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Etiology of reproductive disorders in an organized cattle dairy farm: A case study on leptospirosis and brucellosis

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Abstract
Reproductive disorders and abortions in dairy cattle are generally caused by various infectious agents. Ruling out of the actual etiological agent (s) is an important step towards prevention by designing the suitable control measures. The present study was conducted in a cattle dairy farm which was associated with history of suffering from reproductive disorders and abortions. The study was focused on the two main infectious abortifacient agents *viz*., *Leptospira* and *Brucella* in the infected dairy farm. During the investigation, a total of 68 serum samples were collected and examined for detection of anti-leptospiral antibodies using the Microscopic Agglutination Test (MAT), the gold standard serological test and Rose Bengal Plate Test for detection of anti-brucella antibodies for the diagnosis of these diseases, respectively. The Chi-square test was employed to identify the important risk factors associated with exposure to brucellosis and leptospirosis. The observed seropositivity was 4.4% and 11.8% for brucellosis and leptospirosis respectively. Further, the reproductive disorder status of the animal was significantly (<0.0001) associated with exposure to brucellosis and leptospirosis in a dairy cattle farm. The prevalent predominant serovars observed were Tarsalis, Djasmine, Hebdomadis, Icterohaemorrhagiae, and Grippotyphosa. The present results suggest that, the *Leptospira* and *Brucella* were the causative agents for the reproductive disorders and these zoonotic agents are circulating in a dairy cattle farm and it warrants to design of suitable control measures to prevent the entry and elimination of these pathogens in the farm.

Keywords: Bovine leptospirosis, brucellosis, reproductive disorders, MAT RBPT

Introduction
Reproductive disorders (infertility, retained placenta, abortions, early embryonic death and anoestrum) of animals are one of the main reasons, that curtail the profitability of organized cattle dairy farms. Various etiological agents like viruses, bacteria, and fungi are causing abortions in dairy cattle. Out of them, *Brucella* and *Leptospira* are the bacteria that play a vital role in the development of reproductive disorders in dairy cattle, (Mariya *et al*., 2006 and Menamvar *et al*., 2022) [7, 30]. Generally, Brucellosis and Leptospirosis are neglected zoonotic diseases globally and are often under-reported and not controlled in low-middle-income countries (Balamurugan *et al*., 2021) [6]. Brucellosis is caused by many species of the genus *Brucella*. Out of them, the *B. abortus* and *B. melitensis* are more frequently infecting bovines (Lindahl *et al*., 2019) [23]. Leptospirosis is caused by pathogenic species of the genus *Leptospira* (Ellis, 2015) [13]. The clinical manifestations are ranging from mild to severe forms of infections. Calves up to one month of age are generally affected with the acute form, which is characterized by high fever, petechiation of the mucosa, acute hemolytic anemia, jaundice and haemoglobinuria (Radostits *et al.*, 2000; Prajapati *et al.*, 2018) [39, 38]. In adults, infection with *L. interrogans* serovar Hardjo and Pomona especially in lactating animals results in mastitis with a sudden drop in milk production and blood clots in milk (Thiermann, 1983) [47]. Abortions, stillbirth, premature birth of the weak calf, congenital infection and early embryonic death may occur in pregnant animals (Radostits *et al.*, 2000) [39]. Generally, infection with Pomona is associated with a 'storm' of abortions during the second half of gestation (Howard, 1993; Daniel, 2006) [16, 12]. Most of the time, leptospiruria may be present in up to 30 percent of affected cows without any changes in udder or milk and these animals act as the source of infection for other healthy animals.

In India, the human population stands at greater risk of acquiring these zoonotic diseases because 80% of the Indian population depends on livestock as a source of income and they are

maintaining close contact with the livestock. Hence the prevention and control of these zoonotic diseases are very much crucial and depend on effective surveillance and proper diagnosis. The gold standard for diagnosis of brucellosis is isolation but it requires Biosafety level 3 facilities and is tedious, time-consuming, and imposes a risk of human infection. The best alternative method of diagnosis is by using serological techniques. These are safe, less time-consuming and can be conducted in resource-limited laboratory settings. The present study aims to rule out the etiology of reproductive disorders and abortions occurring in an organized cattle dairy farm located at Korutla, Jagtial, Telangana, India.

