An Abattoir Based Study on Brucellosis, Bovine Tuberculosis and Paratuberculosis in Buffaloes and Cattle at Faisalabad, Pakistan

Research Article

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Abstract

A study was carried out to investigate the prevalence of these diseases at Faisalabad abattoirs in buffaloes and cattle. For this purpose, 400 consecutive animals, including 200 buffaloes and 200 cattle were included in the study. Blood and diseased organ, tissue samples were collected for further processing. For a study on the prevalence of brucellosis, the serum samples were screened by RBPT (Rose Bengal Precipitation Test). The overall prevalence of brucellosis recorded was 10% and 11% in buffaloes and cattle, respectively. The prevalence of brucellosis did not vary between male and females, three age groups and three weight groups each in buffalo and cattle. However, the odds of contracting brucellosis were 3.8 vs 1 for cattle than buffalo at an age below 5 years. On the basis of specific gross and histopathological lesions in different organs/tissues, including lungs, liver, lymph nodes and intestine, the prevalence of bovine tuberculosis and paratuberculosis were assessed. The suspected prevalence of bovine tuberculosis was 1.5% and 1%, while it was 3.75% and 4.1 % in case of paratuberculosis in buffaloes and cattle, respectively. The cattle revealed significant (P = 0.05) difference between age groups on the basis of suspected lesions of tuberculosis in lungs with young cattle showing the lesions more than adult, but it was not so in buffaloes. The odds of contracting paratuberculosis in males were 3.74 vs 1 of buffalo than cattle. The other comparisons were not marked between age groups, sex and weight groups between buffaloes and cattle. The prevalence of these diseases is not unexpected in abattoir and is a threat not only for workers at the slaughterhouse, meat sellers (butchers) but also to the general public.

Keywords: Brucellosis; Tuberculosis; Paratuberculosis; Cattle; Buffalo; Abattoir.

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Introduction

Brucellosis is an infectious disease of animals with zoonotic significance [1]. In humans, most of the cases are occupational involving farmers, veterinarians and butchers [2]. There are many species of *Brucella* involved to cause brucellosis, which manifests in the form of abortion and reproductive disorders [3]. This organism is responsible for causing an abortion, infertility, prolonged calving interval, loss of calves and acute metritis [4] and thus results in economic losses [5]. This organism infects all domestic animals including cattle, buffaloes, sheep, goats and equine. The infection in animals is transmitted by ingestion and through venereal routes [4]. The entry of the organism into the body is through the digestive system, lungs and skin. The organism spread through blood and lymphatic system to different organs, causing a localized disease [1]. Brucellosis is a consistent problem in areas like the Middle East, Africa, Latin America and parts of Asia [6]. Sero-prevalence of brucellosis has been reported to be 3.25% and 4.4% in different areas of Pakistan [7,8]. The main serological tests used for diagnosis of brucellosis are Rose Bengal Plate test (99%), SAT (95.6%) and ELISA (100%), [9].

Bovine Tuberculosis is another serious disease of cattle, buffaloes and other animals. It is caused mainly by *Mycobacterium bovis* in animals. This disease is transmissible from animals to humans and vice versa. The disease is a trade barrier in export of animals and animal products. The disease is present worldwide, including Pakistan and its neighbouring countries. Its prevalence has been reported to be 30-40 % in Calcutta, India [10], 21.4 % in Egypt [11], 8.64 % in Lahore [12] and 2.45-8.48% on farms in Punjab [13].

Paratuberculosis which is also called as Johne's disease is a chronic inflammatory disease of domestic animals. The organism responsible for causing disease is *Mycobacterium avium* subsp. *paratuberculosis* (*M. paratuberculosis*). It has been reported for causing heavy losses in cattle, sheep and goats [14]. The yearly losses from this

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disease are reported of worth US$ 200 to US$ 250 millions in the United States [15]. The clinical manifestation of the disease is weight loss, diarrhoea, decreased milk production, and ultimately death. The routes of transmission of disease are ingestion of food and water contaminated with faeces from *M. paratuberculosis* shedding animals [16,17]. Recently, it has been reported from Pakistan [18].

Brucellosis, tuberculosis and paratuberculosis are diseases of zoonotic importance and are prevalent in Pakistan. Most of the earlier reports from Pakistan on brucellosis and tuberculosis are from field studies. Therefore, there was a dire need to have a data about the situation of these diseases at the abattoir. Thus, it was considered important to have a fresh data about the prevalence of diseases in cattle and buffalo brought for slaughtering.

