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Bovine tuberculosis in a dairy cattle farm as a threat to public health

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Bovine tuberculosis (BTB) remains a disease of economic and public health importance in developing countries. In countries where bovine milk is not pasteurized before use, bovine tuberculosis has emerged as the single major cause of extra-pulmonary human tuberculosis. The aim of this study was to estimate the incidence of bovine tuberculosis in 46 suspected cattle maintained in a private farm at Egypt using the single intra dermal tuberculin test (SITT) and enzyme-linked immunsorbent assay (ELISA) for the detection of the public health hazards through mycobacterial culture of the bovine milk and farm workers sputum. The SITT results revealed that 58.7 and 23.91% of the tested animals were true positive and suspected tuberculin reactors, respectively. ELISA results showed that using the mammalian tuberculin as an antigen, 50% of the bovine serum samples were positive. Combining the SITT and ELISA results, 30.5 and 13.0% of the examined animals were considered to be true and suspect infected with *Mycobacterium bovis*, respectively. Mycobacterial culture identification revealed that 4.35% of the collected 23 bovine milk samples were positive for *M. bovis* isolation. 40% of the examined farm workers were positive by tuberculin and ELISA tests, while their sputum samples were negative for mycobacterial culture. In conclusion, these results showed that 30.5 and 40% of the examined dairy cattle and farm workers were infected with bovine tuberculosis. Presence of *M. bovis* in milk represented a major source of infection to human and other animals. The possible way of bringing down the incidence of BTB in animals and humans is by adopting some strict and uniform control measures for animals and workers at farms.

Key words: Bovine tuberculosis, SITT, ELISA, milk, sputum, public health hazard.

INTRODUCTION

Bovine tuberculosis (BTB) is a zoonotic disease that causes respiratory disorder in both cattle and humans. Active animal tuberculosis outbreaks represent possible sources of infection to both animal and human populations (Ayele et al., 2004; Thoen et al., 2006). Leite et al. (2003) estimated that the proportion of human cases in developing countries due to *Mycobacterium bovis* accounted is 3.1% for all forms of tuberculosis. The Office International des Epizooties classifies BTB as a list B transmissible disease of public health importance and is of high significance to the international trade of animals and animal products (OIE, 1999).

M. bovis infection is certainly an occupational hazard to agricultural workers who may acquire it by inhaling cough spray from infected cattle (Kleeberg et al., 1984). Regassa et al. (2008) reported that the prevalence of BTB was threefold higher in cattle owned by farmers with active tuberculosis (24.3%) than in those owned by farmers who did not have active tuberculosis (8.6%).

Most human tuberculosis cases due to *M. bovis* occur in young individuals and result from drinking or handling contaminated milk. As a result, cervical lymphadenopathy, intestinal lesions, chronic skin tuberculosis (*Lupus vulgaris*), and other nonpulmonary forms are particularly common (Thoen et al., 2006).

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TB is a major opportunistic infection in HIV-infected per-sons. The epidemic of HIV infection in developing coun-tries, particularly countries in which *M. bovis* infection is present in animals and the conditions favor zoonotic transmission, could make zoonotic TB a serious public health threat to persons at risk (Moda et al., 1996; Daborn et al., 1997).

Many methods are available for diagnosis of bovine tuberculosis in animals. Tuberculin testing has tradition-ally been used to determine the prevalence of infection in animals and human. Also, the purified protein derivative of Mycobacterium (PPD-tuberculin) is used for diagnosis of tuberculosis in animals and man (Monaghan et al., 1994).

Serological assays have shown promise as a diagno-stic alternative to skin testing or culture testing for many of these species. Ritacco et al. (1990) and Silva et al. (1999) recorded high specificity of ELISA (94.1 and 95.7%) for diagnosis of BTB. Silva et al. (1999) stated that the specificity and ease of use of the ELISA make it an important tool in the detection of tuberculosis antibodies in cattle in diseases free areas.

The present study was designed to estimate the inci-dence and public health importance of bovine tubercu-losis in dairy cattle maintained in a private farm at Egypt using the single intra dermal tuberculin test (SITT) and ELISA for the detection of a plausible source of infection to farm workers through mycobacterial culture of bovine milk and human sputum samples.

MATERIALS AND METHODS

Sampling and methodology

46 cows reared in a private farm at Egypt complaining of low milk yield, emaciation and anorexia, intermittent diarrhea not responding to antihelmintic treatment, irregular febrile episodes, cough and labored respiration were selected for the study. Sixty-one serum (46 cow and 15 farm workers), 15 sputum (farm workers) and 23 milk (dairy cows) samples were collected. The single intradermal tuber-culin test (SITT) was carried out for screening of tuberculous infection in both humans and animals.

