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Research Article

PREVALENCE OF TUBERCULOSIS, PARATUBERCULOSIS AND BRUCELLOSIS IN ORGANIZED LIVESTOCK FARMS OF TAMIL NADU

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ABSTRACT

Tuberculosis, paratuberculosis and brucellosis are most important diseases affecting livestock and causing severe socioeconomic losses in India. The present study is aimed to determine the prevalence of brucellosis, tuberculosis in cattle, buffaloes, goats and sheep in organized livestock farms of Tamil Nadu, India. Our screening results showed that brucellosis was found 3.13 % positivity in cattle and buffaloes and 100% negativity in sheep and goats. Tuberculosis was found 0.11%, 8.1%and 0.16% positivity in cattle and buffaloes, horses, sheep and goat, respectively. In case of Paratuberculosis, 1.76 % and 2.89% and positivity were recorded in cattle, buffaloes and sheep and goat, respectively in organized livestock farms of Tamil Nadu, India. Since brucellosis, tuberculosis and paratuberculosis are considered under emerging and remerging disease list so continuous periodical screening of livestock needed to prevent spread of those infections and implementation of vaccination policy in high prevalence area.

Keywords: Cattle sheep and Goat, Tuberculosis, Paratuberculosis, Brucellosis.

INTRODUCTION

Tuberculosis, paratuberculosis and brucellosis are the significant zoonotic diseases cause's major public health threats with a great socio-economic losses to the formers and lead to reduced milk and meat production and low reproduction rate due to abortion and loss of draft power, which have a negative effect on income of the farmers (Smith *et al*., 2006). These diseases have been eradicated or controlled in developed countries but remain prevalent in many developing countries where livestock farming plays a significant role in food safety and economic development (Sadiq *et al*., 2013). The *Mycobacterium bovis*, *Mycobacterim paratuberclosis* and *brucella* sp. cause clinically significant diseases that affect livestock industries in developing countries. One of the important factors that increase the risk of zoonotic diseases is the un-diagnosis of infected animals, transportation, failure to separation of affected animals and culling. These animals live in close contact with human and other domestic animals leads to highest prevalence rates among the animals and human (Griffith, 1928, Khan *et al.*, 2009).

Tuberculosis is one of the most significant livestock diseases in the world with huge annual loss of 3 billion dollars in the field of agriculture for implementing control programmes (Garcia *et al.*, 2015). Bovine tuberculosis is a chronic granulomatous inflammatory disease that is predominantly caused by *Mycobacterium bovis* and the pathogen has a broad host range including human and e *M. bovis* causes 10% of the total human TB cases in developing countries and have significant threat to global health (Olea-Popelka *et al*., 2014). Bovine tuberculosis is usually diagnosed based on delayed hypersensitivity reactions using various tuberculin tests such as single intradermal test and other molecular and serological test. Approximately more than 50 million cattle are infected with tuberculosis in the world (Fend *et al*., 2005). The most effective strategy for the control of bovine

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tuberculosis requires identification and removal of the infected animal from the herd. Paratuberculosis is caused by *Mycobacterium avium* sub sp. *paratuberculosis* (MAP) and it is one of the most widespread bacterial diseases infecting a wide range of animal species including ruminants in developed and developing countries (Lavers *et al.*, 2013). The paratuberculosis is considered as globally important bacterial disease and categorized as a List B transmissible disease by the Office International des Epizooties (OIE). The disease is spread by ingestion from contaminated environment, feeding of pooled colostrum containing viable bacteria to calves or contamination of the pasture with infected animal feces. The *Mycobacterium avium* subsp. *paratuberculosis* is the potential source of infection into human being and cause public health problem. These organisms have the ability to survive pasteurization temperature and transmission to human through raw milk, meat and close contact with affected animals.

India has largest livestock numbers in the world. The epidemiological investigation of brucellosis generally depends upon the sero-prevalence studies. Brucellosis, especially caused by *Brucella melitensis* is considered as one of the most common zoonotic diseases worldwide and more than 500000 human cases was reported every year. Brucellosis occurs worldwide in both animals and humans. Bovine brucellosis is endemic in all the states of India and continuously increasing due to increased trade and rapid movement of livestock from one area to another area. The current management practices and herd structure also predispose the endemic brucellosis. This study was designed to assess the prevalence of tuberculosis, paratuberculosis and brucellosis in livestock farms of Tamil Nadu which are the major livestock farming-related zoonotic diseases in India (Naeem *et al.*, 1990; Pappas *et al.*, 2006; Taleski *et al.*, 2002, Sadiq *et al*., 2013).

