Benha Veterinary Medical Journal 43 (2022) 95-99

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Prevalence of tuberculosis in bovine slaughtered animals and suspected patients in Gharbia governorate

Amal, M. Elagdar[1], Abo-bakr, M. Edris[2], Gamal, I. Heikal[3], Samar M. Moustafa[4]

[1]*Veterinary authority, Gharbia governorate, Egypt*

[2]*Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University, Egypt*

[3]*Food Hygiene Department, Animal Health Research Institute-Tanta branch, ARC, Egypt*

[4]*Zoonosis Department, Faculty of Veterinary Medicine, Benha University, Egypt*

ARTICLE INFO

Keywords

Cattle

Mycobacterium bovis

Mycobacterium tuberculosis

PCR.

Received 16/09/2022
Accepted 30/09/2022
Available On-Line 09/10/2022

ABSTRACT

Bovine tuberculosis (bTB) is a chronic disease caused by *Mycobacterium bovis* which leads to high morbidity and mortality in animals and human. The objective of this study is to investigate the prevalence of tuberculosis in bovine slaughtered animals and its prevalence in human patients in Gharbia governorate, Egypt. A total of 600 random animals (300 cows, 150 calves, and 150 buffaloes) were routinely examined in one of slaughter houses of Gharbia governorate, Egypt during the year 2021 for detection of bovine tuberculosis. The suspected tuberculous lesions were collected from different organs of infected animals and the bacteriological examination revealed that the isolation rate was 70%, 80%, 66.7% in cattle, buffaloes and calves, respectively. PCR technique was used to confirm the results of bacteriological examination in 17 positive TB samples in buffaloes and bovine calves. The results showed that 94.1% agreement between bacteriological examination and PCR technique, as well as 35 sputum samples (23 samples from males and 12 females) were collected from suspected patients in the same governorate. The results also showed that 25.7% of patients were positive for TB with a higher prevalence in males (20%) than females (5.7%). PCR technique was used to confirm the results of bacteriological culture on 9 positive human being samples. The results showed that bacteriological culture was 100% consistent with PCR testing for human samples. So, the research documented the occurrence of bovine tuberculosis in slaughtered animals and human in Gharbia governorate which needs to reevaluate the bTB eradication program.

1. INTRODUCTION

Tuberculosis (TB) is a zoonotic disease which is caused by *mycobacterium bovis*, that is marked by a persistent infection and exhausting symptoms (Alonso et al., 2021) and is a member of the *mycobacterium tuberculosis* complex (MTC), which also includes *M. tuberculosis*, the cause of tuberculosis in humans, *Mycobacterium bovis* is the etiologic agent of bovine tuberculosis (bTB). This critical zoonotic illness is present in all countries, with developing nations having a pronounced prevalence. It is regarded as a socioeconomic disease that has a significant negative influence on herd productivity, which causes significant economic losses in agricultural activities. (Furlanetto et al., 2012). According to the World Health Organization (WHO), 1.2 million people died from tuberculosis (TB) disease in 2019, with an additional 208,000 deaths linked to the TB-HIV syndemic. Ten million people (range, 8.9-11.0 million) developed the disease in 2019 (WHO, 2020a). When someone with TB coughs, sneezes, speaks, or exhibits clinical signs, the risk of human TB transmission increases due to the exhaled air being inhaled.

In Egypt, the prevalence of bovine tuberculosis (bTB), which is caused by *Mycobacterium bovis*, ranged from 6.9% to 26.2% in cattle and buffaloes in the 1980s. Control programmes reduced this incidence to 2.6% throughout the 1990s, and the most recent survey, which was conducted in seven governorates in Egypt, revealed that the prevalence of the disease has been reduced to 0.05% (WHO, 1994). According to estimates, 2.1% and 9.4% of cases of pulmonary and extra pulmonary TB, respectively, and 3.1% of all human TB cases globally are caused by *M. bovis* (Ayele et al., 2004).

Serological tests for tuberculosis diagnosis relied on the detection of anti-tuberculous antibodies (or antibodies directed against *M. bovis* antigens) (Garbaccio et al., 2019; Singhla et al., 2019). Detection of *M. bovis* is a key that helps early diagnosis of the disease and remains critical in determining the disease's prevalence and eco-epidemiological standing.

