Egypt. J. Chem. Environ. Health, 2 (2):220-231 (2016)
On line ISSN: 2536-9164.

Prevalence of Mycobacterium in Cattle Milk and Some Milk Products in El sharkia Governorate

Saeid ,I.M., Riad,E.M.and Hassan,M.Gab-ALLa
Animal Health Research Institute –Dokki- Giza- Zagazeg branch

Abstract

The use of raw milk in the production of cheese and other dairy products considered as potential public health risk associated with bovine tuberculosis , as viable mycobacteria (including *M. bovis*) have been found to survive in unpasteurized cheeses. Unpasteurized milk derived from infected cows was regarded as the principal vehicle of infection for humans before the advent of compulsory milk pasteurization.

A total of 459 samples were collected including 200 milk samples, 85 ice-cream samples ,97 kareish cheese samples and 77 yoghurt samples. 100 milk samples were collected from (3) private farms and 100 milk samples were collected from street venders as well as the milk products samples which collected randomly from dairy farms and markets. All milk and milk products samples were prepared and examined by conventional methods (cultural and microscopic procedures) as well as by using real time PCR.

The conventional culture technique showed that, Out of 459 examined milk and milk products samples, 12 samples were positive for *Mycobacterium bovis* with a percentage of 2.6% . Regarding to conventional culture technique on raw milk samples, 5 out of 200 were positive to *Mycobacterium bovis* with a percentage of isolation reached (2.5 %).

While (2) out of 97 kareish cheese samples were positive with a percentage of (2%) and (2) samples out of 85 ice cream samples were positive with a percentage of (2.3%) . While the results of PCR revealed(8) of examined milk samples were positive and (9) milk products samples were positive for mycobacterium bovis using real time PCR.

Key words: Mycobacterium tuberculosis, Real time PCR, Conventional method ,Milk and Milk Products.

Introduction

Tuberculosis is one of the infectious diseases causing highest mortality rates world wide . Eighteen people are affected with TB every minute globally and three of them die per minute (**WHO, 2013).**

Mycobacterium tuberculosis is the etiologic agent of tuberculosis in humans. While ***Mycobacterium bovis*** is the etiologic agent of TB in cows and rarely in humans. Both cows and humans can serve as reservoirs. Humans can also be infected by the

consumption of unpasteurized milk. This route of transmission can lead to the development of **extrapulmonary Tuberculosis. (Tatiana *et al.*, 2014)** .

Spahr and Schafroth (2001) recorded that the use of raw milk in the production of cheese and other dairy products is another potential public health risk associated with tuberculous cattle, as viable mycobacteria (including *M. bovis*) have been found to survive in unpasteurized cheeses. unpasteurized milk derived from infected cows was regarded as the principal vehicle of infection for humans before the advent of compulsory milk pasteurization. Unpasteurized milk and milk products continue to be regarded as the main vehicle for transmission in countries where bovine TB is prevalent and eradication programmes are patchy or non-existent.

The Conventional culture-based detection techniques of such pathogen remain the golden standard technique so it is applied on the collected samples for detection and studying of the mycobacterium isolates even it is time consuming test and have lack in sensitivity and specificity .The sensitivity of the cultural method was discussed by **Taylor *et al.* (2007)** who mentioned that the isolation of mycobacteria and their identification based on phenotypical characters. But due to the fact that these methods are time consuming, their use is on the decline **(Thoen *et al.*, 2006).**

The PCR technique is much faster than culture and reduces the time for diagnosis to 2 days so , PCR method could be used as a rapid screening technique which is complementary to culture method for the routine diagnosis of bovine tuberculosis..

It also provides for the detection of *M. bovis* when rapidly growing *Mycobacterium* spp. are present in the sample and may be able to detect the presence of *M. bovis* in samples even when organisms have become non viable.

The aim of this study is directed mainly to study the prevalence rate of tuberculosis in raw milk and some milk products in El-sharkia governorate in addition to the comparative study on the used diagnostic technique.

Material and Methods

Collection of the samples:

This study was conducted at different localities in El sharkia Governorate in the period between DEC 2015 to FEB 2016

A total of 459 samples were collected including (200) milk samples, (85) ice-cream samples ,(97) kareish cheese samples and (77) yoghort samples). 100 Milk samples were collected from (3) private farms and 100 Milk samples were collected from street venders as well as the milk product samples which collected

randomly from dairy shops and markets , milk and Milk product samples were prepared and examined by conventional methods (cultural and microscopic procedures) as well as using real time PCR.