Material and Methods

The present study was conducted in a cattle dairy farm associated with the complaint of abortions and other reproductive disorders. The farm was located in Korutla, Jagtial district of Telangana state, India. The farm is following a semi-intensive system of rearing. Newborn calves were maintained with mothers, with no separate room for milking. Breeding is achieved by artificial insemination. Bulls were managed separately and not used for natural insemination through mating. Cattle were allowed for grazing in fenced areas around the farm during day time and kept inside the farm during the night. Sanitary measures and health care of animals are monitored by a local veterinary practitioner.

A total of 68 blood samples were collected from a dairy farm. After clotting, the samples were subjected to centrifugation (1500 rpm for 10 min) for the separation of serum. The sera were collected from both male and female animals and animals with different age groups. The collected samples represented the apparently healthy animals and animals with reproductive disorders (Abortions, early embryonic death and anoestrum). The sera were labelled and stored at -20 ºC in the Microbiology Department, College of Veterinary Science, Korutla until further use. The sample was subjected to RBPT and MAT to detect the presence of antibodies against *Brucella* and *Leptospira* serovars organisms.

RBPT

This test is recommended by the WoAH as a suitable screening test for individual and herd levels (OIE 2008) [35]. In the present study, a modified version of WoAH recommended protocol was followed (Blasco 1994 and Lucchese 2016) [10, 25]. The Rose Bengal Plate Agglutination Antigen was obtained from the Institute of Animal Health and Veterinary Biologicals, Hebbal, Bengaluru. The test was conducted by placing 0.03mL (one drop) of test serum on a clean, greasy-free slide with the help of a micropipette. The antigen bottle was shaken to ensure homogenous suspension, 0.03 mL of (one drop) antigen was taken and then added to the test serum on the slide. The antigen and test serum were mixed with a spreader and then the slide was manually rotated for three min. The test was examined for agglutination in bright light. Any degree of agglutination was taken as positive and no agglutination was taken as negative. Data generated from laboratory investigations were recorded and coded using Microsoft® Excel 2016 for Windows 2010.

MAT

MAT is a gold standard test for the diagnosis of leptospirosis (OIE 2008) [35]. The serum samples were transported on ice to the National Institute on Veterinary Epidemiology and Disease Informatics (NIVEDI). The serum samples were screened by MAT in Leptospira Research Laboratory, ICAR-NIVEDI using 18 live *Leptospira* serovars cultures covering the 16 serogroups (Balamurugan *et al*., 2018) [5]. Briefly, the antigen and antibody interaction were examined under the dark-field microscope at 200 magnifications for observing the agglutination reactions. The endpoint (titer) was taken as that dilution which gives 50% agglutination, leaving 50% of the cells free when compared with antigen control (no agglutination should be seen in the antigen control) was considered positive at ≥1:100 dilutions. Data generated from laboratory investigations were recorded and coded using Microsoft® Excel-2016 for Windows 2010.

Results

The observed seropositivity was 4.4% and 11.8% for brucellosis and leptospirosis respectively. Further health status of the animal was significantly (<0.001) associated with exposure to brucellosis and leptospirosis in a dairy cattle farm. The prevalent predominant serovars observed were Tarsalis, Djasmine, Hebdomadis, Icterohaemorrhagiae, and Grippotyphosa.