The polymerase chain reaction (PCR) is a sensitive and fast diagnostic tool that can detect the agent in clinical samples in 48 hours; however, the presence of inhibitors in the samples can interfere with the PCR's performance. (Singh et al., 2004). Instead of amplicon fractionation by

* Corresponding author: manal30490@gmail.com

electrophoresis, the q-PCR approach adds a fluorescent probe in addition to primers to boost the specificity of the amplification of target DNA fragments, provide real-time observation, and streamline and expedite the diagnosis for result visualization. (Parashar et al., 2006).

Control strategies for tuberculosis in cattle rely heavily on ante-mortem and post-mortem inspection to identify infected animals, followed by epidemiologic investigations to identify herds at risk of infection (Humphrey et al., 2014). Abattoir surveillance is critical in bTB eradication programs, and its efficiency and accuracy should be assessed, particularly in endemic areas like Egypt.

Therefore, the aim of this study is to investigate the prevalence of tuberculosis in slaughtered animals and its prevalence in human patients in Gharbia governorate. Also, to compare the diagnosis of TB through using microbiological assays and PCR technique in both animals and human.

2. MATERIAL AND METHODS

1.1. Animal inspection:

The inspected carcasses were routinely examined according to the procedures of Egyptian Guidelines for cattle inspection according to law (517) (GOVS, 1986) and Gracey et al. (1999) at Elsanta abattoir in Gharbia governorate.

1.2. Animal samples:

A total of 600 random animals (300 cattle, 150 buffaloes and 150 bovine calves) were inspected from Jan. 2021 to Dec. 2021. Samples were collected from infected lymph nodes (maxillary, cranial and caudal mediastinal, retropharyngeal, superficial cervical, deep cervical, hepatic, renal, intestinal, internal iliac and mammary LNS) as well as infected organs and tissues that showed tuberculous lesions such as lung, pleura, liver, and kidney. The samples were collected from 60 infected animals (30 cows, 15 buffaloes and 15 bovine calves) and transferred to the laboratory in an ice box as rapidly as possible under strict hygiene measures for bacteriological examination.

1.3. Human samples:

A total of 35 Sputum samples were taken from selected patients attending clinics of respiratory medicine service. Over a three-month period, sputum specimens were collected from all patients suspected to be infected with pulmonary tuberculosis as well as 10 ml of bronchial trap fluid was also collected.

The collected samples were kept at 7 °C and transferred to the laboratory as rapidly as possible under strict hygiene measures. The specimens were analyzed by PCR by an individual blinded to the code. Aliquots from each specimen were stained and cultured for mycobacterium bacilli before being subjected to PCR for IS6110 and the Amplicor system.

2. Confirmation tests

2.1. Staining by Ziehl-Nelsen:

Clinical specimens taken from each macroscopic tuberculous lesion were stained by Ziehl-Nelsen stain according to Krieg and Phillips (1981) and Wentworth (1987).

2.2. Isolation of Mycobacterium species:

2.2.1. Preparation of tissue samples (Gange, 1996):

Organs and/or lymph nodes with obvious tuberculous lesions were cut into small pieces in aseptic environment and the fat was removed using sterile scissors before being placed in sterile mortar with sterile sand. Sputum was diluted with sterile normal saline and blended well. The inspected samples were crushed by the sand by sterile mortar's hand till be pasty. The sample was fully crushed before being mixed with two ml of sterile distilled water to create a suspension. Then, 2 ml of 4% H_2SO_4 were added, incubated for 30 minutes, and then were diluted in 16 ml of sterile distilled water and centrifuged for 20 minutes at 3000 rpm. The supernatant was decanted into 5% phenol and the sediment was used to make direct smear which was then inoculated into two Lowenstein Jensen medium slants, one tube with 4% sodium pyruvate and the other tube with 5% glycerol and then incubated at 37 °C for 3 weeks. Cultures were checked every day for one week and then once a week for 6-8 weeks.