Bacteriological examination of the collected samples:

The milk samples were collected under complete aseptic conditions from dairy cattle in sterile containers, after cleaning and washing the teats and udder, the last strip of milk was collected. Samples were transferred in ice box as soon as possible to the laboratory for bacteriological examination.

A) Preparation of milk samples: (Corner *et al*., 1995)

A total volume of 50 milliliters was collected per sample, each sample was placed in a sterilized flask at the time of collection.The samples were immediately stored on ice in isothermal boxes until their arrival the laboratory. the milk samples were centrifuged for 30 min at 3000 rpm than, freshly used

B) Preparation of milk products samples:

It was done according to **: (A P H A, 1992)** as follow

Ten grams from the prepared milk products samples were emulsified in a sterile ethylene bag with 90 ml of sterile sodium citrate 2% solution at 40˚C stomacher.

Culture of milk samples (Quinn *et al*., 1994) and (Corner *et al*., 1995)

(Except for Middle brook 7H10 agar media was incubated for maximum 24 days).The identification of isolated mycobacteria was initially based on acid-fastness and microscopical ly by using Ziehl – Neelsen stain technique, according to ***collee et al* , (1996)** and the growth characteristics as time and colony features according to **Ernst (1990).**

Real Time PCR

It was carried out according to **(Wards *et al*., 1995)**

- Extraction of DNA (thermo scientific, GeneJET Genomic purification kit).

- Detection of M. tuberculosis complex :

Results and Discussion

Results of the conventional culture technique presented in table (1) showed that the, Out of 459 examined milk and milk products samples, there were 12 samples were positive for *Mycobacterium bovis* wtih a percentage of (2.61%) and all positive samples were harbored the acid fast bacilli. Regarding to conventional culture technique on raw milk samples, 5 out of 200 examined samples were positive to *Mycobacterium bovis* with a percentage of isolation reached (2.5 %). While (2) out of 97 kareish cheese samples were positive by culture method with a percentage of (2 %) and (3) samples out of 85 cream samples were positive with a percentage of (3.5 %) and 2 positive samples out of 77 yogurt samples. .

On the other hand ,the detection of mycobacterium contamination of (97) kareish cheese and (85) cream samples by real time PCR revealed that (4) kareish

cheese samples were positive and harbored mycobacterium microorganisms as well as (3) ice cream positive samples and (2) positive yogurt samples as mentioned in table (2).

Concerning for PCR assay, all samples were tested and confirmed using real time PCR. The obtained results revealed that, 8 out of 200 raw milk samples were positive with percentage of (4 %). While all tested isolates of mycobacterium spp. were confirmed as mycobacterium species with percentage (100%) by using the primers of *Mycobacterium tuberculosis* complex.

Photo (1) showed the amplification plot of 200 tested milk samples and the analysis for the amplification plot in its linear form expressed about (8) samples at cycle 14 which is characteristic for TB and one control positive sample, where the used reference dye is (FAM) and the run is for 45 cycles.

Photo (2) showed the amplification plot of tested milk products samples and the analysis for the amplification plot in its linear form expressed (9) positive samples at cycle 14 and one control positive sample, where the used reference dye is (FAM) and the run is for 45 cycles.

Table (3) showed the comparison between results of culture technique and PCR assay for diagnosis of bovine tuberculosis among milk and milk products samples where 12 samples were positive by Culture technique with percentage of isolation reached (2.6%), while the same tested samples revealed (17) positive samples by RT-PCR with percentage reached (3.7%)..

Milk is an important source of proteins, sugars, lipids and other nutrients for humans. However , these nutrients can also serve as substrates for pathogenic micro-organisms such as Mycobacterium species**(Di Pinto *et al.* , 2006).** Where presence of Tuberculosis which is considered one of the heighest mortality rates world wide among the infectious diseases. Eighteen people are affected with TB every minute globally and three of them die per minute **(WHO, 2013).**

Results recorded in table (1) revealed that, Out of 459 examined milk and milk products samples, 12 samples were positive for *Mycobacterium bovis* (2.6%) by culture technique and all positive samples were harbored the acid fast bacilli.

