



Prevalence and Risk Factors of Bovine Tuberculosis in Cattle and Dairy Farm Workers in Mirpurkhas and Badin Districts of Sindh, Pakistan

Muhammad Qasim Mazari¹, Dildar Hussain Kalhoro^{1,*}, Hasina Baloch¹, Muhammad Saleem Kalhoro², Shahid Hussain Abro¹, Rehana Buriro¹, Asmatullah Kaka¹, Fahmida Parveen¹, Mazhar Hussain Mangi³, Ghulam Murtaza Lochi¹, Abdul Ahad Soomro⁴, Ali Gul Soomro¹, Abdul Ghaffar Abbasi¹ and Sarfraz Hussain Depar¹

¹Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam-70060, Pakistan

²Department of Animal Products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam-70060

³Laboratory of Animal Pathology and Public Health, Key Laboratory of Zoonosis, Ministry of Agriculture, College of Veterinary Medicine, China Agriculture University, Beijing, China.

⁴Central Veterinary Diagnostic Laboratory, Tandojam, Sindh, Pakistan

ABSTRACT

During present study a total of 800 samples were collected from two hundred descriptive (Holstein Friesian) and non-descriptive (Thari) cattle breeds from districts of Mirpurkhas and Badin, 400 samples were taken from each district. Animals were first screened through Single Intradermal Tuberculin Test (SITT) then positive and negative reactors of SITT were further investigated through Rapid BTB Ab test, culture examination and ELISA test. Risk factors such as sex, age, health status, lactating, non-lactating and breed were also investigated. In district Mirpurkhas prevalence was 4%, 15%, 2%, 1%, while in district Badin it was 5%, 18%, 3%, 2% through SITT, Rapid BTB Ab test, culture examination and ELISA test, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via cultural examination was recorded as 1.66%, 2.5% in milk and nasal samples, while in district Badin it was 1.66%, 5%, respectively. The overall prevalence of bovine tuberculosis was 5.5% and 7% in Mirpurkhas and Badin districts through tuberculin, Rapid BTB Ab, Culture examination and ELISA test. Different tests such as SITT, Rapid BTB Ab, culture examination and ELISA test showed that the prevalence of bovine tuberculosis was higher in females, animals above the six years of age, lactating animals, descriptive breed and animals having poor health status. Furthermore, 1% and 2% prevalence was recorded in farm workers at district Mirpurkhas and Badin. It is concluded that disease was prevalent in both districts however; relatively higher prevalence was observed in district Badin.

INTRODUCTION

Bovine tuberculosis is one of the important chronic diseases, which produce major economic losses and huge public health threats causing 10-25% loss in product efficiency in dairy cattle. It is a chronic disease occurs in wild species and domestic animals, after the exposure of disease host can survive for several months, even years without showing clinical signs (De-Lisle *et al.*, 2002). The disease has been associated within the “list-B” disease of

Office International-des-Epizooties (OIE) (OIE, 2009). The WHO has classified it as one of the seventh zoonotic disease, having a potential of infecting human beings (Malama *et al.*, 2013). There are three important types of tuberculosis i.e. Human tuberculosis affecting humans, avian tuberculosis affecting birds and Bovine tuberculosis affecting human's, cattle and buffalo. Human tuberculosis is rarely transferable to animal species and avian tuberculosis is restricted to the birds, whereas, bovine tuberculosis having zoonotic potential, is highly infectious disease affecting diverse group of animals including farm animals, wild life and humans (Katale *et al.*, 2013). *Mycobacterium bovis* is a gram positive, acid fast, aerobic, filamentous and curved rod shaped bacteria. It is very slow

* Corresponding author: drdildarkalhoro@gmail.com
0030-9923/2022/0003-1115 \$ 9.00/0
Copyright 2022 Zoological Society of Pakistan

Article Information

Received 15 September 2019
Revised 17 March 2020
Accepted 10 November 2020
Available online 18 May 2021
(early access)
Published 15 February 2022

Authors' Contribution

MQM, DHK and HB conceived and designed the experiments. MQM and DH performed the experiments. SHA, RB, AK, FP, MHM, GML and AGS analyzed the data. DHK, MQM and AAS wrote the paper. AGA and SHP checked the references.