The slants were examined daily for growth and isolates that grow within seven days are considered rapid grower. While those showed growth thereafter are considered as slow-growers.

2.3. Application of PCR assay:

Application of PCR for identification of IS6110 as species specific gene of *Mycobacterium bovis* at 500 bp and *Mycobacterium tuberculosis* at 541 bp was performed essentially by using primers (Pharmacia Biotech) as shown in the following table:

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
IS*6110* (F)	5′ CGTGAGGGCATCGAGGTGGC'3	500	Dilworth et al. (1996)
IS*6110* (R)	5′ GCGTAGGCGTCGGTGAC '3		
IS*6110* (F)	5′ GTGCGGATGGTCGCAGAGAT '3	541	Figueiredo et al. (2010)
IS*6110* (R)	5′ CTCGATGCCCTCACGGTT'3		

2.3.1. DNA Extraction using QIA amp kit (Shah et al., 2009):

Following overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100 °C for 20 min. 50-200 µl of the culture were accurately placed in Eppendorf tube and stored at -20 ºC till use.

DNA amplification:

2.3.2. DNA amplification of IS6110 gene for M. bovis (Dilworth et al,.1996):

A Thermal Cycler was used for the amplification (Master cycler, Eppendorf, Hamburg, Germany). PCR was carried out precisely in a reaction mix (50 µl) containing 5µl of 10× PCR buffer (Invitrogen), 200 µM dNTP, 2.5 U of recombinant Taq polymeras, 2.0 mM MgCl2, 5 µl of DNA, and 0.2 µM of the primer. Amplification was performed using an initial denaturation step at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 68 °C for 1 min, and elongation at 72 °C for 1 min. Cycling was finished by a final elongation step at 72 °C for 7 min. by using electrophoresis the reaction products were resolved Amplified DNA fragments were analyzed by 1.5% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1× TBE buffer stained with ethidium bromide and captured as well as visualized on UV trans illuminator. The sizes of fragments were determined by using A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH).

2.3.3. Amplification of IS6110 gene for M. tuberculosis (Figueiredo et al., 2010):

The collected sputum samples and bronchial washings (100 p1l) were mixed with 400 ul of lysis buffer containing 15% sucrose, 0.05 M Tris-HCl (pH 8-5) and 0-05M EDTA. PCR mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM $MgCl_2$, 0.01% (wt/vol) gelatin, 0.2 mM of each of the

deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP), 0.2 mM of the primer and 1 U of Taq Polymerase. The DNA amplification was done by initial denaturation one cycle of 95 °C for 5 min followed by 45 cycles of 95 °C for 30 sec, annealing at 60 °C for 1 min and extension at 72 °C for 1 min. The PCR products were analyzed by agarose gel electrophoresis on 2% agarose gel stained with ethidium bromide. For determination of a molecular size marker A 100 bp DNA ladder was used.

3. RESULTS

The recording results in table (1) indicated that the prevalence of tuberculosis in slaughtered animals at Elsanta abattoir in Gharbia governorate based on abattoir inspection. 6.7% of inspected old cows and old buffalos had generalized TB, while the result didn't reveal generalized T.B in inspected bovine calves in. Concerning the localized T.B the high incidence was recorded in lung of bovine calves (86.7%) in comparison to old cows (63.3%) and old buffalos (60%). Moreover, the results in table (2) revealed that the prevalence of bovine TB in slaughtered animals based on bacteriological examination. The results were positive for all cases of generalized T.B in old cows and buffalos with the same percentage (5%). On the other hand, there was a decrease in the incidence of localized T.B. in lung in old cows (46.7%) and old buffalos (53.3), while higher prevalence was recorded in bovine calves (60%).

Table (3) showed the prevalence of bovine tuberculosis in slaughtered animals at Elanta abattoir at Gharbia governorate based on PCR assay. Where the results revealed positive PCR reaction for all localized T.B lung samples for old buffaloes (100%), however, PCR reaction for 8 localized T.B samples out of 9 samples taken from bovine calves (88.9%).

Table (4) showed that the incidence of *Mycobacterium tuberculosis* in sputum samples taken from suspected patients. The results showed that 7 out of 23 samples were positive in males (30.4%) and 2 out of 12 samples were positive in females (16.7%). The results also revealed higher infection percentage in males (20%) than females (5.7%) based on bacteriological examination.