Regarding to conventional culture technique on raw milk samples, 5 out of 200 raw milk samples were positive to Mycobacterium bovis with a percentage of isolation reached (2.5 %).

The use of raw milk in the production of cheese and other dairy products is another potential public health risk associated with tuberculous cattle, as viable mycobacteria (including *M. bovis*) have been found to survive in mature unpasteurized cheeses. **(Spahr and Schafroth**, **2001)**.

Nearly similar results were recorded by **Al- Saqur *et al*. (2009)** in Iraq who detected 3 (4.4%) positive samples out of 68 examined milk samples by microscopical examination. On the other hand , in Egypt **Wahba *et al*. (2013)** found relatively higher results of 3(6%) out of 50 milk samples examined microscopically. Higher results were obtained by **Gad *et al.* (2000)** in Egypt, (5.6%) **Abou-Eisha *et al.* (2002)** found a higher results for isolation of *Mycobacterium bovis* from the milk which was 2 (7.7%) of the 26 tuberculin-positive dairy cattle in Port Said, Egypt, during January 2000 to December 2001) .

Also, similar results were detected in Nigeria by **Ofukwu *et al.* (2008)** who found 4 (1.4%) of the 285 freshly drawn milk positive samples by culture and microscopical examination and low detection percentage of the tubercle bacilli organism in milk samples and in Tanzania, **Kazwala *et al.*(1998)** found that out of 805 milk samples that were collected, 31 (3.9%) were positive by culture. and in Brazil, (**Isabel *et al* ,2008)** found 78 (10%) positive samples out of 780 milk samples examined by culture which is higher than our obtained results. However, the milk samples of 8 tuberculin-reacting dairy cattle were negative for acid fast bacilli culture and the results indicated that cattle and buffaloes still act as potential reservoirs of tuberculosis for man. Furthermore, **Hamid *et al.* (2003)** In Pakistan conducted a study at Lahore and isolated M.bovis from milk samples of four cows out of 16 (25%) with confirmed bovine tuberculosis. In this study (2) out of 97 kareish cheese samples were positive by culture method with a percentage of (2%) and (3) samples out of 85 ice cream samples were positive with a percentage of (3.5%). There are low prevalence rate was obtained by **Centers for Disease Control and Prevention. (2005)** which mentioned that investigation in New York City reported that 1% of culture-positive tuberculosis cases in milk in this area were due to *M. bovis*.

De La Rua-Domenech R.(2006) found that *Mycobacterium bovis*, are highly able to survive in bovine milk and other dairy products where it can be found in the form of viable bacilli in cream, cheese and yogurt produced from raw milk for over 14 days and in butter for over 100 days and mentioned that there are no validated laboratory methods that allow the certification of such untreated milk or dairy products as "free of viable mycobacteria" , and found that *M. bovis* does not multiply in milk or does so very slowly, the large number of mycobacteria that are secreted into the milk .

Higher prevalence rates were detected in Canadian cattle in Ethiopia by **Ameni, *et al.* (2007)** who proved that out of examined 1171 animals there were 548 (46.8%) , that is due to the intensive system of housing at which the imported cattle were kept and leads to lowering the immune system of the cattle.

Moreover, in Iraq, **Al- Saqur *et al*. (2009)** conducted a study on 68 raw milk sample, the positive rates for culture were 7 (10.2%) . Also, similar results in Egypt were obtained by **Hassanain *et al*. (2009)** who mentioned that, in some private farms in Egypt, Mycobacterial culture of milk samples revealed (4.35%) of the collected 23 bovine milk samples were positive for M.bovis isolation. Moreover, Similar results were obtained by **Ben kahla *et al.* (2011)** In Tunisia who proved that, out of 102 SCITT positive cows, 5 were detected as shedders of *M.bovis* in their milk. , Similar results were detected by **Franco *et al*. (2013)** who mentioned that, mycobacteria was isolated from 24 (8%) out of examined 300 milk samples.