Key words

Prevalence, Cattle, Mirpurkhas, Badin Tuberculosis

growing bacteria, having generation time of 16 h, that's why it takes months even years to produce infection in their host (Buddle *et al.*, 2006). Carrier animals show important role in transfer of disease to healthy animals, infective dose through inhalation is very low. Infection can be transferred through short distances about 1-2 meters (AHA, 2009). Clinical signs are emaciation, dyspnea, weakness, variable pyrexia, enlargement of lymph nodes and in advance tuberculosis coughing (Peters, 2010). *M. bovis* infection is transfer to cattle by the inhalation of infectious aerosol, ingestion of contaminated feed, drinking of infected milk. Lesions are associated in upper and lower respiratory tract and lymph nodes (Donnelly and Nouvellet, 2013). Moreover, herds with a better tendency of animal's movement have a great role in disease spreading. Primary route of disease transmission in between herds is the sanitary, poor husbandry, herd size and infected animals (Belchior *et al.*, 2016). There are so many diagnostic techniques such as Enzyme Linked Immunosorbent Assay (ELISA), culture, biochemical, gamma interferon, postmortem, microscopic and tuberculin tests used for the diagnose the Bovine tuberculosis in animals (OIE, 2009). From the milk and saliva samples, diagnosis of *M. bovis* on cultural examination (Lowenstein Jensen agar), Zeheil Nelson staining, microscopic examination and biochemical tests such as Nitrate reduction test, niacin production, urease and tolerance tests are also useful for detection of the organism (OIE, 2004).

Several studies have been reported the prevalence of tuberculosis in large and small ruminants (Ashfaq *et al.*, 2015). Prevalence of bovine tuberculosis in cattle in Punjab was recorded as 1.7% in buffaloes and 5.1% in cattle (Ifrahim, 2001). Higher prevalence of bovine tuberculosis from nasal secretions (12.28%) and milk samples (28.07%) from buffaloes were reported (Jalil *et al.*, 2003). Prevalence of bovine tuberculosis in Punjab recorded as 3% in 2010 (Javed *et al.*, 2010). Though the disease is nearly eliminated in many countries including Slovakia, Sweden, Australia and Canada, it is widespread in Asia, Africa and some Middle East countries (Schiller *et al.*, 2010). According to the World Organization for Animal Health, Turkey is one of the countries where tuberculosis is still present where infection rate in the year 2011 was reported as 22.8% in cattle population (OIE, 2011). Prevalence of bovine tuberculosis in cattle have not been studied in Mirpurkhas and Badin districts, therefore, the current study is designed to evaluate the prevalence of bovine tuberculosis in dairy farms of Mirpurkhas and Badin districts of Sindh Pakistan. Moreover, risk factors associated with the prevalence of disease was also examined.

MATERIALS AND METHODS

During present study a total of 800 samples were collected from two hundred descriptive (Holstein Friesian) and non descriptive (Thari) cattle breeds from districts of Mirpurkhas and Badin, 400 samples were taken from each district. The animals were first screened through Single Intradermal Tuberculin Test (SITT) then both positive and negative reactors of SITT were further investigated through Rapid BTB Ab test and culture examinations and ELISA test. Risk factors such as sex, age, health status, lactating, non-lactating and breed were also investigated.

Single intradermal tuberculin test (SITT)

Centre of the cattle neck was shaved and cleaned with a cotton wool swab (70% alcohol). A fold of skin was pinched with fingers and thickness was measured with the help of Vernier caliper. Avian tuberculin (0.1ml) and mammalian (0.1ml) were injected intradermally at both sides of neck. Injection sites were marked by circle. After 72 h of post inoculation, result was recorded by measuring the thickness of skin. 4 mm or more than 4 in the thickness of skin was considered as positive.

Tuberculin skin test in farm workers

Two hundred farm workers were selected for tuberculin skin test from both districts.

Rapid BTB Ab test

Serum samples were analyzed by rapid BTB Ab test using commercial kit (Lilli Rapid BTB Ab Test Kit by "Lilly dale Diagnostics" England). One drop of serum was transferred into the sample hole "S" and immediately two drops of assay buffer was added. Results were interpreted after 15-20 min (OIE, 2008).

Enzyme linked immunosorbent assay

ELISA is known to be most suitable technique for the detection of antibody (Sayin and Erganis, 2013). In the present study AniGen BTB Ab ELISA 2.0 kit was used. Two hundred blood samples were collected from Mirpurkhas and Badin districts for ELISA test.

Milk samples

The milk samples were collected in sterile bijoux bottles, transferred in ice box and stored at 4°C. Supernatants were discarded after the centrifugation at 3000 rpm for 15 min. Sediments were used for culture as well as microscopy.

Nasal discharge

The nasal samples were collected from both districts with sterile cotton swabs in screw capped test tubes and

stored at 4°C. Same amount of NaOH and N-acetyl was mixed with samples and incubated for 30 min at 37°C. After centrifugation for 15 min at 3000 rpm, supernatants were discarded. Sediments were used for culture as well as microscopy.

Media for mycobacteria

Lowenstein Jensen medium was mixed with 1% of sodium pyruvate and used for bacteriological culture. Thick inoculum of sediments was smeared on the surface medium slopes and incubated at 37°C for 6-8 weeks. Finally, niacin production and urease tests were performed for confirmation. Zeihl Nelson stain was used for the staining of *Mycobacterium*.