Table (5) revealed that the prevalence of *Mycobacterium tuberculosis* for sputum taken from suspected patients based on PCR assay gave positive PCR reaction for all suspected examined samples that previously showed positive in bacteriological examination

Table 1 Incidence of bovine tuberculosis in slaughtered animals based on abattoir inspection at Elsanta abattoir in Gharbia governorate.

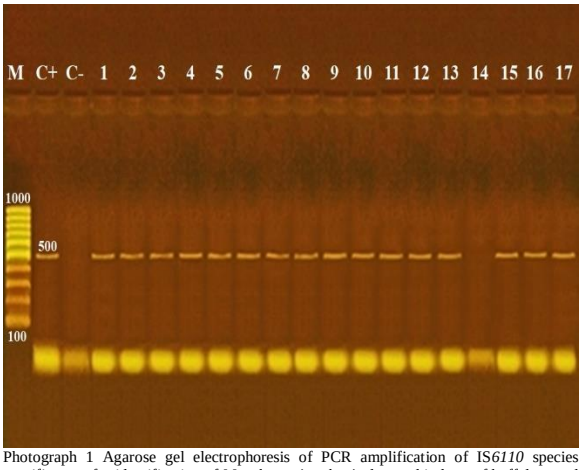
Carcass	Organ	Old cow (n=30)		Old buffalo (n=15)		bovine calves (n=15)		Total (n=60)	
		No.	%	No.	%	No.	%	No.	%
Generalized T.B.		2	6.7	1	6.7	0	0	3	5
Localized T.B.	Lung	19	3.3	9	60	13	86.7	41	68.3
	Liver		16.7	2	13.3	0	0	7	11.7
	Kidneys	1	3.3	0	0	1	6.7	2	3.3
	Udder	2	6.7	0	0	0	0	2	3.3
	Intestine	0	0	1	6.7	0	0	1	1.7
	Head	1	3.3	2	13.3	1	6.7	4	6.7
Total		30	100	15	100	15	100	60	100

* Percentages were calculated according to the total numbers of bovine tuberculosis infection.

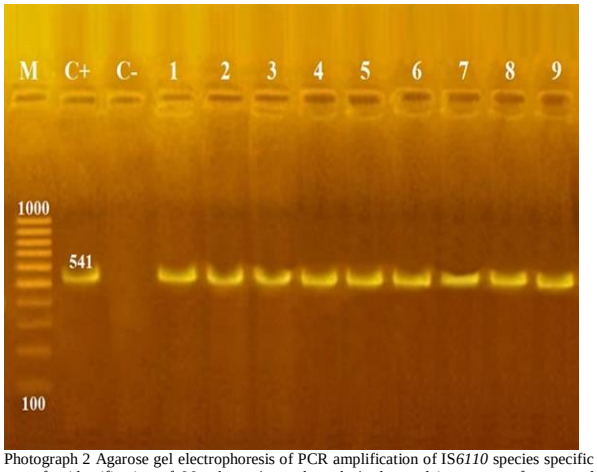
Table 2 Incidence of bovine tuberculosis in slaughtered animals based on bacteriological examination at Elsanta abattoir.

Carcass	Organ	Old cow (n=30)		Old buffalo (n=15)		bovine calves (n=15)		Total (n=60)	
		No.	%	No.	%	No.	%	No.	%
Generalized T.B.		2	6.7	1	6.7	0	0	3	5
Localized T.B.	Lung	14	46.7	8	3.3	9	60	1	51.7
	Liver	3	10	2	13.3	0	0	5	8.3
	Kidneys	1	3.3	0	0	0	0	1	1.7
	Udder	1	3.3	0	0	0	0	1	1.7
	Intestine	0	0	0	0	0	0	0	0
	Head	0	0	1	6.7	1	6.7	2	3.3
+ ve samples		21	70	12	80	10	66.7	43	71.7
- ve samples		9	30	3	20	5	33.3	17	28.3
Total		30	100	15	100	15	100	60	100

* Percentages were calculated according to the total number of tuberculosis lesions.