Tuberculin testing of animals

The single intradermal tuberculin test (SITT) was carried out either on the neck or in the tail of the animals in order to test the diffe-rence in the sensitivity of the site of reaction. A fold of skin was pinched with tips of fingers; its thickness was measured and 0.1 ml (0.5 mg/ml) of the mammalian tuberculin (Central Lab. for Evalua-tion of Vet. Biologics, Abbassia, Egypt) was injected intradermally. Both the injection sites were encircled with indelible ink of different colors. The results were recorded after 72 h post-inoculation by measuring the thickness of skin fold (Monaghan et al., 1994).

Tuberculin testing of farm workers

5 tuberculin units were injected in the skin of the dorsum of the hand. The skin test was read after 48 or 72 h. It is considered posi-tive if the injection was followed by in duration of 10 mm or more in diameter (Sinder, 1982).

Sputum and milk samples mycobacterial examination

The samples were processed for isolation of mycobacteria following standard procedures for homogenization, suspension, centrifuga-tion and decontamination (Vestal, 1977). The processed samples were inoculated on Lowenstein-Jensen (L-J) media with and with-out pyruvate and incubated at 37ºC for a maximum period up to 8 wk. Species level identification was done by CDC Manual (Vestal, 1977).

Enzyme-linked immunsorbent assay (ELISA)

It was performed according to Thoen et al. (1975) and Nasr et al. (2006). The optimal antigen (mammalian PPD) concentration, anti-body and conjugate dilutions were chosen after preliminary checker board titration. In the present study, the optimum conditions were 5 µg/100 µl coating buffer antigen concentration, 1:200 human and animal serum dilutions and 1 : 1000 Horse radish peroxidase- label-ed anti-human-IgG and anti-bovine-IgG (Sigma Co.) as conjugate. The substrate used was OPD. All reaction mixture was set in duplicate, with the mean value being used for recording and calculations. Results were read on SOFTmax PRO ELISA reader (Molecular Device Corporation, California) at a wave length of 405°A. The diagnostic value of the ELISA was evaluated in terms cut off value. The cut off value in the current study was calculated as mean O.D. value of the healthy control subjects plus 3_SD (Daniel and Debanne, 1987).

RESULTS

Mycobacterial culture of milk and sputum samples

1 out of the 23 (4.35%) milk samples was positive for *M. bovis* isolation. The growth of *M. bovis* was promoted with the addition of 1% sodium pyruvate to L-J medium. The isolate produced white, moist, slightly rough and friable colonies with no pigmentation. They showed nega-tive activity for niacin, nitrate reduction, catalase at 68°C, tween hydrolysis, arylsulphatase and thiophen-2 carboxy-lic acid hydrazide (TCH) sensitivity. All the sputum samples of humans were negative for *M. bovis* isolation.

Tuberculin test and ELISA in cattle

The SITT results revealed that 27 (58.7%) and 11 (23.91%) animals gave positive reaction in the neck & tail and neck only, respectively (Table 1). While, 8 animals (17.39%) gave negative reaction in the neck and tail sites. ELISA results showed that using the mammalian tuberculin as an antigen, 23 animals (50%) were positive including 30.5 (14/46), 13.0 (6/46) and 6.5% (3/46) of true positive (positive neck and tail), suspected (positive neck only) and true negative(negative neck and tail) tuberculin reactors, respectively (Table 2). Combining the SITT and ELISA tests for accurate diagnostic potentials, 30.5 and 13.0% of the examined animals were considered to be truly and suspect infected with *M. bovis*. While, 10.9% of the animals were free of tuberculosis (Table 2).

Table 1. Results of comparative diagnosis of bovine tuberculosis in a dairy cattle farm by tuberculin test and ELISA using mammalian PPD tuberculin.

No of examined diary cattle	Tuberculin test								ELISA	
	Neck		Tail		Conclusive tuberculin results					
	+ve	-ve	+ve	-ve	True +ve		True -ve	Total +ve	+ve	-ve
46	38	8	27	19	27	11	8	38	23	23
	82.61%	17.39%	58.7%	41.3%	58.7%	23.91%	17.39%	82.61%	50%	50%

-% is calculated in relation to the total number of the tested animals; -True positive = +ve neck and tail; - ± = Suspected = +ve neck and –ve tail; True negative = -ve neck and tail; - Total positive = true positive and suspected.

Table 2. Relationship between tuberculin test and ELISA in diagnosis of bovine tuberculosis in dairy cattle.

		Tuberculin test			
		True tuberculin positive	Suspected tuberculin	True tuberculin negative	Total
ELISA	+ve	27	11	8	46
		58.7%	23.91%	17.39%	100%
		14	6	3	23
		30.5%	13.0%	6.5%	50%
	-ve	13	5	5	23
		28.2%	10.9%	10.9%	50%

-True positive = +ve neck and tail; Suspected = +ve neck and –ve tail-; -True negative = -ve neck and tail

Tuberculin test and ELISA in human

It was found that 53.3% (8/15) of the examined farm workers were positive for the SITT. ELISA results revealed that 40% (6/15) of the serum samples were positive. The positive ELISA samples were included in the positive tuberculin reactors only (Table 3).