RESULTS AND DISCUSSION

Prevalence of bovine tuberculosis in Mirpurkhas district was recorded as 4%, 15%, 2% and 1% through SITT, rapid BTB Ab, culture examinations and ELISA test, respectively. While in district Badin it was 5%, 18%, 3% and 2% through SITT, rapid BTB Ab, culture examinations and ELISA test, respectively. Overall prevalence of bovine tuberculosis in Mirpurkhas and Badin districts were recorded through SITT, rapid BTB Ab test, Culture examinations and ELISA test 5.5% and 7%, respectively as shown in Table I.

Table I. The overall prevalence of bovine tuberculosis in cattle at Mirpurkhas and Badin districts analyzed through different techniques.

Type of test	Mirpurkhas district		Badin district	
	Total sample	Positive	Total sample	Positive
Tuberculin test (SITT)	100	04	100	05
Rapid BTB Ab test	100	15	100	18
Culture	100	02	100	03
ELISA	100	01	100	02
Total	400	22	400	28

Prevalence of bovine tuberculosis in Mirpurkhas district via tuberculin test (SITT) was recorded as 4.44% in female and 0% in male, while, in district Badin it was 5.55% in female and 0% in male. Prevalence of disease in Mirpurkhas district via SITT was recorded as 6.66%, 2.22% and 0% in above 6 years, 2-6 years and less than 2 years of age, respectively, while in district Badin it was 6.66%, 4.44% and 0% in above 6 years, 2-6 years and less than 2 years of age, respectively. Prevalence of disease in Mirpurkhas district through SITT was

recorded as 6.66%, 2.85% and 0% in poor, satisfactory and good health condition, respectively, while in district Badin it was 8.88%, 2.85% and 0% in poor, satisfactory and good health conditions, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via SITT was recorded as 5%, 3.33% in lactating non-lactating animals, respectively, while in district Badin it was 6.66%, 3.33% in lactating and non-lactating animals, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via SITT was recorded as 6%, 2%, in descriptive (Holstein Friesian) and non-descriptive (Thari) breeds of cattle, respectively, while in district Badin it was 6%, 4% in descriptive (Holstein Friesian) and non-descriptive (Thari) breeds of cattle, respectively Table II.

Table II. Prevalence of bovine tuberculosis in cattle population of districts of Mirpurkhas and Badin in association of risk factors such as sex, age, health status, lactation and breed SITT.

Risk factors	District Mipurkhas		District Badin	
	Total	Tuberculin test (SITT) Positive (%)	Total	Tuberculin test (SITT) Positive (%)
Sex				
Female	90	04 (4.44%)	90	05 (5.55%)
Male	10	00 (0%)	10	00 (00%)
Total	100	04 (4%)	100	05 (05%)
Age				
≤ 2 years	10	00 (0%)	10	00 (00%)
Above 2 to 6 years	45	01 (2.22%)	45	02 (4.44%)
> 6 years	45	03 (6.66%)	45	03 (6.66%)
Total	100	04 (4%)	100	05 (05%)
Health status				
Poor	45	3 (6.66%)	45	4 (8.88%)
Satisfactory	35	01 (2.85%)	35	01 (2.85%)
Good	20	00 (0%)	20	00 (00%)
Total	100	04 (4%)	100	05 (05%)
Lactating	60	03 (5%)	60	04 (6.66%)
Non-lactating	30	01 (3.33%)	30	01 (3.33%)
Total	90	04 (4.44%)	90	05 (5.55%)
Breed				
Descriptive (HF)	50	03 (06%)	50	03 (06%)
Non-descriptive (Thari)	50	01 (02%)	50	02 (04%)
Total	100	04 (04%)	100	05 (05%)

Prevalence of bovine tuberculosis in Mirpurkhas district via rapid BTB Ab test was recorded as 15.55% in female and 10% in male, while in district Badin it was

Table III. Prevalence of bovine tuberculosis in cattle population of districts of Mirpurkhas and Badin in association of risk factors such as sex, age, health status, lactation and breed through Rapid BTB Ab test.

Risk factors	District Mipurkhas		District Badin	
	Total	Rapid BTB Ab Positive (%)	Total	Rapid BTB Ab Positive (%)
Sex				
Female	90	14 (15.55%)	90	17 (18.88%)
Male	10	01 (10%)	10	01 (10%)
Total	100	15 (15%)	100	18 (18%)
Age				
≤ 2 years	10	01 (10%)	10	00 (00%)
Above 2 to 6 years	45	06 (13.33%)	45	08 (17.77%)
> 6 years	45	08 (17.77%)	45	10 (22.22%)
Total	100	15 (15%)	100	18 (18%)
Health status				
Poor	45	09 (20%)	45	11 (24.44%)
Satisfactory	35	05 (14.28%)	35	07 (20%)
Good	20	01 (5%)	20	00 (00%)
Total	100	15 (15%)	100	18 (18%)
Lactating				
Lactating	60	11 (18.33%)	60	13 (21.66%)
Non-lactating				
Non-lactating	30	03 (10%)	30	04 (13.33%)
Total	90	14 (15.55%)	90	17 (18.88%)
Breed				
Descriptive (HF)	50	08 (16%)	50	10 (20%)
Non- descriptive (Thari)	50	07 (14%)	50	08 (16%)
Total	100	15 (15%)	100	18 (18%)