Photograph 1 Agarose gel electrophoresis of PCR amplification of IS*6110* species specific gene for identification of *Mycobacterium bovis* detected in lung of buffaloes and cow calves. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *M. bovis* for IS*6110* gene. Lane C-: Control negative. Lanes from 1 to 8: Positive buffalo lung for *M. bovis*. Lanes from 9 to 13 and 15, 16 & 17: Positive bovine calf lung for *M. bovis*. Lane 14: Negative bovine calf lung for *M. bovis*.



Photograph 2 Agarose gel electrophoresis of PCR amplification of IS*6110* species specific gene for identification of *Mycobacterium tuberculosis* detected in sputum of suspected patients. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *M. tuberculosis* for IS*6110* gene. Lane C-: Control negative. Lanes from 1 to 9: Positive patient's sputum for *M. tuberculosis*.

Table 3 Incidence of bovine tuberculosis in slaughtered animals at Elsanta abattoir in Gharbia governorate based on PCR assay.

Sex	Total examined samples	+ve T.B.		-ve T.B.	
		No.	%	No.	%
Old buffaloes	8	8	100	0	0
bovine calves	9	8	88.9	1	11.1
Total	17	16	94.1	1	5.9

Table 4 Prevalence of *Mycobacterium tuberculosis* in sputum samples from suspected patients

Patient Sex	Total examined specimens	+ve T.B		-ve T.B	
		No.	%	No.	%
Males	23	7	30.4	16	69.6
Females	12	2	16.7	10	83.3
Total	35	9	25.7	26	74.3

Table 5 Prevalence of *Mycobacterium tuberculosis* for sputum taken from suspected patients based on PCR assay.

patient Sex	Total examined specimens	+ve T.B		-ve T.B	
		No.	%	No.	%
Males	7	7	77.7	0	0
Females	2	2	22.2	0	0
Total	9	9	100	0	0

4. DISCUSSION

The main purpose of this study is to determine the prevalence of bovine tuberculosis in slaughtered animals and human beings at Elsanta slaughterhouse. The routine Post-mortem inspection of slaughtered cattle revealed 60 slaughtered animals infected with tuberculosis in the examined abattoir. So, the postmortem examinations are not accurate enough and confirmed by bacteriological examination to identify the positive TB results. The results agree with those obtained by Amin et al. (2015) who showed that isolation rate of *Mycobacterium bovis* was 75% and 58.3% in cattle and buffaloes, respectively, in Egypt, and also the results agreed with bacteriological examination isolation rate for *M. bovis* recorded by Hasanen et al. (2017) that was 86.9% and 57.1% for cattle and buffaloes, respectively in Egypt. Also, these results agreed with Vordermeier et al. (2012) and Bermingham et al. (2009), This may be due to cattle suffer from poor housing, mal-nutrition or may be kept under intensive or semi-intensive production system so they became more susceptible to infection (Nega et al., 2012). These results were higher than that obtained by Khan et al. (2014) who showed that 14.04% and 13.33% for buffaloes and cattle respectively as well as that obtained by Singhla and Boonyayatra (2022). Since buffaloes spend a lot of time wallowing in the mud to reduce thermic stress, which can be a possible source of spreading *M. bovis* among the herd, these results may be attributable to the fact that buffalos are more adapted to defend themselves from the heat than cattle. Since buffalos are more resilient to adverse environmental circumstances than cattle, farmers may give buffaloes less grain and less frequent herd health care than they do with cattle.

Moreover, genetic variations between cattle and buffalo were also in consideration *M. bovis* occurrence (Carneiro et al., 2019). Additionally, a difference in the number of investigated animals could account for some of the results.

PCR technique was applied to confirm the results of bacteriological examination. So, the study was consistent with the results obtained by Amin et al. (2015) and Hasanen et al. (2017). PCR has been evaluated for the detection of *M. bovis* from a range of specimens and seems to have sensitivity equal to or greater than that of the culture method, but in short time (Beige et al., 1995). PCR technique in the present study was a rapid and accurate method for diagnosis of TB within 3 days, while bacteriological culture took several weeks.

