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RESEARCH ARTICLE

Monitoring the Health Status and Herd-Level Risk Factors of Tuberculosis in Water Buffalo (*Bubalus bubalis*) Dairy Farms in Pakistan

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ABSTRACT

Bovine tuberculosis is a chronic disease caused by *Mycobacterium bovis*, a bacterium that infects a wide range of animal species, including humans. In this study, we determined the prevalence of tuberculosis in domesticated water buffalo (*Bubalus bubalis*) and assessed potential risk factors at four government dairy farms in Punjab, Pakistan. Tuberculin skin testing was performed on 627 water buffaloes. Risk factors such as age, body weight, milk yield, lactation status, the total number of animals, and the presence of small ruminants at the farms were recorded due to their potential association with tuberculosis transmission. The impact of *M. bovis* infection on complete blood cell counts (CBC) was also assessed. In total, 27 (4.3%) animals were positive for tuberculosis in the four dairy farms. Mean corpuscular hemoglobin concentrations were lower in infected animals compared to non-infected animals, whereas the number of platelets was higher in infected animals compared to non-infected animals. The results revealed that the increase in body weight, age of the animals, and water buffalo density on the farm can increase the disease occurrence. Binary logistic regression analysis, including farm and other variables, revealed that the assessed herd-level risk factors of bovine tuberculosis transmission had a statistically non-significant association for monitoring water buffaloes. The current study had emphasized revealing different possible risk factors in native husbandry practices. The study suggests regular tuberculosis screening for proper control of *M. bovis* infection in water buffalo herds. This will ultimately help to further reduce the cases of zoonotic bovine tuberculosis.

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INTRODUCTION

The bovine dairy industry has gained substantial economic impact around the world, including Pakistan, and a variety of management issues, including infectious diseases, are an important threat to this industry. Therefore, proper monitoring of infectious diseases, especially zoonoses, is necessary for the protection of livestock and public health. Bovine tuberculosis (TB), caused mostly by the bacterium *Mycobacterium bovis* (OIE, 2020) is a zoonotic disease endemic in bovine herds. Bovine TB is a chronic, contagious disease that causes weakness and tubercle formation primarily in the respiratory tract but can cause granulomas virtually anywhere in the body (OIE, 2018). Bovine TB reduces the productive efficiency of infected animals up to 25% (Radostits *et al.*, 2000). The health status and livelihood of downgraded backyard dairy farmers and consumers of milk can be affected by the disease (OIE, 2018). The risk of Bovine TB is higher in developing countries, where control measures are not well implemented to manage the diseases spread. In resource-limited countries with higher bovine TB incidence, several infection risk-factors have been identified, including herd management, lack of efficient veterinary facilities, and poor surveillance systems (Bonsu *et al.,* 2000; Ravi *et al.,* 2018). Tuberculosis is transmitted from one animal to the others through colostrum/milk, ingestion of flies, feces of infected animals, aerosol, and droplets (Acha and Szyfres, 2001; Garcia, 2006). Zoonotic TB transmission to humans has been generally credited to the ingesting of contaminated animal/dairy products however through direct contact has also been reported (Sichewo *et al.,* 2020). Bovine tuberculosis is a threat to cattle and buffaloes in government-owned as well as privately-owned farms worldwide (Ali *et al.,* 2004). The prevalence of this disease is almost 4.03% in dairy herds of Spain (Francisco *et al.,* 2007), 2.71% in India (Singh *et al.,* 2004), 5.2% in southern Chile (Abbate, 2020) and around 11.7% in Pakistan (Javed *et al.,* 2012). Regarding diagnosis, polymerase chain reaction (PCR) can easily confirm the presence of *M. bovis* from nasal swabs and milk specimens (Akhtar *et al.,* 2015). However, a more widely used diagnostic tool is the Tuberculin test (delayed-type hypersensitivity reaction). The test can be completed by injecting *M. bovis* purified protein derivatives (PPD) in the skin of the cervical region. The test site is observed for swelling and thickness after 72 hours of the injection (OIE, 2018). Some other diagnostic techniques include serum immunoglobin G-test and bacterial isolation from fecal, urine, milk, and saliva samples (OIE, 2014). The disease remains a public health threat in low-income nations like Pakistan and, regrettably, due to the paucity of national bovine tuberculosis surveillance and control. Considering the socio-economic importance of bovine tuberculosis, this study aimed to investigate the epidemiology of tuberculosis based on different risk factors such as age, body weight, milk yield, lactation status, the total number of animals, and the presence of small ruminants at a water buffalo *(Bubalus bubalis)* dairy farm. The impact of *M. bovis* infection on the complete blood cell counts (CBC) in the water buffaloes was also evaluated.