MATERIALS AND METHODS

This study was undertaken in organized livestock farms of Tamil Nadu (Table - 1). Samples were collected from cattle, buffalo, sheep and goats, horses of different breeds including the cross bred animals and both male and female sex from unvaccinated animals. The antigen for intradermal test and brucellosis antigen and reference samples were procured from Division of Biological Products, ICAR-Indian Veterinary Research Institute, Izatnagar Nagar, Bareilly (U.P.). The Central University Laboratory, TANUVAS have both Institutional Biosafety Committee approval as well as Animal Ethical Committee approval (Approval number: 24/SA/IAEC/2022) to handle pathogenic organism for diagnosis of field samples. The samples were collected as swabs from nasal discharge for tuberculosis besides faecal sample and rectal pinch for paratuberculosis diagnosis. Moreover, for diagnosis of brucellosis, milk samples were collected for Abortus Bang Ring Test (ABRT) and serum samples for Rose Bengal Plate Agglutination Test (RBPT) and Serum Tube agglutination Test (STAT). The samples were collected from different age groups and both the sexes and blood was taken from suspected animals randomly. In the laboratory, serum was separated by centrifugation at 1500 rpm for 15 minutes from clotted blood and stored in refrigerator at 4^{o}C until laboratory tests were performed. Milk samples were individually collected in sterile tubes and nasal discharge was taken on cotton swabs and kept dipped in tubes containing saline solution. All the samples were kept refrigerated at 4°C until processed.

Smears were made from nasal discharges, rectal pinch and subjected to Acid Fast Staining for the detection of Acid Fast organisms (Schaeffer and Fulton 1933). Single intra dermal Tuberculin Test, and Johnin Test were performed for tuberculosis and paratuberculosis in live animals as per the procedures described by Dacso (1990) with slight modification. The test involved the intradermal injection of bovine tuberculin in the neck region and in some animal caudal fold area and the immune response were measured 3 days later. In the injection site, 12-15 cm from the top of the neck was shaved without inflicting wound. Skin thickness was measured by using caliper. Using a McLintock syringe, 0.1 ml of PPD was injected into the sites. A correct injection was confirmed small palpable swelling at each site of injection. The skin-fold thickness was re-measured after 72 hours post injection. When the difference was more than 4 mm between by 72 hours post injection and 0 hour the animal was declared a reactor (figure.1,2,3,4,5, & 6). The same protocol was followed for intra dermal Johnin test. The serum samples collected from animals were subjected to RBPT and SAT to detect Anti-brucella antibodies based on the method described by Morgan *et al*., (1969) with slight modification. The positive reactions revealed agglutination (granulation) and the negative reactions showed a cloudy suspension without agglutination. The highest degree of dilution of serum in a tube showing agglutination was the titer. The milk samples were subjected to ABRT based on the methods described by Alton *et al*. (1998).

RESULTS AND DISCUSSION

Prevalence of Tuberculosis was 0.11% in cattle and buffaloes (1/851), 0.16% in sheep and goats (3/1829); and 8.11% in horses (3/37). In case of paratuberculosis, 1.76% in cattle and buffaloes (15/851); and 2.89% in sheep and goats (53/1829). In the present study,3.13% of the total samples (30/957) in cattle and buffaloes and 0% in sheep and goat (0/2448) were found positive for anti-brucella antibodies by using RBPT and (3/2448) ABRT. The overall prevalence of tuberculosis, paratuberculosis and brucellosis was 0.25%, 2.5%, 3.13% positivity in cattle and buffaloes and 100% negativity in sheep and goat in organized livestock farms of Tamil Nadu in the present study. The current study reports very low prevalence of tuberculosis, paratuberculosis and brucellosis in cattle, buffaloes, sheep, goats and horses due to periodical screening and culling of affected animals in organized livestock farms. Mukherjee (2006) reported a prevalence of tuberculosis in two dairy

herds at 15.76% in Northern India and 0.65–1.85% in Western India.

Thakur *et al.* (2016) recorded 14.31% (overall animal prevalence) and 16.67% (farm prevalence) of tuberculosis in Himachal Pradesh. In southern states of India such as Tamil Nadu and Karnataka, the rate of prevalence was 34.58% and 30–35% respectively (Filia *et al.*, 2016; Javad *et al.*, 2006). The ELISA may be used as a complement to the tuberculin test especially for anergic tuberculosis cattle (Sayin and Erganis, 2013). Brucellosis in horses, donkey and mules mainly causes abscess in tendon, bursae and joints. Abortion and other reproductive disorders are very rare in both male and female horses. Mixed farming and joint breeding of horse, cattle and pig was found as main risk factor for equine brucellosis (Dorneles *et al*., 2023). Thakur *et al* (2016) screened 541 milk samples against tuberculosis and they found 71 (13.12%) animals were positive. The mycobacteria sp. Were isolated from 3 (4.22%) lymph node aspirates but not from any cultured milk and blood samples. Twenty eight numbers (39.43%) of lymph node aspirate and 5 numbers of (9.25%) milk samples were positive for *Mycobacterium* tuberculosis complex (MTC) by PCR amplification but blood samples from suspected animals were found to be negative. They used multiplex-PCR for species differentiation and they found out of 28 lymph node aspirate, *Mycobacterium bovis* was detected in 18 (64.28%) and MTB in 8 (28.57%), whereas 2 aspirate samples (7.14%) were positive for both the species and they found all five milk MTB positive samples were positive for *M. bovis.*

Table 1. Prevalence of Tuberculosis, Paratuberculosis and Brucellosis in organized livestock farms, in Tamil Nadu.

