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Brief communication (Original)

Certain risk factors associated with positive SCCIT test for tuberculosis in cattle at two cities in Pakistan

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Background: Bovine tuberculosis is a significant disease in animals and monitoring is important in preventing spread and transmission to man.

Objective: We identified risk factors for a positive tuberculin test (SCCIT) in cattle at Faisalabad and Okara, Pakistan.

Methods: Fifty-nine herds (230 cattle) at Faisalabad and 38 (291 cattle) at Okara were included in the study. The risk factors studied included area, farm, nature of villages, location, total cattle, presence of other animals, other species (buffalo, small ruminants, equines or pets), age, sex, live weight, calving number, lactation length, lactation status, total milk produced, and per day milk.

Results: Our results showed an association between age, live weight, calving number, lactation length, and total number of cattle at the farm with positive tuberculin test. The analysis of variance technique also showed association of all of these factors except lactation length with positive skin test. The results of logistic regression analysis also suggested an association of age, live weight, calving number, total animals at the farm, and total cattle at the farm with positive SCCIT test in cattle.

Conclusions: The prevalence of tuberculosis in cattle is around 9% at herd level and 2% at animal level with stronger risk factors being live weight, total animals, and total cattle at the farm.

Keywords: Cattle, Pakistan, risk factors, tuberculosis

Tuberculosis in animals is a disease caused by *Mycobacterium tuberculosis* complex organisms and is a zoonosis. The disease in animals is of high significance in the international trade of animals and animal by-products, and is a global problem [1]. The prevalence of tuberculosis in large ruminants in Pakistan varied from time to time and place to place. In 1974, a prevalence of 0.54% (1/186) at a Rohri private buffalo farm and 5.3% (128/2407) in buffalo in and around Quetta was recorded by tuberculin testing [2]. In 1988–89, 7.3% (11/150) of buffaloes showed a positive tuberculin test at Lahore Abattoir [3]. In 2000–1, a study conducted in and around Faisalabad using tuberculin tests revealed prevalence of 1.7% in buffalo and 5.1% in cattle [4]. In 2003, 6.9% (57/815) of buffalo at Lahore showed positive reaction to tuberculin [5]. In 2006, at two farms, 2.45 and 8.48% buffalo showed positive tuberculin tests [6]. The organism in a herd can spread through the respiratory route, which is the most common mode of transmission. The other route of infection, which is less common, may include colostrum or milk given to calves. The risk factors for tuberculosis in animals has been studied widely and include age, gender, breed, body condition, immune status, genetics of animals, herd size, type of cattle (beef/dairy), intensity of farming system and housing, manure (use of slurry), feeding, introduction of new animals in the herd, movement of animals, contact between animals, culling rate, other species at the farm, wildlife contacts, and environment/climate [7]. In Pakistan, few studies have been conducted to determine the association of risk factors with tuberculosis in buffalo. Cattle, being a different species, may differ in susceptibility from buffalo. To our knowledge, this is the first such study

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to be conducted to investigate the association of possible risk factors for tuberculosis in cattle around Faisalabad and Okara.