All collected samples were tested by using real time PCR. The obtained results of molecular detection of *Mycobacterium tuberculosis* complex by Real time PCR. were shown in table (2) revealed that, 8 out of 200 raw milk samples were positive with percentage of (4 %). On the other hand ,the detection of mycobacterium contamination of (97) kareish cheese and (85) ice cream samples and (77) yogurt samples by real time PCR revealed that (4) kareish cheese samples, (3) ice cream and 2 yogurt samples were positive and harbored mycobacterium microorganisms and this result is lower than that obtained in Tunisia in 2011 (3.1%) which was attributed to the small quantity of produced milk that sold at retail and may be consumed raw or used for producing fermented dairy products **(Cadmus and Adesokan, 2007 , Jorado *et al*.,2009 and Ben Kahla *et al.*, 2011).**

Gertman *et al.*(1990) and Jha *et al*.(2007) recorded a percentage of isolation as high as 34% and 24% respectively from raw milk samples. These exaggerated percentage of isolation may attributed to the fact that raw milk samples representing bulk tank milk which could be contaminated by few number of infected cows. Moreover, real time PCR is more accurate and faster than conventional method for TB diagnosis. early diagnosis of TB disease is crucial in initiating treatment and interrupting the strain transmission. Similar results were obtained by **Silaigwana *et al.* (2012)** who detected 5.5% positive milk .

On contrast **Ereqat *et al*., (2013)** failed to detect any positive cases among 30 examined milk samples and the higher detection results of real time PCR was discussed by **Al- Saqur *et al*. (2009)** who proved that only three bacilli in milk samples sufficient to be detected by real time PCR. and 7 (10.3%) positive samples were recorded which are higher than that obtained by culture, as the PCR give high sensitivity and specificity for the Mycobacterium. Higher results were obtained by **Leite *et al.* (2003)**, who conduct a study on (128) bovine milk samples from retail markets in the State Sâo Paulo, out of them there were 23 (18%) positive milk samples by PCR. and **Srinand *et al.* (2000)** who examined and identified , *M. bovis* in 32.6% of 46 pools of milk from cattle of tuberculin test-positive herds by PCR. In

contrast, the 70 Argentine cattle showed a modest prevalence of M. bovis shedding in milk (1.4%).

Low results obtained than our results by **El-Gedawy *et al.* (2014)** who examined One hundred bulk tank milk samples were collected from three dairy farms at Sharkia Province, Egypt, to isolate M.bovis by PCR, and found only the percentage was 1%, and the detection of M. bovis in milk samples.

Concerning the use of molecular diagnosis , Liebana *et al.*, (1995) described a simple, rapid method for the extraction of DNA from bovine tissue samples was developed and used in a PCR designed for the diagnosis of tuberculosis. Tissues from 81 cattle from tuberculosis-infected herds (group 1) and 19 cattle from tuberculosis-free herds (group 2) were tested in this PCR, and the results were compared with those of conventional culture. The PCR assay detected 71.4% of the culture-positive animals from group 1. Tissues from all animals in group 2 were negative in the PCR assay and by culture. The described method could be used as a rapid screening technique which would be complementary to culture of tissue specimens for the routine diagnosis of bovine tuberculosis. The PCR technique is much faster than culture and reduces the time for diagnosis from several months to 2 days. It also provides for the detection of *M. bovis* when rapidly growing *Mycobacterium* spp. are present in the sample and may be able to detect the presence of *M. bovis* in samples even when organisms have become un viable.. As early diagnosis of TB disease is crucial in initiating treatment and interrupting the strain transmission.

Conclusion :

- The PCR technique is much faster than culture and reduces the time for diagnosis So, the rapid detection and early diagnosis of mycobacterium spp. is essential and important especially toward the public health issue.
- The rapid detection of mycobacterium spp. Is essential and important toward the public health issue .

Table (1) Results of bacteriological examination of (459) milk and milk products samples by conventional culture technique:

Type of samples	**No. of tested samples**	Cultural method	
		Positive	%
milk products			
1- kareesh cheese	**97**	**2**	**2.0**
2- ice cream	**85**	**3**	**3.5**
3- youghort	**77**	**2**	**2.6**
raw milk	**200**	**5**	**2.5**
Total	**459**	**2.60**	

Egypt. J. Chem. Environ. Health, 2 (2):220-231 (2016)
On line ISSN: 2536-9164.