18.88% in female and 10% in male animals, respectively. Prevalence of disease in Mirpurkhas district via rapid BTB Ab test was recorded as 17.77%, 13.33% and 10% in above 6 years, 2-6 years and less than 2 years of age, respectively, while in district Badin it was 22.22%, 17.77% and 0% in above 6 years, 2-6 years and less than 2 years of age, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via rapid BTB Ab test was recorded as 20%, 14.28% and 5% in poor, satisfactory and good health condition, respectively, while in district Badin it was 24.44%, 20% and 0% in poor, satisfactory and good health condition animals, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via rapid BTB Ab test was recorded as 18.33%, 10% in lactating and non-lactating animals, respectively, while in district Badin it was 21.66%, 13.33% in lactating and non-lactating animals, respectively. Prevalence of disease in Mirpurkhas district via rapid BTB Ab test was recorded as 16%, 14%,

in descriptive (Holstein Friesian) and non-descriptive (Thari) breeds of cattle, respectively, while in district Badin it was 20%, 16%, in descriptive (Holstein Friesian) non-descriptive (Thari) breeds of cattle, respectively as summarized in Table III. Prevalence of bovine tuberculosis in Mirpurkhas district through cultural examination was recorded as 1.66%, 2.5% in milk and nasal samples, respectively, while in district Badin it was 1.66%, 5% in milk and nasal samples, respectively as shown in Table IV.

Table IV. Prevalence of bovine tuberculosis in cattle at Mirpurkhas and Badin districts by culture examination.

Source of sample	District Mipurkhas		District Badin	
	No of sample	Positive (%)	No of sample	Positive (%)
Milk	60	01 (1.66%)	60	01 (1.66%)
Nasal swab	40	01 (2.5%)	40	02 (5%)
Total	100	02 (2%)	100	03 (3%)

Table V. Prevalence of bovine tuberculosis among cattle at Mirpurkhas and Badin Districts in relation to the various risk factors such as sex, age, health status, lactation and breed analyzed by culture examination.

Risk factors	District Mipurkhas		District Badin	
	Total	Culture examination Positive (%)	Total	Culture examination Positive (%)
Sex				
Female	90	02 (2.22 %)	90	03 (3.33%)
Male	10	00 (0 %)	10	00 (0 %)
Total	100	02 (2 %)	100	03 (3 %)
Age (years)				
≤ 2	10	00 (0 %)	10	00
Above 2 to 6 years	45	00 (0 %)	45	00 (0 %)
> 6 years	45	02 (4.44 %)	45	03 (6.66 %)
Total	100	02 (2 %)	100	03 (3 %)
Health status				
Poor	45	02 (4.44 %)	45	03 (6.66 %)
Satisfactory	35	00 (0 %)	35	00 (0 %)
Good	20	00 (0 %)	20	00 (0 %)
Total	100	02 (2 %)	100	03 (3 %)
Lactating				
Lactating	60	02 (3.33 %)	60	03 (5 %)
Non-lactating				
Non-lactating	30	00 (0 %)	30	00 (0 %)
Total	90	02 (2.22 %)	90	03 (3.33 %)
Breed				
Descriptive (HF)	50	02 (4 %)	50	03 (06 %)
Non- descriptive (Thari)	50	00 (0 %)	50	00 (0 %)
Total	100	02 (2 %)	100	03 (3 %)

Table VI. Prevalence of bovine tuberculosis among cattle at Mirpurkhas and Badin Districts in relation to the various risk factors such as sex, age, health status, lactation and breed analyzed by ELISA.

Risk factors	District Mirpurkhas		District Badin	
	Total	Elisa test	Total	Elisa test
		Positive (%)		Positive (%)
Sex				
Female	90	01 (1.11%)	90	02 (2.22%)
Male	10	0 (0%)	10	00 (0%)
Total	100	01 (01%)	100	02 (02%)
Age (years)				
≤ 2	10	0 (0%)	10	0 (0%)
Above 2 to 6 years	45	0 (0%)	45	0 (0%)
> 6 years	45	01 (2.22%)	45	02 (4.44%)
Total	100	01 (01%)	100	02 (02%)
Health status				
Poor	45	01 (2.22%)	45	02 (4.44%)
Satisfactory	35	00 (00 %)	35	0 (0%)
Good	20	00 (00%)	20	0 (0%)
Total	100	01 (01 %)	100	02 (02%)
Lactation				
Lactating	30	01 (3.33 %)	30	02 (6.66%)
Non-lactating	60	0 (0%)	60	0 (0%)
Total	90	01 (1.11%)	90	02 (2.22%)
Breed				
Descriptive	50	01 (02%)	50	02 (04%)
Non- descriptive	50	0 (0%)	50	0 (0%)
Total	100	1 (01%)	100	02 (02%)