Methods

This study was conducted in cattle at Faisalabad and Okara, Pakistan. Faisalabad is the third largest city of the country and Okara is south of Faisalabad. Both cities have an agricultural base with a large population of dairy animals. Fifty-nine farms/herds (230 cattle) in Faisalabad and 38 herds/farms (291 cattle) in Okara were included in the study. The present study was conducted between May and July 2007. Farms with single cattle were included. However, at least two buffalo were also present at the premises. Herd size varied from three to eighty-five animals. The status of tuberculosis in cattle was determined by single comparative cervical intradermal tuberculin test (SCCIT) using bovine (50,000 IU/mL) and avian (25,000 IU/mL) PPDs produced at an authorized laboratory at the Istituto Zooprofilattico Umbria e Marche, Italy. Protocols described in the World Organization for Animal Health (OIE) Manual of Standards for diagnostic tests and vaccines were followed [8]. The risk factors studied included age, sex, breed, live weight, calving number, lactation status, lactation length, total milk produced per day, area of village, location of village (east, west, north, south), farm, combination of animals at the farm, total animals, total cattle, presence of other species, and which other species (buffalo, small ruminants, equines or pet) are also present. These risk factors related to farming practices in Pakistan. The data thus collected were analyzed by frequency analysis and logistic regression procedures after adjusting for the clustering variable, i.e., farm. The results were summarized by using a Mantel–Haenszel chi-square statistic. The odd ratios and 95% confidence limits were also determined to interpret the results [9]. Analysis of variance was used to analyze the data on age, live weight, calving number, per day milk production, lactation length, total number of cattle and total number of animals at the farm. Means were compared by Tukey's test using a general linear model procedure. Bivariate logistic regressions procedure was controlled for clustering variable (farm) and independently each variable was included in the model with result as an outcome variable. The significant results of different variables are presented in **Table 4**. The multivariate logistic regression analysis was controlled for farm + age, farm + age + breed, and farm + age + breed + calving. All these analyses revealed significant association of same factors so the results after controlling for farm + age + breed are presented in **Table 4**. The Hosmer and Lemeshow goodness of fit test was applied to test the fitness of logistic model. The *p* values obtained are indicated in **Table 4**. The data analysis was conducted on personal computers using SAS statistical software, version 9.1 [10]. This study was formally approved by the donor agency and Faculty Scrutiny and Ethical Review Committee.

Results

Crossbred and Sahiwal cattle were kept at 86% and 14% of the farms, respectively with males at 17%. Most cattle (41%) were producing between 10 and 15 liters of milk, while 23% produced between 5 and 10 liters. Fifty-nine percent of the cattle were lactating. Less than 10 animals were present at 40% of the farms, 10 to 20 animals at 37% of the farms. Less than five cattle were present at 54% of the farms and 5 to 10 cattle at 37% of the farms. Other than cattle, animals of other species were present at 99% of farms and included buffalo at 96%, pets at 44%, and small ruminants and equines at 8% of the farms.

The prevalence of tuberculosis based on SCCIT test at herd and animal levels under different conditions is presented in **Table 1**. Prevalence of 2.3% at animal level and 9.3% at herd level was noted. Prevalence of 13.8% at herd level and 2.6% at animal level was observed when herds with less than 10 animals were excluded. However, prevalence of 50% at herd level and 4% at animal level was observed when herds with less than 10 cattle were excluded. There was only one farm where cattle were the only species and all the cattle reacted negatively to SCCIT test. Similarly, no cattle reacted positively to skin test at farms where small ruminants were present. The prevalence was 2.1 times higher at animal level and 2.4 times at herd level when pets were present at the farm. However, the prevalence was 1.1 and 1.6 times at herd and animal levels, respectively when equine animals were present and the prevalence was 2.0 and 7.7 times higher at herd and animal levels, respectively when buffalo were present.

The results of bivariate frequency analysis revealed significant association between age of the cattle ($p < 0.01$), their live weight ($p < 0.005$), calving number ($p < 0.01$), lactation length ($p < 0.05$), and total number of cattle ($p < 0.01$) at the farm with

positive skin tests (**Table 2**). The prevalence was 1.3 times higher in Sahiwal and it was 2.4 times higher in Okara than Faisalabad.

Tukey's test revealed nonsignificant differences between means of SCCIT test-positive and -negative cattle for lactation length and daily milk production (**Table 3**). However, the means of live weight, age, calving number, total number of cattle at the farm, and total number of animals at the farm were significantly ($p < 0.05$) higher for SCCIT test positive than negative cattle.

The bivariate logistic analysis revealed significant association of age, live weight, calving, total cattle and total animals at the farm (**Table 4**). The multivariate logistic analysis after controlling for age + breed + farm showed a significant association between live weight, total animals and total cattle at the farm, while multivariate logistic analysis including all variables in the model with backward elimination procedure revealed significant association between live weight and total cattle at the farm.