Table (2) Illustrated results of real time PCR assay on raw milk and milk products

Type of samples	Number of tested samples	Real time-PCR	
		positive samples	%
milk products 1-kareish cheese 2-cream 3-yougort	 97 85 77	 4 3 2	 4.1 % 3.5 % 2.6 %
milk samples	200	8	4 %
Total	459	17	3.7 %

Table (3) Comparison between results of culture technique and PCR assay

Types of Samples	Number of tested samples	Culture technique		RT-PCR	
		Positive		Positive	
		Number	%	Number	%
Milk and Milk products samples	459	12	2.6%	17	3.7 %

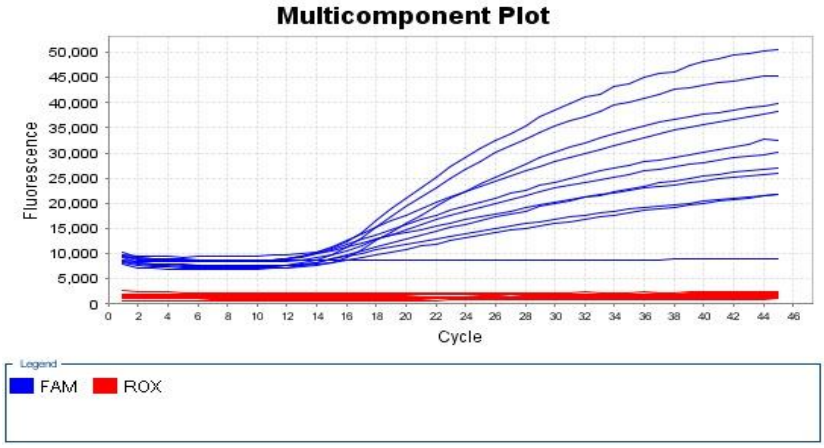

FIG.(1): The amplification plot of suspected milk products samples. Analysis for the amplification plot in its linear form: Nine positive samples at cycle 14 and one control positive sample, one negative samples .The used reference dye is (FAM). The run is for 45 cycles.

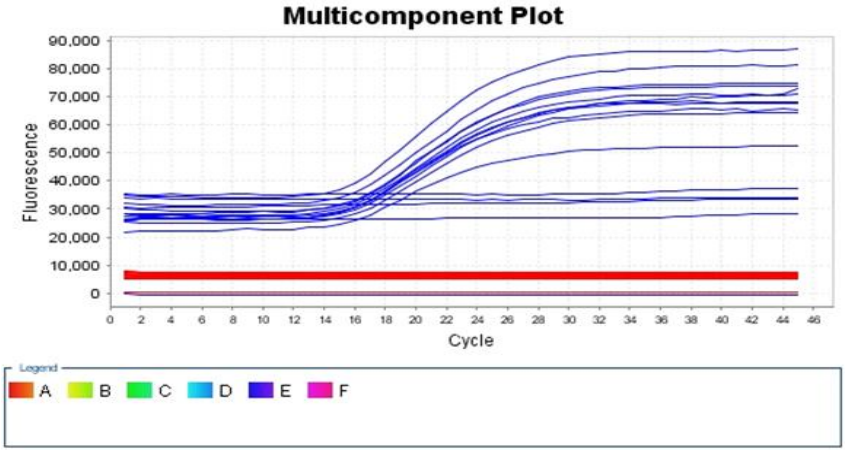

FIG. 2. Results of PCR assay showed (8) positive samples out of (200) tested raw milk samples and one control positive samples and (2) negative samples and one control negative samples.

References

Abou- Eisha, A.M.; El-Attar, A.A. and El-Sheary, M.N. (2002): Bovine and atypical mycobacterial infections of cattle and buffaloes in Port Said Province, Egypt. Assiut Veterinary Medical Journal 47(93):152-162

AL-Saqur, I.M.; AL-Thwani, A.N. and Al-Attar, I.M. (2009): Detection of *Mycobacteria spp.* In cow's milk using conventional methods and PCR. Iraqi Journal of Veterinary Sciences, Vol. 23, Supplement Ⅱ, 2009 (259-262) Proceeding of the 5th Scientific Conference, College of Veterinary Medicine , University of Mosul .

Ameni, G. and Erkihun,A. (2007): Bovine tuberculosis on small scales dairy farms in Adama town, Central Ethiopia, and farmer awareness of the disease. Rev.Sci. tech., 26(3):711-9.

Anon. (2008) : Zoonotic tuberculosis and food Safety Authority of Ireland, Dublin ; http:// www .fsai. ie/resources publications.html [Accessed 01 May 2009] .