Materials and Methods

The study was conducted at the Faisalabad abattoir to investigate the prevalence of brucellosis, tuberculosis and paratuberculosis in slaughtered cattle and buffaloes. A total of 400 consecutive animals, including 200 cattle and 200 buffaloes, were included in the study. The animals were screened for the presence of brucellosis by Rose Bengal Plate Test (RBPT) procured from Veterinary Research Institute, Lahore following the standard procedure [19]. For this purpose, blood samples from these animals were collected and serum was separated and stored in a freezer till tested by RBPT.

The data about each animal, including its tentative age, weight and sex were also recorded. The age of the animal was assessed by an eruption of permanent teeth. To assess the weight of the animal, the girth was measured and then their tentative weights were estimated by using the method as described earlier [20]. The judgment of butchers about the weight was also recorded. The weight of the animals judged by butchers was correlated well with the results of the girth of the animal.

The tissues showing lesions suspected for tuberculosis and paratuberculosis were collected. These tissues were fixed in buffered formalin. Tissue sections of 5 micron meters thick were cut and stained with haematoxylin and eosin staining procedure and observed under microscope.

Results and Discussion

Brucellosis

Two hundred animals each of buffalo and cattle were tested for brucellosis by RBPT. The sero-prevalence of brucellosis was found to be 10% and 11% in buffalo and cattle, respectively (Table 1). The results revealed that 7.6% of buffaloes of more than 10 years of age reacted positively to RBPT, while 16.2 % reacted positively in age group of 5-10 years. The sero-prevalence was low (3.64 %) in buffalo of below 5 years of age. In cattle, 15.6% reacted positively in the age group of above 10 years, 9.1% in the age group within the range of 5-10 years and 12.5% at age below 5 years. The odds of contracting the disease were 3.8 and 2.2 in cattle than buffaloes at an age below 5 years and at more than 10 years of age. However, the odds of contracting disease were 1.9 in buffalo than cattle at age 5-10 years (Table 1).

The results with reference to animal sex revealed a significant (p<0.05) association of brucellosis in male in buffaloes (Table 2). In buffaloes, 20% males and 7.5% of females reacted positively in RBPT showing a significant association of disease with sex. However, in cattle such significant association was not observed, although it was 11.4% positive reaction in male versus 9.2% in females. The odds of contracting disease in females were 1.49 in cattle than buffalo with an overall odds ratio (OR) of 1.11 (Table 2).

Tuberculosis

Gross lesions of abscessation with creamy yellow coloured exudate and microscopically with the characteristic granuloma formation were considered suspect for tuberculosis. The microscopic lesions seen were having central caseation with occasional calcification, macrophages, giant cells and lymphocytes making a rim around the central necrotic area with fibrous encapsulation. The prevalence of tuberculosis on this basis was 1.5% in buffaloes and 1.0% in cattle. The lesions suspected for tuberculosis were noted in significantly higher (p<0.005) percentage of younger animals of less than 5 years of age in cattle (Table 1). However, in buffaloes, non-significant difference was seen among age groups, although, such lesions were observed in higher percentage of buffaloes of 5-10 years of age. The odds of contracting disease were 1.11 in cattle than buffalo.

With respect to sex, not a single male of cattle or buffalo showed such lesions and these lesions were observed only in females (Table 2). The odds of contracting disease in female were 1.22 in buffalo than cattle with an overall OR of 1.51 (Table 2). The results of lesions suspected for tuberculosis in animals of different weight groups revealed non-significant difference (Table 3). However, animals having weight less than 400 kg showed lesions both in cattle and buffaloes. The odds of contracting tuberculosis in animals of less than 400 kg weight were 1.19 in cattle while in buffalo the odds ratio was 1.51.

Paratuberculosis

Gross lesions of corrugated thickening of the last part of the small intestine (Ileum) and variable parts of the large intestine (caecum and part of the colon) with thickening of ileocaecal valve were considered as suspected for paratuberculosis. Such lesions under microscope revealed intense granulomatous chronic inflammatory reaction with predominant involvement of macrophages. The results on the prevalence of paratuberculosis on the basis of suspected lesions revealed 4.5% prevalence in buffaloes and 2.5% in cattle with non-significant difference between age groups in each cattle and buffaloes. However, the prevalence was relatively higher in buffaloes of more than 10 years (6.1%) than buffaloes of less than 5 years (3.63%) and between 5 to 10 (3.75%) years of age, however, it was otherwise in cattle (Table 1). The odds of contracting disease were 1.84 in buffaloes than cattle (Table 1). Similarly, non-significant difference was observed in each cattle and buffaloes with respect to sex of the animal (Table 2). The prevalence was 5% in males and 4.4% in female buffalo, while it was 1.4% in male and 4.0% in female cattle (Table 2). The odds of contracting disease were 3.74 in buffalo males than cattle males, while these were 1.44 in buffalo females than cattle females (Table 2) with an overall OR of 1.84. The prevalence of paratuberculosis did not vary between animals of different weight