Table 1. Prevalence of bovine tuberculosis at herd/farm level and animal level

Parameters	Herds/Farms			Cattle		
	Negative n	Positive n (%)	95% confidence limits	Negative n	Positive n (%)	95% confidence limits
Total herds/animals						
	88	9 (9.3)	4.62 to 16.34	509	12 (2.3)	1.25 to 3.88
10 or more cattle at the farm						
	7	7 (50.0)	25.13 to 74.87	192	10 (4.0)	2.54 to 8.65
10 or more buffalo + cattle at the farm						
	50	8 (13.8)	6.62 to 24.51	409	11 (2.6)	1.38 to 4.51
Other animals at the farm						
Yes	87	9 (9.4)	4.67 to 16.50	506	12 (2.3)	1.26 to 3.91
No	1	0 (0.0)	0	3	0 (0.0)	0
Small ruminants kept with Cattle						
Yes	8	0 (0.0)	0.00 to 31.23	35	0 (0.0)	0.00 to 8.20
No	80	9 (10.1)	0	474	12 (2.5)	1.34 to 4.16
Pet kept with cattle						
Yes	43	6 (12.2)	OR = 2.37	302	9 (2.9)	OR = 2.06
No	51	3 (5.6)	[reciprocal = 0.42]	207	3 (1.4)	[reciprocal = 0.49]
Equine kept with cattle						
Yes	30	4 (11.8)	OR = 1.55	247	6 (2.4)	OR = 1.06
No	58	5 (7.9)	[reciprocal = 0.65]	262	6 (2.2)	[reciprocal = 0.94]
Buffalo kept alone or with other animals at cattle farms						
No	4	0 (0.0)	0.00 to 52.71	16	0 (0.0)	0.00 to 170.75
Alone	36	3 (7.7)	1.99 to 19.52	148	3 (2.0)	0.51 to 5.31
With other animals	48	6 (11.1)	0	345	9 (2.5)	0

OR = odds ratio

Table 2. Bivariate analysis of frequencies of different parameters in cattle with positive and negative reactions to a SCCIT test

Parameters studied	Negative reactors n	Positive reactors n	%	Mantel–Haenszel chi-square
1. Factor related with cattle				
Lactating status of Cattle				
Heifer	105	0	0	$p > 0.32$
Dry	26	2	7.14	
Lactating	245	10	3.92	
Pregnant	47	0	0	
Sex				
Male	86	0	0	$p > 0.11$
Female	423	12	2.76	
Age of Cattle (years)				
<3	171	0	0	$p < 0.01$
3–6	193	5	2.53	
6.1–10	104	3	2.8	
>10	41	4	8.89	
Breeds				
Sahiwal	141	4	2.76	$p > 0.66$; OR = 1.30
Cross breed	368	8	2.13	
Weight (kg)				
<200	50	0	0	$p < 0.005$
200–299	105	0	0	
300–399	162	1	0.61	
400–499	143	10	6.54	
>500	49	1	2	
Milk Production (Litres/day)				
0	127	0	0	$p > 0.11$
1–4.9	11	0	0	
5–9.9	93	6	6.06	
10–15	171	6	3.39	
>15	21	0	0	
Number of calves produced				
<1	191	0	0	$p < 0.01$
2–3	125	6	4.58	
4–5	70	3	4.11	
>6	37	3	7.5	
Lactation length (months)				
0	113	0	0	$p < 0.05$
1–2.9	65	1	1.52	
3–6	160	8	4.76	
6.1–9	65	2	2.99	
>9	20	1	4.76	
2. Factors related with surrounding of cattle				
Location to village				
East	102	2	1.92	$p > 0.94$
North	70	3	4.11	
South	51	0	0	
West	286	7	2.39	
Total number of cattle at the farm				
<5	118	1	0.84	$p < 0.01$
5–10	247	3	1.2	
11–20	104	5	4.59	
>20	40	3	6.98	

Table 2. Bivariate analysis of frequencies of different parameters in cattle with positive and negative reactions to a SCCIT test

Parameters studied	Negative reactors n	Positive reactors n	%	Mantel–Haenszel chi-square
Total animals kept at the farm				
<10	100	1	0.99	$p > 0.06$
10–20	160	3	1.84	
21–30	102	2	1.92	
31–40	106	3	2.75	
>40	41	3	6.82	
Small ruminants				
No	474	12	2.47	$p > 0.34$
Yes	35	0	0	
Area				
Okara	282	9	3.09	$p > 0.17$; OR = 2.41
Faisalabad	227	3	1.3	
Companion animals at the farm				
Yes	506	12	2.32	$p > 0.70$
No	3	0	0	
Pet as companion animal				
Yes	302	9	2.89	$p > 0.27$; OR = 2.06
No	207	3	1.43	
Buffalo as companion animal				
No	16	0	0	$p > 0.53$
Yes	493	12	2.38	
Buffalo as companion animal				
No	16	0	0	$p > 0.50$
Alone	148	3	1.99	
With other animals	345	9	2.54	
Equine as companion animal				
Yes	247	6	2.37	$p > 0.91$; OR = 1.06
No	263	6	2.23	