MATERIALS AND METHODS

This epidemiological study was carried out among the water buffaloes (n=627) from the four government livestock farms of Punjab, Pakistan. The tuberculin skin test injection was given to the animals older than 2-years-old in the left side of their neck. The delayed-type hypersensitivity reaction was read after 72 hours of administering intradermal tuberculin injections. The measures described by Aagaard *et al.* (2003) were applied to categorize the animals as healthy or infected. A total (5 ml) of blood was collected from the jugular vein in a glass test tube containing 0.5 ml of 1% anticoagulant EDTA. These blood samples were transported to the clinical pathology laboratory, College of Veterinary and Animal Sciences, Jhang, Pakistan for further analysis. The blood samples were analyzed for hematological parameters including red blood cell (RBC) counts (10^{12}/l), hemoglobin (Hb) concentration (g/dl), packed cell volume (PCV %), white blood cell (WBC) counts (10^9/l), and differential white cell counts (lymphocyte, granulocyte) through automated hematology analyzer (Sysmex Poch-100i, Germany). This equipment runs on impedance technology with hydrodynamic focusing. Poch-65 XL reagent pack was used as an easy-to-use reagent system. Aspiration volume was set as 15µl whole blood. The data related to age, body weight, lactation status (dry, lactating), milk yield, management practices on the farms, the total number of water buffaloes, and the presence of other animals at the farm were collected by circulating the standard questionnaire to farm manager at each farm.

Statistical analysis: We performed binary logistic regression analyses. The logistic analysis was also applied using backward as well as forward elimination methods to infer the connection of diverse risk factors with tuberculosis in water buffaloes using SAS statistical software (SAS, 2007). The t-test was applied to compare the average values of hematological parameters (RBCs, WBCs, PCV, Hb, MHC, and DLC) between healthy and infected water buffaloes.

RESULTS

The results of the study revealed that out of a total of 627 water buffalos, 27 (4.3%) were found positive for TB from the four government-owned livestock farms, the prevalence varied from 3.5 to 5.0% with 100% farms having tuberculin positive animals. The distribution of water buffaloes concerning different factors regarding bovine tuberculosis revealed non-significant differences between different groups for age, body weight, milk yield, lactation status, and the total number of animals at the farm as shown in Table 1. Binary logistic regression analysis, including farm and other variables, revealed a statistically non-significant association with the disease prevalence between different groups. However, the data showed that with the increase in body weight, age of the animals, and the total number of water buffaloes on the farm, the possibility of disease occurrence can increase with (OR: 1.002, 1.056, 1), respectively. In contrast, with the presence of small ruminants at the farm and an increase in animal lactation number, there will be fewer chances of occurrence of disease (Table 2). The statistical analysis was applied to continuous variables, both with and without division into groups showed non-significant results (Table 2). Relatively, higher disease prevalence was noted in animals with heavy body mass, high producing and at farms with a stock density more than 500. The hematolo-gical parameters in healthy and infected animals (n=54) showed a non-significant difference in all parameters; however, the Mean corpuscular hemoglobin concentration (MCHC) was significantly lower, whereas the platelet count was found significantly higher in infected animals (Table 3). The information about the probability and distribution of data related to water buffalo's body weight, age, milk yield, and lactation number for the presence of bovine tuberculosis in four different government-owned livestock farms shown in Fig 1-4.

DISCUSSION

An overall prevalence of 4.3% was recorded for water-buffalo TB from the four government-owned farms as check through the intradermal tuberculin skin test. The findings of the present study reflected a slightly varying prevalence ranging from 3.45 to 4.98% between the different buffalo farms. A similar type of study was carried out in Ethiopia using a comparative tuberculin test, where the prevalence was recorded as 19 and 1.6% at, the herd and individual levels respectively. In central Ethiopia, the herd and animal level prevalence rates of disease were 58.5 and 39.3%, respectively (Tulu *et al*., 2021). In India, the data reported a prevalence of bovine tuberculosis as 51% (18/35) at the herd level and 4.1% (19/460) at the animal level (Laval and Ameni, 2004). The overall 45.6% (herd level) and 11.3% animal level disease prevalence have also been reported in selected areas of Bangladesh (Islam *et al.,* 2020). A recent study reported bTb prevalence of 20.6% in different regions of Bangladesh (Islam *et al*., 2021). In the current study certain possible risk factors, including age, body weight, the total number of animals, and lactation status of the animals were assessed. All these measured risk factors were found statistically non-significant by binary logistic regression analysis. The risk of a bovine TB occurrence is known to vary between herds and the nature of the disease is not uniform as it can be "persistent, sporadic and recurrent". In the present study, the disease was relatively more prevalent in aged animals and the chances of bovine TB may increase with the age of the animals and can be considered as a risk factor for *M. bovis* infection in water buffaloes. It has also been previously reported that *M. bovis* infection increases, as the age of the animal increases (Inangolet *et al.*, 2008; Humblet *et al.*, 2009). Similarly, Cleaveland *et al.* (2007) and Phillips *et al.* (2002) suggested that aged animals were more susceptible to tuberculosis. The results of live body weight showed that the heavier animals were more affected or the infection occurs in heavier animals and those later become weak probably due to the effect of the disease. The results revealed that due to the intensification of the herd, the probability of infection non-significantly increases. The high stock density plays a key role to increase the risk of TB transmission (Kaneene *et al*., 2002). In addition to this as the herd size increases at any premises, the animal-to-animal spread can rise due to overpopulation (Ghebremariam *et al.,* 2016). In contrast, there will be fewer chances of occurrence of disease with the presence of small ruminants at the farm. The findings are in line