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Table 1. Results relevant to age groups examined for TB and PTB

Animal	Age in years						Total		Statistics Chi - Square
	< 5 yrs		5-10 yrs		>10 yrs		Total		
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	
	n	n	n	n	n	n	n	n	
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	
Reaction to RBPT antigen									
Buffaloes	2 (3.6)	53	13 (16.3)	67	5 (7.7)	60	20 (10)	180	NS
Cattle	6 (12.5)	42	11 (9.2)	109	5 (15.6)	27	22 (11)	178	NS
	OR=3.8		OR=1.9		OR= 2.2		OR=1.1		
Granulomatous lesions in Lungs suspected for Tuberculosis									
Buffaloes	0	55	2 (2.5%)	78	1 (1.54%)	64	3 (1.5)%	197	NS
Cattle	2 (4.1%)	46	0	120	0	32	2 (1.0%)	198	Likelyhood chi sq. = 5.646; P=0.05
	OR= 0		OR= 0		OR= 0		OR=1.51		
Intestinal Corrugation suspected for Paratuberculosis									
Buffaloes	2 (3.63%)	53	3 (3.75%)	77	4 (6.1%)	61	9 (4.5%)	191	NS
Cattle	2 (4.1%)	46	3 (2.5%)	117	0	32	5 (2.5%)	195	NS
	OR = 0.87		OR = 1.52		OR = 0		OR = 1.84		

Table 2. Results relevant to sex examined for TB and PTB

	Sex of the animal				Total		Statistics
	Male		Female		Total		
	+ve n (%)	-ve n	+ve n (%)	-ve n	+ve n (%)	-ve n	
Reaction to RBPT antigen							
Buffalo	0	32	12 (7.5)	148	20 (10)	180	OR= 0
Cattle	8 (11.4)	62	14 (10.8)	116	22 (11)	178	OR=1.07
	OR= 0		OR= 1.49		OR= 1.11		
Tuberculous Lungs suspected for Tuberculosis							
Buffalo	0	40	3 (1.8)	157	3 (1.5)	197	NS
Cattle	0	72	2 (1.5)	128	2 (1.0)	198	NS
	OR= 0		OR= 1.22		OR= 1.51		
Intestinal Thickening (Corrugation) suspected for Para Tuberculosis							
Buffalo	2 (5.0)	38	7 (4.4)	153	9 (4.5)	191	OR=1.15
Cattle	1 (1.4)	71	4 (3.1)	126	5 (2.5)	195	OR=2.25
	OR= 3.74		OR= 1.44		OR= 1.84		

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Table 3. Results relevant to weight group examined for TB and PTB

| | Weight of Animals (kg) | | | | | | Total | | Statistics |
| | <400 | | 400-500 | | >500 | | | | |
	+ve n (%)	-ve n	+ve n (%)	-ve n	+ve n (%)	-ve n	+ve n (%)	-ve n	
Tuberculous Lungs suspected for Tuberculosis									
Buffalo	2 (2.1%)	93	1 (1.3%)	74	0	30	3 (1.5%)	197	NS
Cattle	2 (2.5%)	78	0 (0%)	90	0	30	2 (1%)	198	NS
	OR= 1.19		OR= 0		OR= 0		OR=1.51		
Intestinal Thickening (Corrugation) suspected for Para Tuberculosis									
Buffalo	4 (4.2%)	91	5 (6.6%)	70	0	30	9 (4.5%)	191	NS
Cattle	2 (2.5%)	77	3 (3.3%)	87	0	30	5 (2.5%)	195	NS
	OR=1.69		OR= 2.07		OR= 0		OR= 1.84		

groups, each in cattle and buffaloes (Table 3). The odds of contracting disease in animals of less than 400 kg weight were 1.69 in buffalo than cattle, while these were 2.07 in buffaloes than cattle in weight group 400-500 kg body weight (Table 3)

Discussion

Brucellosis has been reported from all over the world. A study in the mid nineties from Pakistan reported prevalence of 23% on Government farms; 4.9% of private farms and 1.6% in rural areas [21]. Other studies reported a prevalence of 10 % [22] and 15.8% of livestock farms in Punjab in cattle and buffaloes, respectively [23]. The results of the present study revealed an overall prevalence of 10% in buffaloes and 11% in cattle. These results at the abattoir suggest that disease is hovering around 10-15% in the field, thus is a concern for future development of livestock in the country and is also a potential public health risk. Most of the studies in Pakistan used Rose Bengal Plate Test so results are comparable. The sensitivity of RBPT is reported to be very high (>99%) but the specificity may be low [24]. The results of the present study also suggest that both cattle and buffalo are equally susceptible to brucellosis. However, in some countries low prevalence of 1.9% in cattle and 1.8% in buffaloes has been reported [25].