Ben kahla.; Boschiroli, M.L.; Souissi, F.; Cherif, N.; Benzarti, M.; Boukadida, J. and Hammami, S. (2011): isolation and molecular characterization of *Mycobacterium bovis* from raw milk in Tunisia. African Health Science Vol., 11 , No., 51, pp., 52-55.

Cadmus, S.I.B. and Adesokan H.K. (2007): phenotypic characterization and spoligotype profiles of *Mycobacterium bovis* isolated from unpasteurized cow's milk in Ibada,Nigeria. Tropical veterinarian, 25 (2): 65-72.

Center for Disease Control and Prevention"CDC". (2005): Human tuberculosis caused by *Mycobacterium bovis*—New York City, 2001-2004. Morb. Mortal. Wkly. Rep., 54**:** 605-608.

Collee, J.G; Fraser, A.G.; Marmion, B.P. and Simmons, A. (1996): Mackie, McCortney, Practical Medical Microbiology 14th ed. 838-841. Churchill Livingstone, New York Edinburgh,London.

Corner, L.A.; Trajstman, A.C. and Lund, K.L. (1995): determination of the optimum concentration of decontamination for the primary isolation of *Mycobacterium bovis*.New Zealand Veterinary Journal, Vol., 43, No., 1 , p. 129-133

De La Rua-Domenech, R. (2006): Human *M. bovis* infection in the United Kingdom: incidence, risks, control measures, and review of the zoonotic aspects of bovine tuberculosis. Tuberculosis. 86: 77–109.

Di Pinto, A.; Ciccarese, G.; Forte, T.V.; Bijo, B.; Shehu, F. and Tantillo, G. (2006) : Detection of mycobacterium tuberculosis complex in milk using polimerase chain reaction (PCR) Food Control., 17:776–780. doi: 10.1016/j.foodcont.2005.04.019.

El-Gedawy, A.A.; Ahmed, H.A. and Awadallah, M.A.I. (2014): Occurrence and molecular characterization of some zoonotic bacteria in bovine milk, milking equipments and humans in dairy farms, Sharkia, Egypt. International Food Research Journal. 21 (5): 1813-1823.

Ereqat, S.; Nasereddin, A.; Levine, H.; Azmi, K.; Al-Jawabreh, A.; Greenblatt, Ch.; Abdeeb, Z. and Bar-Gal, G. (2013): First time detection of *Mycobacterium bovis* in livestock tissues and milk in the west Bank, Palestinian Territories. PLOSNTDs 7 (9) .

Franco, M.M.J.; Paes, A.C.; Ribeiro, M.G.; Pantoja, J.C.F.; Santos, A.C.B.; Miyata, M.; Leite, C.Q.F.; Motta, R.G. and Listoni, F.J.P. (2013): Occurrence of mycobacteria in bovine milk samples from both individual and collective bulk tanks at farms and informal markets in the southeast region of Sao Paulo, Brazil. BMC Veterinary Research 9(85).

Gad, E.W.A.; EL-Abeedy, A.; Mettias, K.N. and Manal, A. (2000): The present state and public health importance of tuberculosis of bovine udder. J .Egypt. Vet. Med.Ass., 60 (6). 189-194.

Gertman M.I., Galatova L.V. and Petrov A.A. (1990):"Isolation of L forms of mycobacteria from cow's milk." Veterinarya Moskova, 6:30-31

Hamid, H.; Das, P. and Suleman, A. (2003): Bovine tuberculosis in Dairy Animals at Lahore, Threat to the Public Health. Published online in *VETSCAN.COM*, an online magazine.

Hassanian, N.; Hassanian, M.; Soliman, Y.A.; Ghazy, A. and Ghazy, Y. A. (2009): Bovine tuberculosis in dairy cattle farm as a threat to public health. Afr. J. Microbiol. Res., Vol., 3(8) PP. 446-450

Isabel N., Marta A., Susana P., Nora M.,Maria A.D., Marta O.R., Marıa T.,Claudia L., Wellman R., Vicente G., Dolores K.,Luis A., Lucy M.,Carlos R.and Jacobus H. de W.(2008):" Human Mycobacterium bovis infection in ten Latin American countries" Tuberculosis 88, 358–365.

Jha, V.C.; Morita, y.; dhakal, M.; Beseni, B.; Sato, T.; Nagat, A.; Kato, M.; Kozawa, K.; Yamamoto, S. and Kimura, H. (2007): isolation of Mycobacterium species from milking buffaloes and cattle in Nepal. 12(6): 520-2 : 21624773 .