S.No	Name of farms/source	TB No. of positive/total number of animals tested Intra dermal skin test/acid fast staining			JD No. of positive/total number of animals Intra dermal skin test/acid fast staining			BRUCELLOSIS No. of positive/total number of animals STAT&RBPT		
		Cattle/ Buffaloes	Horses	Sheep and goats	Cattle/ buffaloes	Horses	Sheep and goats	Cattle/ buffaloes	Horses	Sheep and goats
1	MSRS, Pottaneri	0	0	3/449	0	0	18/449	0	0	0/449
2	Animal quarantine and certification services	0	3/37	0	0	0	0	0	0	0
3	KCRS, Sathiyamangalam	0/93	0	0	0/93	0	0	0/93	0	0
4	RRC, Pudukottai	0	0	0/87	0	0	2/87	0	0	0/99
5	PGRIAS, Kattuppakkam	30/68	0	0/292	4/68	0	2/292	0/42	0	0/292
6	SBRS,OOTY	0	0	0/1001	0	0	31/1001	0	0	0/1001
7	Burgur Cattle Research station, Burgur	1/108	0	0	3/108	0	0	0/108	0	0
8	ILFC,MMC	0/33	0	0	0/33	0	0	0	0	0
9	PCRS Manamadurai	0/53	0	0	0/53	0	0	30/30	0	0
10	GOSALA Tiruvallur	0/65	0	0	7/65	0	0	0/140	0	0
11	Private farm, Thenkasi	0	0	0	0	0	0	0/45	0	0
12	Animal health camps, Villupuram Vellore, Tiruvannamalai, Kanchipuram	0/484	0	0	1/484	0	0	0/499	0	0/499
13	Total	1/851	3/37	3/1829	15/851	0	53/1829	30/957	0	0/2448
14	Positive Percentage	0.11%	8.1%	0.16%	1.76%		2.89%	3.13%	0	0

Table 2. Prevalence of tuberculosis in cattle, buffaloes, horses, sheep and goats in organized livestock farms in Tamil Nadu.

S.No	Species	Number of samples tested	Number of sample positive	Positive percentage (single intra dermal skin test)
1	Cattle and Buffaloes	851	1	0.11%
2	Horses	37	3	8.1%
3	Sheep and goats	1829	3	0.16%

Table 3. Prevalence of paratuberculosis in cattle, buffaloes, horses, sheep and goats in organized livestock farms of Tamil Nadu

S. No	Species	Number of samples tested	Number of sample positive	Positive percentage single intra dermal skin test)
1	Cattle and Buffaloes	851	15	1.76%
2	Sheep and goat	1829	53	2.89%

Table 4. Prevalence of brucellosis in cattle, buffaloes, sheep and goats in organized livestock farms in Tamil Nadu.

S.No	Species	Number of samples tested	Number of sample positive	Positive percentage (RBPT &STAT)
1	Cattle and Buffaloes	957	30	3.13%
2	Sheep and goat	2448	0	0%

Figure 1. Intra dermal skin test for screening TB in cattle

Figure 2. Intra dermal skin test for screening JD in cattle

Figure 3. Measurement of skin thickness before intra dermal test in sheep.

Figure 4. Measurement of skin thickness after intra dermal test in caudal fold of tail of sheep (positive for JD)

Figure 5. Intra dermal skin test for screening of JD in calf

Figure 5. Intra dermal skin test for screening of JD in buffalo.

Trangadia *et al*. (2013) reported that the maximum sero reactivity (31.25%) was found in greater than 6 years age group in all farms and sex wise and age wise similar trends were also noticed. The wide variation in prevalence of bovine tuberculosis could be due to breed type, screening tests used, management practices followed in the farming system, contact between animals and transport of animals, different herds sharing common facilities and grazing areas (Ameni *et al*., 2011). The met-analysis of bovine tuberculosis showed critical need for the development of a national surveillance programme and the implementation of an effective control strategy for bovine tuberculosis in India. The increasing cattle population and demands on milk production and an inability to cull potentially diseased cows need for a vaccine that can reduce the burden of infection and transmission. The recent reports suggest that the BCG vaccine may have considerable utility in this critical condition (Ameni *et al*., 2017). India has 300 million cattle population and also has the highest burden of tuberculosis infected animals in the world exceeding even at the lower confidence interval and the total number of dairy cattle in the United States (USDA, 2016) and *M. bovis* may not necessarily be the only causative agent of bovine tuberculosis in all reactor animals (Sweetline Anne *et al*., 2017).