Table 1: Distribution of water buffaloes concerning different risk factors regarding bovine tuberculosis (TB).

		TB Bovine tuberculosis (TB)						Chi-square df (P-value)
		Absent		Present		Total		
		Count	%	Count	%	Count	%	
Farm Name	BRI	191	31.8	10	37.0	201	32.1	0.409NS
	Dera Chal	84	14.0	3	11.1	87	13.9	df = 3
	Khushab	158	26.3	7	25.9	165	26.3	(0.938)
	R. Gulaman	167	27.8	7	25.9	174	27.8	
	Total	600	100.0	27	100.0	627	100.0	
Bodyweight								3.379NS
	300–500	263	43.8	7	25.9	270	43.1	df = 1
	>500	337	56.2	20	74.1	357	56.9	(0.066)
	Total	600	100.0	27	100.0	627	100.0	
Age group	<4 years	2	0.3	0	0.0	2	0.3	0.306NS
	4–8 years	359	59.8	15	55.6	374	59.6	df = 2
	>8 years	239	39.8	12	44.4	251	40.0	(0.858)
	Total	600	100.0	27	100.0	627	100.0	
Milk yield	<5.0	220	36.7	5	18.5	225	35.9	3.730NS
	5.1 - 6.0	167	27.8	10	37.0	177	28.2	df = 3
	6.1 - 7.0	104	17.3	6	22.2	110	17.5	(0.292)
	7.1+	109	18.2	6	22.2	115	18.3	
	Total	600	100.0	27	100.0	627	100.0	
Total Animal								1.631NS
	1-100	27	4.5	0	0.0	27	4.3	df = 3
	101-500	88	14.7	4	14.8	92	14.7	(0.652)
	>500	241	40.2	10	37.0	251	40.0	
	Total	600	100.0	27	100.0	627	100.0	
Lactation Number	<5	338	56.3	14	51.9	352	56.1	0.766NS
	5.0-10.0	221	36.8	10	37.0	231	36.8	df = 2
	>10	41	6.8	3	11.1	44	7.0	(0.682)
	Total	600	100.0	27	100.0	627	100.0	

NS = Non-significant (P>0.05); df = Degrees of freedom.

Table 2: Binomial logistic regression procedure for various parameters showing association for bovine tuberculosis

Parameter	B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I. for Exp(B)	
							Lower	Upper
Body Weight	.002	.003	.883	1	0.347	1.002	.997	1.007
Age	.055	.057	.925	1	0.336	1.056	.945	1.181
Milk yield	-.102	.347	.086	1	0.769	.903	.458	1.782
Small ruminants	.408	.432	.893	1	.345	.665	.285	1.551
Total Animal	.000	.001	.001	1	0.973	1.000	.999	1.001
Lactation Number	-.013	.073	.031	1	0.861	.987	.856	1.139
Status	-.273	.404	.456	1	0.499	.761	.345	1.680
Constant	-6.781	2.269	8.930	1	0.003	.001		