Table 3. Means (±SD) and 95% confidence limits of different parameters studied in positive and negative reactor cattle

Parameters	mean	SD	95% Confidence limit Lower	Upper
Age of cattle				
Positive	7.58 A	2.78	5.82	9.35
Negative	4.77 B	2.95	4.51	5.03
Live weight of cattle (kg)				
Positive	425.00 A	39.88	399.66	450.34
Negative	331.63 B	11.58	321.91	341.35
Number of calf produced				
Positive	4.08 A	2.02	2.79	5.37
Negative	2.25 B	2.21	2.04	2.46
Daily milk produced (liter)				
Positive	9.08	2.97	7.19	10.97
Negative	7.44	5.84	6.89	8
Lactation length of cattle				
Positive	5.5	2.75	3.37	4
Negative	3.68	3.29	3.76	4.24
Total number of cattle at the farm				
Positive	14.75 A	6.28	10.76	18.74
Negative	09.18 B	6.05	8.66	9.71
Total number of animals at the farm				
Positive	33.33 A	18.16	21.79	44.87
Negative	22.82 B	14.75	21.54	24.11

Table 4. Results of logistic regression analysis

Parameters	Odds ratio	95% confidence limits		Hosmer and Lemeshow goodness of fit P
		Lower	Upper	
Bivariate logistic regression results of individual variable with result as an outcome variable				
Age of Cattle	1.268	1.090	1.476	0.6582
Live weight of cattle	1.008	1.002	1.014	0.1538
Number of calving	1.272	1.065	1.519	0.2392
Total animals at the farm	1.035	1.006	1.065	0.9274
Total Cattle at the farm	1.139	1.042	1.245	0.8761
Multivariate logistic regression results controlling for age, breed and farm with result as an outcome variable				
Live weight of cattle	1.007	1.000	1.013	0.7012
Total animals at the farm	1.035	1.002	1.069	0.7366
Total cattle at the farm	1.144	1.032	1.268	0.7533
Multivariate logistic regression results including all variables in model with result as outcome variable				
Live weight of cattle	1.009	1.002	1.016	0.8562
Total cattle at the far	1.136	1.030	1.253	

Discussion

There were 97 herds in total, among these, 96 had cattle and buffalo as dairy animals, and at one farm, only cattle were present. In Pakistan, the buffalo is the main dairy animal and cattle are the supporting dairy animals because local cattle produce less milk than buffalo. If we look at the latest scenario, the selection of dairy animals is changing and probably cattle are going to take over from buffalo as the main dairy animal. The main reason for this change can be linked with the milk production potential of crossbred cattle. This is reflected in the present data that indicated crossbred cattle were present at 86% of the farms and the local breed of cattle was present at only 14% of the farms. The results also revealed that 10 or more cattle were present only at 14 farms, while 10 or more cattle plus buffalo were present at 58 farms. This shows that in most cases, the herd size is small. This situation is quite different from the farm size in previous decades. Furthermore, it appears that the dairy industry in Pakistan is progressing at a good pace and herd size will increase in coming years [11].