Out of eight positive samples, six sera showed agglutination against a single serovar (5 samples – Tarsalis and 1 sample – Grippotyphosa, and two serum samples showed cross-reactivity with more than one serovar). The seropositivity of bovine leptospirosis was 12.07% (7/58) in females which is higher than in males (10% (1/10)). Further host factors (gender and age) were not significantly (p=0.7) associated with the exposure of leptospirosis. The observed seropositivity in the animals with >2 years of age was 13.73% (7/51) and 5.88% in the animals with < 2 years of age. The seropositivity of 77.8% was observed in animals with history of reproductive disorders and antibodies against *Leptospira* serovars were not detected in apparently healthy animals. Additionally, the reproductive disorder status of the animal was significantly (P=0.00001) associated with exposure to leptospirosis (Table 1). The seropositivity of brucellosis in females was 5.17% (3/58), whereas antibodies against *Brucella* organism were not detected in male. The seropositivity of brucellosis was 5.88% (3/51) in animals of > 2 years of age. Further, no antibody against Brucella organism was detected in animals of < 2 years of age. 22.3% of seropositivity was observed in animals with reproductive disorders compared with 1.69% in apparently healthy animals. Further, the occurrence of bovine brucellosis was significantly (p=0.01) associated with the presence of animals with reproductive disorders (Table 2) and other animal-level host factors like age and sex were not significantly associated with the exposure of brucellosis in the farm.

Table 1: Univariate analysis of animal level risk factors associated with the exposure of leptospirosis in cattle

Variables	Total no. of samples	Number of samples Positive for leptospirosis	Percentage of positivity (%)	Chi-square	P value*
Age					
>2 years	51	7	13.73	0.12	0.72
<2 years	17	1	5.88		
Sex					
Female	58	7	12.07	0.132	0.71
Male	10	1	10		
Symptoms					
Apparently healthy	59	1	1.69	19.49	0.00001
Reproductive problems	9	7	77.8		

Table 2: Univariate analysis of animal-level risk factors associated with the exposure of brucellosis in cattle

Variables	Total no. of samples	Positive for leptospirosis	Percentage of positivity (%)	Chi-square	P value*
Age					
>2 years	51	3	5.88	-	-
<2 years	17	0	0		
Sex					
Female	58	3	5.17	-	-
Male	10	0	0		
Symptoms					
Apparently healthy	59	1	1.69	6.2	0.01
Reproductive problems	9	2	22.22		

Discussion

In India, dairy farming is one of the important sources of income for farmers. Brucellosis and leptospirosis cause high economic losses to the farmer not only due to abortions but also due to the repeat inseminations and birth of weak calves. *Brucella* is one of the major causative agents of abortions and reproductive disorders in a dairy farm (Anderson ML *et al*., 1990) [2]. The seropositivity of brucellosis by RBPT in the present study is 5.17% (3/58), which is concurrent with the similar observation (6.20%) noticed by Trangadia *et al*., 2010 [49] while screening the dairy farm from the western region of India and similarly Ramesh *et al*., 2013 observed 8.57% of positivity in an organized dairy farm in Uttarakhand. On the contrary higher prevalence of brucellosis was observed in an organized dairy farm in southern (30.48%), central (42.31%), and northern regions (17.07%) of India (Trangadia *et al*., 2009) [49] and 45% of prevalence was observed in south India (Sarangi *et al*., 2021) [44].

A higher seroprevalence of brucellosis in female animals in the present study was supported by various studies (Suresh *et al.*, 1993; Asfaw *et al*., 1998; Anuradha and Ganesan 2006; Muma *et al.*, 2007; Tolosa *et. al*, 2008; Bayemi *et al.*, 2009 and Islam *et al*., 2013) [46, 4, 3, 33, 48, 9, 18]. Generally, male and female animals are equally susceptible to brucellosis, the differences observed may be due to the low sample size (only 10 males were tested in the present study), as the farmer is opting artificial insemination breeding method. Another aspect is erythritol, a polyhydric acid found in higher concentration in the placenta and foetal fluids of females than in seminal vesicles and testis of males can be responsible for females being more susceptible than males (Radostits *et al.*, 2000) [39]. Females are mostly sent out for grazing in free-range pastures due to the absence of handling problems compared to males, frequent mixing with unknown herds and flocks of sheep and goats (Renukaradhya *et al.*, 2002) [42] might have contributed to higher prevalence