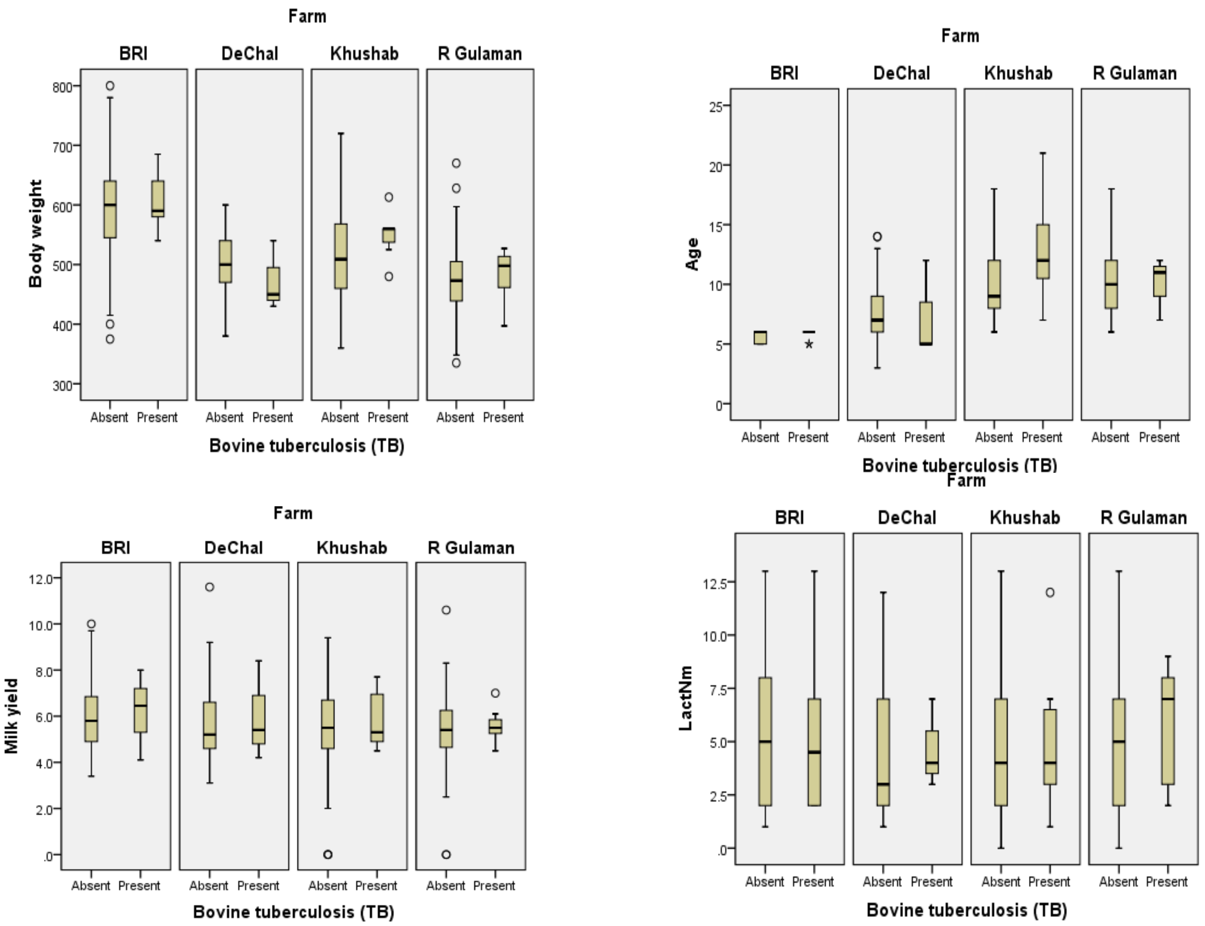

Fig 1-4: Box plots showing the distribution of data related to water buffalo's body weight, age, milk yield, and lactation number for the presence of bovine tuberculosis in four different government-owned livestock farms in Punjab, Pakistan.

with a previous study conducted by Javed *et al.* (2011). The possible reason for this may be the grazing nature of small ruminants, which served to actively reduce the bacterial load on soil and vegetation. In the present study, the MCH was found to be significantly lower, while platelets were observed to be significantly higher in infected animals, which is suggestive of chronic infection. In water buffaloes, a significantly lower hemoglobin concentration was observed in infected ones (Javed *et al.*, 2010). The possible reason for the decrease of Hb level can be the failure of iron utilization, bone marrow suppression, and chronic inflammation. Another study by Kumar *et al.* (2007) showed a decreased Hb concentration in positive reactors. In the current study, the leukocyte count was found as relatively high, but not significantly elevated. Some other studies also suggested that the mean of the TLC in TB-positive animals was either normal or non-significantly elevated in comparison to healthy/control animals (Akpan *et al.*, 2012; Shareef *et al.*, 2012). The findings of the study helped in better understanding of the disease prevalence and some possible risk factors for infection as the risk factors may operate at regional, animal, and herd-level and may diverge across areas due to differing farm management practices, farm structures, bovine TB control program, regional disease incidences, and the relative importance of specific risk factors by area. It can be concluded from the current study that all the studied farms were found positive for bovine TB with different prevalence rates. The current study provides evidence about the disease prevalence in indigenous water buffaloes and provides insight to investigate different correlated risk factors in native husbandry practices. The study suggests regular TB screening along with the identification and control of risk factors of bovine TB in the country to reduce the TB cases of water buffalo.

Author's contribution: AR and MTJ conceived the idea of research; AR collected the samples and was involved in sample testing and/or lab activity; SHE drafted the skeleton; MTJ analyzed the data; MZA, IA, and MKR prepared the manuscript; MR, TH drafted the details of the manuscript and helped in data editing, data analysis and construction of tables; AS and GHD did proofreading, added references and checked reference styling etc. All authors read and interpreted the data, critically and approved the final version.

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