Concerning, bacteriological examination results of suspected patients' sputum, nearly similar results were recorded by Horton et al. (2016), who found that in low- and middle-income countries, men have a significantly higher TB prevalence than women. PCR technique was used in the current study confirmed the results of bacteriological examination in positive human samples. The results showed that bacteriological examination was 100% consistent with PCR technique for examined human samples, and this agree with the study conducted by Sánchez-Carvajal et al. (2021), who found higher agreement between both assays ($\kappa = 0.83$).

Also, according to the World Health Organization (WHO), the ratio of male to female tuberculosis (TB) prevalence worldwide is 1.85. (WHO, 2009).

The prevalence of TB in men and women varies greatly between nations, and in certain places, the frequency is higher in women. males appear to make up the bulk of adult TB cases in Africa and Eastern Europe (Lawson et al., 2010; Uwizeye et al., 2011) Other causes have also been offered, including male social behaviors and the differential between male and female vulnerability to tuberculosis (Uplekar et al., 2001; Neyrolles et al., 2009).

Different methods can cause spread of tuberculosis. The bacteria can spread in exhaled air, sputum, urine, feces, and pus. Depending on the species involved, aerosol inhalation can transmit the disease through direct touch or contact with contaminated animal excrement (Philips et al., 2003).

According to Joardar et al. (2003) the third of the world's human populations are infected with tuberculosis The zoonotic nature of tuberculosis makes it potentially contagious to people, making it a serious public health concern with significant economic implications for the livestock business (O'Reilly and Daborn, 1995).

Although *M. bovis* is not the primary cause of human tuberculosis, it can still infect humans through direct contact with infected animals as well as through the consumption of raw milk, meat, and other products from contaminated animals (Malama et al., 2013; Verma et al., 2014).

5. CONCLUSION

The recorded results revealed higher disagreement in the diagnosis of tuberculosis by postmortem examination at abattoir and bacteriological examination may lead to false condemnation of slaughtered carcass. Moreover, the results showed that typical agreement between the bacteriological examination and PCR technique for diagnosis of bovine TB.O However, PCR is more expensive than bacteriological examination and require skilled personnel. The prevalence of *Mycobacterium tuberculosis* in human being is higher in males than females. Tuberculosis is a very important zoonotic disease that needs to be considered in all country because of its risk on animal and human health.

5. REFERENCES

1. Alonso,N , Griffa ,N , Moyano,R,D, Mon,M,L ,Olivieri M.A.C , Barandiaran S , Vivot M.M , Fiorini G , Canal A.M, Santangelo M.P , Singh M , Romano M.I,(2021). Development of a lateral flow immunochromatography test for the rapid detection of bovine tuberculosis. Journal of Immunological Methods, Elsevier. 491: 112941.1-5
2. Amin R.A, Nasr E.A, El-Gaml A.M, Saafan E.M (2015). Detection of Tuberculosis in slaughtered food animals by using recent technique. Benha Veterinary Medical Journal, 2:129-134.
3. Ayele WY, Neill SD, Zinsstag J,(2004): Bovine tuberculosis: an old disease but a new threat to Africa. Int J Tuberc Lung Di. ;8(8):924–93.