The observed seropositivity of brucellosis in > 2 years of age was not significantly associated and is supported by different researchers (Kumar *et al*., 2016, and Sanogo *et al*., 2012) [21, 43] and Age is known as one of the intrinsic factors influencing brucellosis seropositivity (Megersa *et al*., 2011) [29]. This may be not only due to the presence of maternal antibodies, which decreases as age advances but also due to the higher likelihood of contact with infected animals happening with aged animals (Sanogo *et al*., 2012) [43]. Brucellosis is commonly associated with sexual maturity than with age (Radostits *et al*., 2000) [39]. However, Kazi *et al*. (2005) [20] reported that the high prevalence of brucellosis among old animals might be related to maturity with advancing age, thereby the organism may have propagated to remain as a latent infection or it may cause disease.

The reproductive disorder status of the animal is significantly associated with the occurrence of brucellosis on the farm. Similar results were also found in Haryana and Punjab (Chand and Chhabra, 2013) [11]; in South India (Sarangi *et al*., 2021) [44], in Pakistan (Ali *et al*., 2017) [1], in Uganda and Kenya (Magona *et al*., 2009; Muendo E *et al*., 2012) [26, 32]. Reproductive disorders like metritis and abortions were also reported from *Brucella* sero-positive farms in Zimbabwe (Hussain *et al*., 2008; Matope *et al*., 2011) [17, 28]. Improper handling and disposal of postpartum material may aggravate the brucellosis incidence in the farm. The absence of separation of infected animals from healthy animals would have further contributed to the brucellosis prevalence in the farm (Sarangi *et al*., 2021) [44].

Leptospirosis is a globally important bacterial zoonosis caused by pathogenic bacterial species of the genus *Leptospira* (Fernandes *et al*., 2014; Faine *et al*., 1999) [15, 14] affecting humans and a wide range of animals worldwide. In the present study, 11.8% of seropositivity was observed and the prevalent predominant serovars detected were Tarsalis, Djasmine, Hebdomadis, Icterohaemorrhagiae, Grippotyphosa. The present study results were in agreement with previous studies (Rani *et al*., 2013 and Sonali Menamvar *et al*., 2022) [41, 30] and in concordance with the results of Sritharan 2012 [45], who reported 36.4% of seroprevalence in Telangana. This could be due to variations in the sample size and sample source. In the present study, samples were collected from an organized dairy farm where efficient management practices were conserved to control the disease spread.

Further univariate analysis revealed that age and gender were not significantly associated with the exposure of leptospirosis in dairy cattle. These results were in agreement with earlier reports (Sonali Menamvar *et al*., 2022; Balamurugan *et al*., 2013 and 2016; Jain *et al*., 2019) [30, 8, 7, 19]. Further, the reproductive problem's status of the animal is significantly associated with the exposure of leptospirosis in bovines in the studied cattle dairy farm. Moori *et al*., 1967 [31] reported 7.7% of prevalence in cattle dairy farms presented with reproductive disorders. Similarly Oliveira *et al*., 2021 [36] revealed that highly reactive herds presented 14% more embryonic death than the herds with low seroreactivity against leptospirosis. Libonati *et al*., 2018 [22] reported that estrus repetition in dairy, which is one of the most important reproductive disorders was strongly associated presence of *Leptospira* infection. The observation was similar to the present study results. In bovine leptospirosis reproductive disorders like embryonic loss and estrus repetition were arises due to the presence of leptospirae in the uterus and this changes the uterine environment, which disturbs the estrus cycle and also negatively affects the implantation and survival of the embryo and further *Leptospira* invade the embryo directly which severely damage the embryo and results in the death of the embryo (Loureiro and Lilenbaum 2020 and Orjuela *et al*., 2022) [24, 37].

In conclusion, the present results suggest that *Leptospira* and *Brucella* were the causative agents along with other possible organisms for the reproductive disorders and these zoonotic agents are circulating in a dairy cattle farm and it warrants to design of suitable control measures to prevent the entry and elimination of these pathogens in the farm.

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Conflict of interest

The authors have declared no conflict of interest

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