Through culture examination prevalence of bovine tuberculosis in Mirpurkhas district was recorded as 2.22% in female and 0% in male animals, while in district Badin it was 3.33% in female and 0% in male animals, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via culture examination was recorded as 4.44%, 0% and 0% in above 6 years, 2-6 years and less than 2 years of age, respectively, while in district Badin it was 6.66%, 0% and 0% in above 6 years, 2-6 years and less than 2 years of age, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via culture examination was recorded as 4.44%, 0% and 0% in poor, satisfactory and good health condition, respectively; while in district Badin it was of 6.66%, 0% and 0% in poor, satisfactory and good health condition, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via culture examination was recorded as 3.33%, 0% in lactating and non-lactating animals, respectively, while in district Badin it was 5%,

0% in lactating and non-lactating animals, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via culture examination was recorded as 4%, 0%, in descriptive (Holstein Friesian) and non-descriptive (Thari) breeds of cattle, respectively, while in district Badin it was 6%, 0%, descriptive (Holstein Friesian) and non-descriptive (Thari) breeds of cattle, respectively (Table V).

Prevalence of bovine tuberculosis in Mirpurkhas district via ELISA test was recorded as 1.11% in female and 0% in male, while in district Badin it was 2.22% in female and 0% in male animals, respectively. Prevalence of Bovine tuberculosis in Mirpurkhas district via ELISA test was recorded as 2.22%, 0% and 0% in above 6 years, 2-6 years and less than 2 years of age, respectively, while in district Badin it was 4.44%, 0% and 0% in above 6 years, 2-6 years and less than 2 years of age, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via ELISA test was recorded as 2.22%, 0% and 0% in poor, satisfactory and good health condition, respectively, while in district Badin it was 4.44%, 0% and 0% in poor, satisfactory and good health condition animals, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via ELISA test was recorded as 3.33%, 0% in lactating and non-lactating animals, respectively, while in district Badin it was 6.66%, 0% in lactating and non-lactating animals, respectively. Prevalence of bovine tuberculosis in Mirpurkhas district via ELISA test was recorded as 2%, 0%, in descriptive (Holstein Friesian) and non-descriptive (Thari) breeds of cattle, respectively, while in district Badin it was 4%, 0%, in descriptive (Holstein Friesian) non-descriptive (Thari) breeds of cattle, respectively (Table VI).

District Badin 100 workers were tested by tuberculin test in which 2% workers were positive reactor of tuberculin test while in district Mirpurkhas also 100 workers were tested in which 1% workers were positive reactor of tuberculin test and total prevalence in both district were 1.5% (Table VII).

Table VII. Prevalence of tuberculosis in cattle owner farmers at Mirpurkhas and Badin districts by Tuberculin test.

No of worker checked	n	No of positive reactor (%)
Badin	100	02 (2 %)
Mirpurkhas	100	01 (01 %)
Total	200	03 (1.5 %)

DISCUSSION

During present study a total of 800 samples were collected from two hundred descriptive (Holstein Friesian)

and non descriptive (Thari) cattle breeds from districts of Mirpurkhas and Badin, 400 samples were taken from each district. Out of 400 samples in Mirpurkhas district 4%, 15% 2% and 1% were found positive by (SITT) tuberculin, rapid BTB Ab, culture examination and ELISA test, respectively, while in Badin district 5%, 18%, 3% and 2% were positive against bovine tuberculosis through (SIIT) tuberculin, rapid BTB Ab, culture examination and ELISA test, respectively. The variation in results between tests is because of immune system's sensitivity and specificity towards the tuberculin test in different animals and chronic nature of tested animals (Konch *et al.*, 2017). Present study was designed to detect prevalence of bovine tuberculosis through SITT as 4%, 5% in Mirpurkhas and Badin districts, respectively. The findings of the study are in line with the reports of Jalil *et al.* (2003), who reported 7.3% prevalence in Lahore. Present findings are lower than findings of Khan *et al.* (2008), Thakur *et al.* (2010), Kumar *et al.* (2013), who reported 12% prevalence of bovine tuberculosis in Lahore Pakistan, 14.31% in Himachal Pradesh India, 23.63% in Punjab area of India through SITT.