4. Bermingham M. L., More S. J., Good M., Cromie A. R., Higgins I. M., Brotherstone S.& Berry D. P.. 2009Genetics of tuberculosis in Irish Holstein Friesian dairy herds. J. Dairy Sci. 92, 3447–3456. (doi:10.3168/jds.2008-1848). Crossref, PubMed, ISI, Google Scholar
5. Carneiro PAM, Takatani H,Pasquatti TN, Silva CBDG, Norby B,Wilkins MJ, Zumárraga MJ, Araujo FR and Kaneene JB (2019)Epidemiological Study ofMycobacterium bovis Infection inBuffalo and Cattle in Amazonas,Brazil. Front. Vet. Sci. 6:434.5-7.
6. Dilworth, J.; Goyal, M.; Young, D. and Shaw, R. (1996): Comparison of polymerase chain reaction for IS6110 and Amplicor in the diagnosis of tuberculosis. Thorax, 51: 320-322.
7. Figueiredo, J.; Carvalho, R.; Silvestre, F.; Lilenbaum, W.; Fonseca, L.; Silva, J. and Paschoalin, V. (2010): Detection of Mycobacterium bovis and in nasal swabs from tuberculous cattle by a multiplex PCR. Brazilian J. Microbiol., 41: 386-390.
8. Furlanetto, L.V., Figueiredo, E.E.S., Conte Júnior, C.A., Silva, F.G.S., Duarte, R.S., Silva, J.T., Lilenbaum, W., & Paschoalin, V.M.F. (2012). Prevalence of bovine tuberculosis in herds and animals slaughtered in 2009 in the state of Mato Grosso, Brazil. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 64(2), 274–280.
9. Garbaccio, S.G., Garro, C.J., Delgado, F., Tejada, G.A., Eirin, M.E., Huertas, P.S., Leon, E. A., Zumarraga, ´M.J. (2019). Enzyme-linked immunosorbent assay as complement of intradermal skin test for the detection of mycobacterium bovis infection in cattle. Tuberculosis. 117, 56–61.
10. GOVS (1986): General Organization for Veterinary Services, law (517).
11. Gracey, J.F.; Collins, D.S. and Huey, R.J. (1999): Meat Hygiene, 3rd ed., W.B. Saunders Company Ltd, Pp. 669-678.
12. Grange, J.M. (1996): Human and bovine tuberculosis-new threats from an old disease. BritishVet. J., 125: 3-8.
13. Hasanen F.S, Hassan M.A, Moawad E (2017). Studies on Tuberculosis in Slaughtered Animals at Menufia Governorate. Benha Journal of Applied Sciences (BJAS), 2: 99-102.
14. Horton KC, MacPherson P, Houben RMGJ, White RG, Corbett EL (2016) Sex Differences in Tuberculosis Burden and Notification in Low-and Middle-Income Countries:A Systemic Review and Meta-analysis.PLoS Med 13(9): e1002119.
15. Humphrey, H.M., Orloski, K.A. & Olea-Popelka, F.J. (2014). Bovine tuberculosis slaughter surveillance in the United States 2001–2010: assessment of its traceback investigation function. BMC Vet Res 10 (1)182: 1-10.
16. Beige J, Lokies J, Schaberg T, Finckh U, Fischer M, Mauch H, Lode H, Köhler B, Rolfs A. (1995). Clinical evaluation of a Mycobacterium tuberculosis PCR assay. J Clin Microbiol. 33(1):90–95.
17. Joardar, S.N., Ram, G.C. and Goswami, T.K. (2003) Mycobacterium bovis AN5 Antigens Vary in their ability to induce nitric oxide production in blood monocytes of experimentally infected cattle. Veterinary Immunology and Immunopathology, 93, 61-68.
18. Khan J, Ayaz S, AbdElsalam N.M, Ullah R and Shah T (2014). Prevalence of tuberculosis in buffalo and cattle. Journal of Pure &Applied Microbiology, 8:721-726.
19. Krieg, R. and Phillips, G. (1981): Manual of methods for general microbiology. ASM Press, Washington DC, USA.
20. Lawson L, Yassin MA, Onuoha AN, Ramsay A, Anderson de Cuevas RR, Theobald S, Davies PDO, Cuevas LE (2010): Yield of smear microscopy and radiological findings of male and female patients with tuberculosis in Abuja, Nigeria. Hindawi Publishing Corporation Tuberculosis Research and Treatment (9): 241659:1-5.
21. Malama, S., Muma, J.B. and Godfroid, J. (2013) A Review of Tuberculosis at the Wildlife-Livestock-Human Interface in Zambia. Infectious Diseases of Poverty, BioMed Central Ltd 2:13.2-5.
