-1</sup>) than the maximum permissible limits (10/100 mL<sup>-1</sup>) in raw drinking water of livestock of dairy receiving disinfection only (Warrington, 2001). It seems that heavily contaminated water might also helped in transmission of infection to farm animals, eventually leading to abortions. Treatment of affected animals for brucellosis is not cost effective so it can be controlled by adopting the prevention and control strategies including screening of animals, segregation of positive animals and vaccination of healthy animals etc (OIE, 2008).

## CONCLUSION

From this investigation, it can be deduced that *Brucella* might be associated with abortion in dairy animals even without apparent septicaemia or any other manifestations. The presence of anti-*Brucella* antibodies in sera samples of animals can be used as an indicator of infection in farm animals. Being a zoonotic disease, all the protective and preventive measures should be applied for its control in animals as well as human beings. On the basis of above facts along with availability of suitable vaccine and diagnostics, a control programme for this disease should be started as early as possible.

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