Whereas, through rapid BTB Ab test, a positive percentage of 15% and 18% was observed in Mirpurkhas and Badin districts. Higher prevalence was recorded through this test as compared to other tests. Our results are comparable with previous findings of Rahman and Samad (2008) and Islam *et al.* (2007) who reported 30% prevalence of rapid BTB Ab test, whereas, 2% and 3% prevalence were observed in district Mirpurkhas and Badin through culture examinations. Present results are in line with the findings of other authors who reported 4.28% prevalence in Lahore, Pakistan (Qamar and Tehmina, 2013). ELISA was also used to detect prevalence of bovine tuberculosis as 1%, 2% in Mirpurkhas and Badin districts, respectively. Present findings of study agree with the Buyuk *et al.* (2017) who reported 3% prevalence of bovine tuberculosis in cattle by ELISA test. However, current findings are lower than findings of Ratan *et al.* (2018) and Silva (2001) who reported 23.7% and 47% prevalence of bovine tuberculosis in cattle through Elisa technique, respectively. Hassanain *et al.* (2009) reported 50% prevalence in Egypt. During present study prevalence of bovine tuberculosis indicated that it mostly affects the female as compared to the male in all tests that agrees with previous studies in other laboratories (Noorrahim *et al.*, 2015; Mondal *et al.*, 2014; Buyuk *et al.*, 2017) who reported that females were more effected as compared to male, through all tests. Higher prevalence of the disease may be associated with stress produced from high milk producer animals (Mukherjee, 2006).

Disease prevalence was higher in older animals as compared to the younger animals. Present results are in

line with those of Noorrahim *et al.* (2015), Chandra *et al.* (2007), Javed *et al.* (2012) and Ratan *et al.* (2018) who stated that older animals were more susceptible than younger animal by all tests. Older animals were highly affected due to prolonged closed confinement with positive reactors (Mackay and Hein, 1989). Higher prevalence of bovine tuberculosis was observed in lactating as compared to the non-lactating (dry animals). Current findings are in agreement with those of Arshad *et al.* (2012), Khan *et al.* (2008), Sayin and Ergani (2013) and Jalil *et al.* (2003) who reported that the disease is highly prevalent in lactating than non-lactating animals. It has been suggested that lactating animals are at higher risk due to greater yield (Amin *et al.*, 1992; Rodwell *et al.*, 2001), whereas higher prevalence was observed in HF animals as compared to Thari animals through all tests. Present result are in line with those of Trangadia *et al.* (2013), Tahmid Uddin *et al.* (2014), Khan *et al.* (2008) and Buyuk *et al.* (2017), who suggested that descriptive breeds were more effected. Higher potential production of HF directly associated with stress therefore, animal active towards disease (Trangadia *et al.*, 2013). Present study was also designed to detect the prevalence of bovine tuberculosis through SITT in humans as 1%, 2% in Mirpurkhas and Badin districts, respectively. The findings of the study are in line with the reports of Muller *et al.* (2013), who reported 2.8% prevalence in Africa.

CONCLUSION

This study reported that prevalence of bovine tuberculosis in both districts, but higher prevalence was observed in Badin as compared to Mirpurkhas district, whereas, bovine tuberculosis cases were higher in female than male animals. Farm workers, Lactating, descriptive breeds, animals with age group of >6 years were shown higher rate of infection than young animals. while, higher prevalence was observed through rapid BTB Ab test as compared to other tests.

ACKNOWLEDGEMENTS

The authors are highly thankful to the Central Veterinary Diagnostic Laboratory, Tandojam, Directorate of Veterinary Research and Diagnosis, Government of Sindh, Pakistan

Statement of conflict of interest

The authors have no conflict of interest to declare.