22. Nega, M., Mazengia,H. and Mekonen, G. 2012. Prevalence and zoonotic implications of bovine tuberculosis in Northwest Ethiopia. Int J Med Sci 2(9): 188–192.
23. Neyrolles O, Quintana-Murci L: Sexual inequality in tuberculosis. PLoS Med. 2009, 6 (12): e1000199-10.1371/ journal. pmed. 1000199.
24. O'Reilly, L.M., Daborn, C.J., 1995. The epidemiology of Mycobacterium bovis infections in animals and man – a review. Tubercle Lung Dis. 76, 1–46.
25. Parashar, D., Chauhan, D.S., Sharma, V.D., & Katoch, V.M. (2006). Applications of real-time PCR technology to mycobacterial research. Indian Journal of Medical Research 124(4), 385–398.
26. Phillips, C.J.C., Foster, C.R.W., Morris, P.A. and Teverson, R. (2003) The Transmission of Mycobacterium bovis Infection to Cattle. Research in Veterinary Science, 74, 1-15.
27. Sánchez-Carvajal JM, Relaño Á., Ruedas-orres A, Jurado-Martos F, Larenas-Muñoz F, Vera E, Gómez-Gascón L, Cardoso-Toset F, Rodríguez-Gómez IM, Maldonado A, Carrasco L, Tarradas C, Gómez-Laguna J and Luque I (2021) Real-Time PCR Validation for *Mycobacterium tuberculosis* Complex Detection Targeting IS6110 Directly from Bovine Lymph Nodes. Front. Vet. Sci. 8:643111.1-9. doi: 10.3389/fvets.2021. 643111
28. Shah, D.; Shringi, S.; Besser, T. and Call, D. (2009): Molecular detection of food borne pathogens, Boca Raton: CRCPress, In Liu, D. (Ed). Taylor & Francis group, Florida, USA. Pp. 369-389.
29. Singh SK, Verma R, Shah DH. Molecular finger printing of clinical isolates of Mycobacterium bovis and Mycobacterium tuberculosis from India by restriction fragment length polymorphism (RFLP). J Vet Sci. (2004); 5(4): 331–335.
30. Singhla, T., Boonyayatra, S., Chulakasian, S., Lukkana, M., Alvarez, J., Sreevatsan, S., Wells, S.J. (2019). Determination of the sensitivity and specificity of bovine tuberculosis screening test in dairy herds in Thailand using a Bayesian approach. BMC Vet. Res. 15 (1), 149.1-7. doi: 10.1186/s12917-019-1905-x.
31. Singhla, T., and Boonyayatra, S., (2022). Prevalence, Risk Factors, and Diagnostic Efficacy of Bovine Tuberculosis in Slaughtered Animals at the Chiang Mai Municipal Abattoir, Thailand. Front. Vet. Sci., Sec. Veterinary Epidemiology and Economics 9: 846423, 1-7.
32. Uplekar MW, Rangan S, Weiss MG, Ogden J, Borgdorff MW, Hudelson P (2001): Attention to gender issues in tuberculosis control. Int J Tuberc Lung Dis. 5 (3): 220-224.
33. Uwizeye CB, De SG, Gilca R, Schwartzman K, Gasana M (2001): Tuberculosis may be underestimated in Rwandan women. Int J Tuberc Lung Dis. 15 (6): 776-781.
34. Verma, A.K., Tiwari, R., Chakraborty, S., Neha, Saminathan, M., Dhama, K., and Singh, S.V. (2014) Insights into bovine tuberculosis (bTB), various approaches for its diagnosis, control, and its public health concerns: An update. Asian Journal of Animal and Veterinary Advances, 9, 323-344.
35. Vordemeier M, Ameni G, Berg S, Bishop R, Robertson B. 2012. The influence of cattle breed on susceptibility to bovine tuberculosis in Ethiopia. Comp Imm Microb Inf. Dis 35: 227–232.19.
36. Wentworth, B. B. (1987): Diagnostic procedures for bacterial infections. 7th ed., American Public Health Association, Washington DC, USA.
37. WHO (1994) Zoonotic tuberculosis (*Mycobacterium bovis*): memorandum from a WHO meeting (with the participation of FAO). Bull World Health Organ, 72: 851–857.
38. WHO (2009) Global Tuberculosis Control: A Short Update to the 2009 Report. 2009, World Health Organization, Geneva, Switzerland.
39. WHO (2020). Global Tuberculosis report, Geneva, Switzerland.