The present results showed a higher prevalence in males (20% in the case of buffaloes and 11.4% in the case of cattle) than the females (7.5% in the case of buffaloes and 9.2% in the case of cattle) in both buffaloes and cattle. As male animals are used for AI and natural mating, thus the spread of disease is quite obvious and it may someday become a major obstacle in the dairy industry. However, these are the results of a study conducted on one abattoirs and thus cannot be taken for most parts of the country as previous studies carried out showed results otherwise as to the sex of the animal is concerned. A higher prevalence in females has been reported [26] reported. The prevalence of brucellosis in different age groups revealed variable results in cattle and buffaloes. However, the result showed that in cattle and buffalo of less than

5 years, cattle have 3.8 times more chances of contracting the disease than buffaloes, and these chances decrease to 2.2 at age more than 10 years. Further studies are required to confirm these results as it may be a chance variation and may be related to the source of animals to be brought to slaughter.

Tuberculosis is undoubtedly one of the serious diseases of animals and is of zoonotic importance. This disease is present all over the world including Pakistan and its neighbouring countries. The results of the present study showed a prevalence of 1.5% in buffaloes and 1.0% in cattle which was almost similar to a low prevalence of 1.76% observed in cattle [27] around Faisalabad city. Earlier farm based study from Punjab, Pakistan revealed a prevalence of 2.5-8.5% in buffaloes [13]. These results suggest that the prevalence of tuberculosis is lower in the field than on Government Livestock farms. One of the reasons could be that in more than 70% of cases the livestock is kept in less than 3 animal number. This low number of animals at home, which are also chained and with no grazing or very limited grazing has protected the animals from being infected.

In buffaloes, the animals showed Tuberculous lesions significantly higher (p<0.005) in animals more than 5 years of age, which is quite obvious due to its chronic nature but it was interesting to see lesions in younger animals in cattle. Our study confirms the finding of the earlier study where disease was more prevalent in older buffaloes [13]. However, the present results of high prevalence in young cattle may be considered as chance prevalence and further studies are required to rule it out. It was interesting to note that not a single male of buffalo or cattle showed lesions suspected for tuberculosis. It may be possible that as the females have to bear more natural stress, i.e., pregnancy, lactation, etc. than the males which make them more susceptible to tuberculosis. The weight of animals suspected of having tuberculosis was less than 500 kg in buffaloes and less than 400 kg in cattle suggesting debilitation caused by the disease. The owners eventually sold their sick, emaciated animals for slaughter. This slight variation in weight of buffalo and cattle is quite a natural variation as buffalo are normally

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heavier than local cattle breeds in Pakistan. It is quite possible that these animals might have weighed more than 400-500 kg earlier, but owing to tuberculosis, they lost the weight. These results of low weight in tuberculosis suspected animals were similar to previous findings in buffaloes from Pakistan in which majority of tuberculin positive reactors had weight less than 500 kg [13].

Paratuberculosis has worldwide distribution. Since, it is reported from all over the world and has recently been reported from Pakistan as well [18]. An overall prevalence of 4.5% was observed in buffaloes and 2.5% in cattle. This prevalence is less than as reported in a study at Lahore abattoir that reported 12.8% and 12.4% prevalence in buffalo and cattle, respectively. The difference in two studies can be due to the method of diagnosis as the later study used a PCR based method of diagnosis and the former study only used lesions as a tool for diagnosis of disease. A study from India reported 2% of buffaloes showing lesions of paratuberculosis in the intestine [28]. The disease prevalence was relatively higher in older animals which is quite obvious that this is a chronic disease whose incubation period can be as long as 2 years or in some case even more. Similarly, higher prevalence at old age has been reported by [29,30]. The results of the present study revealed that there are more chances of buffalo being infected the most than the cattle (OR = 1.84).

Further studies are required involving PCR based methods of diagnosis to draw meaningful conclusions about these issues. However, the present study has highlighted that the diseased animals having zoonotic diseases are coming to the same slaughterhouse where normal animals are being slaughtered. This causes a serious risk to butchers, workers and veterinarians working in these slaughter houses along with meat sellers and consumers. Further as the environment of the slaugherhouse is being contaminated there are every chances that the meat from healthy animals may become contaminated which is another serious concern. Thus, we recommend building of special slaughterhouses for animal slaughtering those are having zoonotic diseases. All the animals before slaughtering must be first quarantined and screened and then moved to slaughterhouses according to their diseased or disease free status. Slaughterhouse workers should be provided with incentives along with education and training.

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