The seroprevalence of JD reported in cattle and buffalo of Uttar Pradesh and Punjab states were 31.9 % and 23.3%, respectively. However, that was lower in Gujarat (13.39%), Andhra Pradesh (16.26%) and Karnataka (15.14%). The difference in prevalence pattern of JD could be due to diversity in topography and environment, animal movements, animal rearing system and husbandry practices followed in different states of India (Lall 1963, Trangadia *et al.*, 2012, Gupta *et al.*, 2012). The control and management of Johne's disease has focused mainly on dairy cattle due to the higher prevalence of disease within the dairy industry. The absence of a successful treatment and effective vaccine for JD and negative economic impacts leads to potential increase in prevalence of JD and next several years provide incentive for effective control strategies in infected herds (Lu *et al*., 2016, Garcia *et al*., 2015). Ritter *et al*. (2015) found that the choice of testing policy for the control of Johne's disease is dependent on a producer's motivation and goals for disease control. In this study the prevalence of paratuberculosis is 2.89 % in sheep and goats which indicate the important of continuous annual screening of JD in small ruminant were important.

The seroprevalence of brucellosis has been reported in various states of India. Maansi and Upadhyay (2015) have recorded 7.25 % prevalence in bovines (12.77 % in cattle and 3.55% in buffaloes) in Uttar Pradesh. The various reports from Punjab recorded as worst affected bovine population with constant presence of 11.23% overall prevalence rate (Dhand *et al.*, 2005). Aulakh *et al*., (2008) recorded 17.68% prevalence of bovine brucellosis in Punjab. Rahman *et al.* (2012) studied the seroprevalence of brucellosis in swine in Bangladesh. They found 6.7% and 4.8% were positive by RBPT and STAT respectively. High brucellosis seroprevalence rates in domestic swine herds have been reported in Wallus and Fatuna Islands. The Milk ring test and milk-ELISA conducted on the samples of the same state revealed a prevalence of 4.35% and 5.38%, respectively (Kumar, 2016) and 29.61% of prevalence in cattle and buffaloes were reported in Uttarakhand. Organized farms showed (41.30% on serological basis and 27.02% through milk tests) greater burden as compared to non-organized or small herds (4.34% on serological basis and 3.06% through milk tests as reported by Maansi and Upadhyay, 2015, Muhammad *et al.*, 2012). Mehra *et al.* (2000) reported 6.5% prevalence in cattle from organized farms, compared to 5.1% from unorganized sector. The buffaloes might show some natural resistance to brucellosis but few reports also showed higher seropositivity in buffaloes rather than cattle (Jagapura *et al*, 2013; Krishnamoorthy *et al*., 2015 and Kushwaha *et al.* 2016). Poor hygiene and management practices like improper disposal of aborted material causes rapid spread of infection from one animal to another in organized farms (Manish *et al*., 2013).

Pillai *et al.* (1991) first reported the presence of *B. canis* infection with 2.18% prevalence in Tamil Nadu, whereas the present study reports only less prevalence in cattle and buffaloes (3.13%). In case of sheep and goats, none of the animal was found positive for brucella

antibodies. This could be attributed to strict biosecurity measures and periodic screening and culling of affected animals and screening of newly purchased animals in organized farms. The current data revealed low prevalence of these diseases in organized farms in Tamil Nadu and this prevalence was lower than previously reported observations. The study was undertaken in well-organized farms where periodical screening and culling of positive animals have been undertaken. This profound reduction in occurrence of TB, Brucellosis and Johne's diseases in recent years may be associated with different measures taken against the diseases in livestock farms. However, further indepth detailed field level studies are needed with increased number of samples need to control tuberculosis, paratuberculosis and brucellosis and for implementing effective vaccination policy in high prevalent areas.

CONCLUSION

The current data revealed low prevalence of these diseases in organized farms in Tamil Nadu and this prevalence was lower than previously reported record. The study was under taken in well organized farms where periodical screening and culling of positive animals have been undertaken. This profound reduction in occurrence of TB, Brucellosis and Johne's diseases in recent years may be associated with different measures taken against the diseases in livestock farms. However further in depth detailed field level studies are needed with increased number of samples to ascertain the prevalence of Tuberculosis, Paratuberculosis and Brucellosis and also to chalk out suitable control strategies.

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