Jordao J.C.M, Lopes F.C.M., David S., Farache F.A and Leite C.Q.F (2009):"Detection of non-tuberculous mycobacteria from water buffalo raw in Brazil." Food microbiology, 26 (6):658-661.

Kazwala, R.R.; Daborn, C.J.; Kusiluka, L.J.M.; Jiwa, S.F.H.; Sharp, J.M. and Kambarage, D.M. (1998):"Isolation of Mycobacterium species from raw milk of pastoral cattle of the Southern Highlands of Tanzania" , Tropical Animal Health and Production. 30, 233-239

Leite, F.; Anno, I.; Leite, S.; Roxo, E.; Morlock, G. and Cooksey, R. (2003): Isolation and identification of mycobacteria from livestock specimens and milk obtained in Brazil. Mem. Inst. Oswaldo Cruz. vol., 98 319-323.

Liebana E., Alicia A., Ana M. and Vilafranca M. (1995):" Simple and Rapid Detection of *Mycobacterium tuberculosis* Complex Organisms in Bovine Tissue Samples by PCR." J. of Clinical Microbiology p.33-36.

Ofukwu, R.A.; Oboegbulem, S.I. and Akwuobu, C.A. (2008): Zoonotic Mycobacterium species in fresh cow milk and fresh skimmed, unpasteurized market milk (nono) in Makurdi, Nigeria: implications for public health. Journal of animal and plant sciences. vol. 1, issue 1:21-25.

Quinn P.J.; Carter M.E.; Markey B.K. and Carter G.R. ,(1994):"Clinical veterinary microbiology".Wolf publishing an imprint of Mosbyear book Europe limited .London, England.Printed in Spain by Grafos,S.A.Arte Sobre Papel.Pp.332-344

Spahr,U.andSchafroth,K.(2001): " fate of *Mycobactreriun avium subsp.Paratuberculosis* in Swiss hard and semihard cheese manufactured from raw milk. " Appl. Environ. Microbiol., 67:4199-4205

Silaigwana, B.;Green,E. and Ndip,R.N.(2012): Molecular detection and drug resistance of *Mycobacterium tuberculosis complex* from cattle at a dairy farm in the Nkonkobe region of South Africa: a pilot study. International Journal of Environmental Research and Public Health. 9(6):2045-2056.

Srinand, S.; Bookout, J. B.; Ringpis, F.; Perumaalla, V. S.; Ficht, T.A.; Adams, L.G.; Hagius, S.D.;.; Bricker, B.J.; Kumar, G.K.; Rajasekhar, M.; Srikrishna,

I. and Barathur, R.R. (2000): A multiplex approach to molecular detection of Brucella abortus and/or *Mycobacterium bovis* infection in cattle.Journal of Clinical Microbiology. 38(7):2602-2610.

Tatiana K., Tatyana A., Boris N., Arseny K., Alexander A.(2014): "Latent tuberculosis infection: What we know about its genetic control?" Tuberculosis (94): 462-468

Taylor, G.M.; Worth, D.R.; Palmer, S.; Jahans, K. and Glyn Hewinson, R. (2007): Rapid detection of *Mycobacterium bovis* DNA in cattle lymph nodes with visible lesions using PCR. BMC Veterinary Research. 3 :12–23.

Thoen, C.; Lobue, P. and de Kantor, I. (2006): The importance of *Mycobacterium bovis* as a zoonosis.Vet. Microbiol., 112**:**339-345.

Wahba, N.; Nasr, S.; Saad, N.; Nasr, E. A. and Elsherif, W. (2013): Detection of bovine tuberculosis in milk and serum of tuberculin reactors dairy farm animals in Assuit City, Egypt. Basic Res. J. Anim. Sci., Vol., 1 pp 1-6 .

Wards, L.J.; Brown, J.C. and Davey, G.P. (1995): " Detection of dairy Leuconostoc strains using polymerase chain reaction." Letters in Applied Microbiology 20, issue 4 : 204-208 .

WHO (World Health Organization.) (2006): Bringing Vets and Medics together to deal with the bovine tuberculosis in the United Republic of Tanzania. In: The Control of Neglected Zoonotic, p. 5.

WHO; World Health Organization. (2013): Tuberculosis and gender, E-Focus: Newsletter of the WHO Global TB Programme .