REFERENCES

- Amin, S., Khan, M.A., Hashmi, H.A., Khan, M.S., Ahmad I. and Bhatti, M.A., 1992. Detection of buffalo tuberculosis by using short thermal test and isolation of causal organisms from lymph nodes. *Buff. J.*, **8**: 83-7.
- Animal Health Australia, 2009. Bovine tuberculosis case response manual, managing an incident of bovine tuberculosis. *Tub. Cas. Res. Man. Fin. J.*, 04-11-09, pp. 1-9.
- Arshad, M., Ifrahim, M., Ashraf, M., Rehman, S.U. and Khan, H.A., 2012. Epidemiological studies on tuberculosis in buffalo population in villages around Faisalabad. *J. Anim. Pl. Sci.*, **22**: 246-249.
- Ashfaq, M., Razzaq, A. and Muhammad, G., 2015. Economic analysis of dairy animal diseases in Punjab: A case study of Faisalabad district. *J. Anim. Pl. Sci.*, **5**: 25-35.
- Belchior, A.P.C., Lopes, L.B., Goncalves, V.S.P. and Leite, R.C., 2016. Prevalence and risk factors for bovine tuberculosis in Minas Gerais State, Brazil. *Trop. Anim. Hlth. Prod.*, **48**: 373-378. <https://doi.org/10.1007/s11250-015-0961-x>
- Buddle, B.M., Wedlock, D.N. and Denis, M., 2006. Progress in the development of tuberculosis vaccines for cattle and wildlife. *Vet. Microbiol.*, **112**: 191-200. <https://doi.org/10.1016/j.vetmic.2005.11.027>
- Buyuk, F., Bozukluhan, K., Saglam, A.G., Gokce, G., Celebi, O., Celik, E. and Sahin, M., 2017. The prevalence estimates of *Mycobacterium bovis* infection in cattle with ELISA. *J. Hell. Vet. Med. Soc.*, **68**: 541-546. <https://doi.org/10.12681/jhvms.16050>
- Chandra, H.A.V., Yukio, M., Dhakal, M., Besnet, B., Sato, T., Nagai, A., Kato, M., Kozawa, K., Yamamoto, S. and Kimura, H., 2007. Isolation of *Mycobacterium* spp. from milking buffaloes and cattle in Nep. *J. Vet. Med. Sci.*, **69**: 819-825. <https://doi.org/10.1292/jvms.69.819>
- De Lisle, G.W., Bengis, R.G., Schmitt, S.M. and O'Brien, D.J., 2002. Tuberculosis in free-ranging wildlife detection, diagnosis and management. *Rev. Sci. Tech. OIE.*, **21**: 317-334. <https://doi.org/10.20506/rst.21.2.1339>
- Donnelly, C.A. and Nouvellet, P., 2013. The contribution of badgers to confirmed tuberculosis in cattle in high-incidence areas in England. *PLoS Curr.*, **5**: <https://doi.org/10.1371/currents.outbreaks.097a904d3f3619db2fe78d24bc776098>
- Hassanain, N.A., Hassanain, M.A., Soliman, Y.A., Ghazy, A.A. and GhazyI, Y.A., 2009. Bovine tuberculosis in a dairy cattle farm as a threat to public health. *Afri. J. Microbiol. Res.*, **3**: 446-450.
- Ifrahim, M., 2001. *Epidemiological studies on tuberculosis in cattle and buffalo population in villages around Faisalabad*. M.Sc. Hons. thesis Department of Veterinary Microbiology, University of Agriculture Faisalabad, Pakistan.
- Islam, M.M., Siddique, M.A.R., Haque, M.A., Baki, M.A., Majumder, S., Parrish, J.J. and Shamsuddin, M., 2007. Screening some major communicable diseases of AI bulls in Bangladesh. *Livest. Res. Rural Dev.*, **19**: 1-9.
- Jalil, H., Das, P. and Suleman, A., 2003. *Bovine tuberculosis in dairy animals at Lahore, threat to the public health*. Metropolitan Corporation Lahore, Pakistan. pp. 1-11.
- Javed, M.T., Ahmad, L., Feliziani, F., Pasquali, P., Akhtar, M., Usman, M. and Cagiola, M., 2012. Analysis of some of the epidemiological risk factors affecting the prevalence of tuberculosis in buffalo at seven livestock farms in Punjab Pakistan. *Asian Biomed.*, **6**: 35-42. <https://doi.org/10.1016/j.actatropica.2010.04.004>
- Javed, M.T., Shahid, A.L., Farooqi, F.A., Akhtar, M., Cardenas, G.A., Wasiq, M. and Cagiola, M., 2010. Risk factors associated with the presence of positive reactions in the SCCIT test in water buffalo around two cities in Punjab, Pakistan. *Acta Trop.*, **115**: 242-247.
- Katale, B.Z., Mbugi, E.V., Karimuribo, E.D., Keyyu, J.D., Kendall, S., Kibiki, G.S., Godfrey, P., Faussett, A. L., Michel, R.R., Kazwala, VAN., Helden, P. and Matee, M.I., 2013. Prevalence and risk factors or infection of bovine tuberculosis in indigenous cattle in the Serengeti ecosystem, Tanzania. *Vet. Res.*, **9**: 267. <https://doi.org/10.1186/1746-6148-9-267>
- Khan, I.A., Khan, A., Mubarak, A. and Ali, S., 2008. Factors affecting prevalence of bovine tuberculosis in Nili Ram buffaloes. *Pak. Vet. J.*, **1**: 28-34.
- Konch, P., Dutta, B., Goswami, S., Barua, A.G. and Saikia, G.K., 2017. *Prevalence of bovine tuberculosis in Assam India*, **5**: 143-146.
- Kumar, H., Randhawa, S.N.S., Gupta, M.P., Brar, A.P.S., Kaur, K., Folia, G. and Chand, N., 2013. Bovine and caprine tuberculosis in Punjab. *Ind. Vet. J.*, **90**: 26-29.
- Mackay, C.R. and Hein, W.R., 1989. A large proportion of bovine T cells express the T cell receptor and show a distinct tissue distribution and surface phenotype. *Int. Immunol.*, **1**: 540-545. <https://doi.org/10.1093/intimm/1.5.540>
- Malama, S., Muma, J.B. and Godfroid, J., 2013. A