The prevalence of 9.3% at herd level and 2.3% at animal level shows 5% and 0.3% lower prevalence of tuberculosis in cattle than buffalo at herd and animal levels, respectively, in the same area [11]. These values were much lower than the values from a neighboring country where 51% and 4.1% of prevalence at herd and animal level, respectively, have been reported [12]. A study from Ethiopia reported 48% and 19% prevalence at herd and animal level, respectively [13]. Similarly, 47% prevalence at herd level has been reported from Uganda [14]. One of the reasons for low herd prevalence in Pakistan can be linked to small herd size. However, 50% and 4% prevalence at herd and animal level was found when herds with less than 10 cattle were excluded, while it was 14% and 2.6%, respectively when herds with less than 10 dairy animals (cattle and buffalo) were excluded. These findings also confirm the earlier reports that the herd prevalence is higher than the prevalence at animal-level [11, 15-17]. As observed in buffalo [11], ten positive cattle were from nine positive herds, confirming the earlier point of view that the transmission of the disease within positive herds is not serious. These findings can be linked to reasons cited earlier [11] and the fact that the risk of transmission through direct contact from the infected bovine animals is low [15, 18, 19]. The management practices adopted by farmers in Pakistan as cited by Javed et al. [11] may be the other reasons for low prevalence. The management practices included keeping animals tied in the open (kept indoor only during nights in winter) and fed chopped green fodder with mixing of wheat straw in group of two to four animals. The small herd sizes may contribute to low prevalence at herd and animal level. The low prevalence at herd and animal level is an ideal situation under which to have a test and slaughter strategy to control the disease in any country [20], but it demands the political will of the Government to control the disease (tuberculosis) in animals.

The results of bivariate frequency analysis and stratified frequency analysis (results not shown) after

controlling for breed and farm, revealed an association of age, live weight, calving number, lactation length, and total number of cattle at the farm. The analysis of variance confirmed the association of age, live weight, calving number, and total cattle at the farm, but also suggested the association of the total number of animals as a potential risk factor. However, the analysis of variance failed to suggest association of lactation length as potential risk factor. The results of analysis of the variance technique were confirmed by bivariate logistic regression analysis. This suggests that age, live weight, number of calves, total animals at the farm, and total cattle at the farm are potential risk factors for tuberculosis in cattle. However, when factors like age, breed, and farm were controlled, then live weight, total animals, and total cattle at the farm showed significant association with a positive skin test. Finally, the multivariate logistic regression analysis only revealed significant associations of live weight of cattle and total cattle at the farm. A previous study in buffalo in the same area revealed significant association of lactation status of buffalo, presence of cattle at the farm, total cattle at the farm and cattle, plus other animals at the farm [11]. Previously, the presence of cattle at a buffalo farm proved a potential risk factor [11], but the presence of buffalo at a cattle farm was not found associated with a positive skin test in cattle (present results). Another study at buffalo farms found a significant association of age (log OR = 1.164), live weight (log OR = 1.007), total milk produced (log OR = 1.002), presence of cattle at the farm (log OR = 2.447), lactation length (log OR = 0.98), and number of other animals at the farm (log OR = 0.999) with tuberculosis [21]. Thus, there is similarity in results except for the presence of other animals at the farm and lactation length results, which differ in both studies. To clarify this variation requires further study. A significant association of age has also been reported earlier where it was stated that animals of more than 10 years of age are at higher risk of infection (OR: 1.9) [22] and that older animals are more susceptible to tuberculosis [23, 24]. A higher risk of infection in cattle kept with sheep was found by another study [22], but could not be confirmed in the present study. An association of herd size with tuberculosis was not reported after one study [22], but is reported after many others [11, 16, 23]. However, Javed et al. [11] reported a protective effect of herd size with tuberculosis in buffalo that was not found in cattle in this study with logistic odds of 1.13. The role of airflow, as suspected earlier [11] in spread of tuberculosis in buffalo, could not be proven in cattle. We were also unable to find association of breed with tuberculosis. This was similar to earlier results in buffalo in the same area [11].

The role of purchase and culling of animals could not be investigated during this study as indicated earlier [14]. However, water as a source of infection, as indicated previously [14], may be a factor in the present study because fresh water is mostly offered to the animals in buckets. Although, buckets may be contaminated by an infected animal drinking the same water. Buckets are taken back to the hand pump where fresh water is added. This may reduce or even eliminate Mycobacteria. The role of birds and humans in tuberculosis of cattle could not be studied during present investigations, but has been reported in India [25].

It can be concluded that the prevalence of tuberculosis in cattle in Pakistan is around 9% at herd level and 2% at animal level, and the prevalence increases with increase in herd size. Strong risk factors identified were live weight, total number of animals, and total number of cattle at the farm.

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