- review of tub Infectious diseases of poverty 2.1 erculosis at the wildlife-livestock-human interface in Zambia.* pp. 13. <https://doi.org/10.1186/2049-9957-2-13>
- Mondal, M.A.H., Parvin, M.S., Sarker, S.C., Rahman, A.K.M.A. and Islam, M.T., 2014. Prevalence and risk factors of bovine tuberculosis in cattle in Mymensingh Sadar. *Bangladesh J. Vet. Med.*, **12**: 179-183. <https://doi.org/10.3329/bjvm.v12i2.21283>
- Mukherjee, F., 2006. Comparative prevalence of tuberculosis in two dairy herds in India. *Rev. Sci. Tech. OIE*, **25**: 1125-1130. <https://doi.org/10.20506/rst.25.3.1717>
- Muller, B., Dürr, S., Alonso, S., Hattendorf, J., Laisse, C.J., Parsons, S.D. and Zinsstag, J., 2013. Zoonotic *Mycobacterium bovis* induced tuberculosis in humans. *Emerg. Infect. Dis.*, **19**: 6899. <https://doi.org/10.3201/eid1906.120543>
- Noorrahim, M.S., Shahid, K.M., Shah, A., Shah, M. and Rafiullah, H.A., 2015. Prevalence of tuberculosis in livestock population of district Charsadda by Tuberculin Skin Test (TST). *J. Entomol. Zool. Stud.*, **2**: 15-19.
- OIE, 2004. Office international des épizooties, 2004. *Manual of diagnostic tests and vaccines for terrestrial animals: 2004*. OIE.
- OIE, 2008. *Office international des epizooties terrestrial manual*. Chapter 2.4.7, *W. H. O.*
- OIE, 2009. *Manual of standards for diagnostic tests and vaccines, Bovine tuberculosis O.I.E.*, pp. 683-697.
- OIE, 2011. *World animal health information database*. [http://www.oie.int/wahis_2/public/wahid.php/Disease information/statusdetail](http://www.oie.int/wahis_2/public/wahid.php/Disease%20information/statusdetail).
- Peters, D., 2010. *Farm animals, signs and symptoms of tuberculosis in cattle*. UK. pp.1-8.
- Qamar, M.F. and Azhar, T., 2013. Detection of *Mycobacterium* from bovine milk in Lahore, Pakistan. *Sci. Int.*, **25**: 2.
- Rahman, M.M. and Samad, M.A., 2008. Prevalence of bovine tuberculosis and its effects on milk production in Red Chittagong cattle. *Bangladesh J. Vet. Med.*, **6**: 175-178. <https://doi.org/10.3329/bjvm.v6i2.2332>
- Ratan Das, Dandapat, P., Chakrabarty, A., Nanda, P.K., Bandyopadhyay, S. and Bandyopadhyay, S., 2018. A cross-sectional study on prevalence of bovine tuberculosis in Indian and crossbred cattle in Gangetic delta region of West Bengal, India. *Int. J. One Hlth.*, **4**: 1-7. <https://doi.org/10.14202/IJOH.2018.1-7>
- Rodwell, T.C., Whyte, I.J. and Boyce, W.M., 2001. Evaluation of population effects of bovine tuberculosis in free-ranging African buffalo (*Syncerus caffer*). *J. Mammal.*, **82**: 231-238. [https://doi.org/10.1644/1545-1542\(2001\)082<0231:EOP EOB>2.0.CO;2](https://doi.org/10.1644/1545-1542(2001)082<0231:EOP EOB>2.0.CO;2)
- Sayin, Z. and Erganis, O., 2013. Diagnosis of bovine tuberculosis by PPD-ELISA and sonication-ELISA. *Isr. J. Vet. Med.*, **68**: 180-184.
- Schiller, I., Oesch, B., Vordermeier, H.M., Palmer, M.V., Harris, B.N., Orloski, K.A., Buddle, B.M., Thacker, T.C., Lyashchenko, K.P. and Waters, W.R., 2010. Bovine tuberculosis. A review of current and emerging diagnostic techniques in view of their relevance for disease control and eradication. *Transb. Emerg. Dis.*, **57**: 205-220. <https://doi.org/10.1111/j.1865-1682.2010.01148.x>
- Silva, E., 2001. Evaluation of an enzyme-linked immunosorbent assay in the diagnosis of bovine tuberculosis. *Vet. Microbiol.*, **78**: 111-117. [https://doi.org/10.1016/S0378-1135\(00\)00282-0](https://doi.org/10.1016/S0378-1135(00)00282-0)
- Tahmid, A.S.M.T., Akter, M.R., Khatun, M.N., Mannan, M.A., Rahman, M.M. and Kabir, S.L., 2014. Investigation of bovine tuberculosis in rangpur division of Bangladesh. *J. Life Sci. Res.*, **1**: 1-4.
- Thakur, A., Sharma, M., Katoch, V.C., Dhar, P. and Katoch, R.C., 2010. A study on the prevalence of bovine tuberculosis in farmed dairy cattle in Himachal Pradesh. *Vet. World*, **3**: 409-414. <https://doi.org/10.5455/vetworld.2010.408-413>
- Trangadia, B.J., Rana, S.K. and Srinivasan, V.A., 2013. Prevalence of bovine tuberculosis in organized dairy farm. *Ind. J. Vet. Patthol.*, **37**: 72-74.