DISCUSSION

The incidence of BTB has been observed to be rising in many parts of the world whereas the standard of living is poor especially in Asia and Africa (Cadmus et al., 1999; Ameni et al., 2003). This may be attributed to lack of organized and practicable test for mass screening.

In the present study, the SITT results showed that 58.7% of the investigated animals were true positive Lower incidence of bovine tuberculosis by the SITT was recorded by Asseged et al. (2000); Bonsu et al. (2001) and Kang'ethe et al. (2007). This study was conducted in smallholder farm with smaller herds, Cook et al. (1996) indicated that breed of cattle, housing and gathering of animals at watering and grazing sites have influenced the prevalence of BTB.

Results of the present investigation revealed that 82.6% (38/46) of the examined animals gave positive SITT, while 43.5% (20/46) of them were positive for ELISA. The current available assays developed for skin testing of animals for mycobacteria have low sensitivity and suboptimal specificity (Neill et al., 1994; Costello et al., 1997), probably due to the use of complex bacterial extracts containing antigens expressed by pathogenic and environmental mycobacteria (Koo et al., 2005).

ELISA results showed that 50% of the investigated animals were positive. This result agreed with Asiak et al. (2007) who recorded seroprevalence of 45.7% of BTB in slaughtered cattle. On the other hand, lower results were reported by many investigators (Ritacco et al., 1990; Silva et al., 1999; Otero et al., 2003). Ritacco et al. (1990) stated that the low sensitivity of ELISA limits its usefullness as a diagnostic tool for BTB eradication campaigns. However, it could be helpful in epidemiological surveillance if its efficiency to identify infected herds is demonstrated.

The SITT and ELISA results showed that 58.7 and 30.5% of the investigated animals were true positive for cutaneous testing and ELISA, respectively, whereas positive plus suspect results were 82.6% for the SITT and 50% for serological analysis. Similar SITT and higher ELISA results were recorded by Saegerman et al. (1995) who reported positivity of 54% for cutaneous testing and 74% for serological analysis, whereas positive plus suspect results were 80% for cutaneous testing and 95% for ELISA. On the other hand, lower result of cutaneous

Table 3. Results of comparative diagnosis of bovine tuberculosis in farm workers by tuberculin test and ELISA using mammalian PPD tuberculin.

		Tuberculin test		Total
		Positive	Negative	
ELISA	+ve	8	7	15
		53.3 %	46.7%	100%
		6	-	6
		(40%)	-	(40%)
	-ve	2	7	9
		13.3%	46.7%	60%

- * Number of tested farm workers = 15; - % is calculated in relation to the total number of the tested humans.

testing and higher record of ELISA was obtained by Akcay and Izgur (2002) and Plackett et al. (2008). Plackett et al. (2008) stated that the evident low specificity and sensitivity of the ELISA make it of little value as an alternative to the tuberculin test, but it can detect some anergic cattle at the cost of increasing the number of false positive reactors. This may be acceptable in some circumstances and would justify the use of the ELISA as a complement to the tuberculin test or to an *in vitro* assay of T-cell immunity.

Milk is one of the most important links between bovine tuberculosis and human beings especially children (Leite et al., 2003; Srivastava et al., 2008) . One out of the 23 collected milk samples (4.35%) gave culture of *M. bovis*. Higher results were recorded by Ameni et al. (2003) and Khan et al. (2008). On the other hand, lower result was stated by Kazwala et al. (1998). Ali et al. (2005) examined 105 cattle milk samples, but did not find any milk sample positive for acid fast bacilli. Grange and Yates (1994) reported that tuberculosis in cattle was principally a pulmonary disease; only 1% of the tuberculous cows excrete tubercle bacilli in their milk, which shows that cows transmit the disease by exogenous route. Kleeberg et al. (1984) indicated that one cow with tuberculous mastitis can excrete enough viable tubercle bacilli to contaminate the milk of up to 100 cows when milk pooling and bulk transportation is used. Some investigators have pointed out the risk of human infection through using raw milk for producing cream, butter or dahi (curd) among cattle owners and herdsmen in community (Cotter et al., 1996; Bonsu et al., 2001).

Because most of the farmers either sell their milk to local people or pool milk in units for selling milk products without heat treatment, risk of milk contamination with *M. bovis* is a potential major health hazard to consumers. Furthermore, the cattle owners' poor understanding of BTB exacerbates the situation. Le Jeune and Rajala-Schultz (2009) stated that physicians, veterinarians and dairy farmers who promote, or even condone, the human consumption of unpasteurized milk and dairy products may be at risk for subsequent legal action.

In this study, 40% of the examined farm workers were positive by tuberculin and ELISA tests, while all their sputum samples were negative for *M. bovis* isolation. In humans, *M. bovis* is the major cause of extra-pulmonary tuberculosis (Dankner et al., 1993; Bonsu et al., 2001). Ameni et al. (2003) reported that the frequency of extra pulmonary tuberculosis cases was high (87.5%) in rural areas of the district. Thoen et al. (2006) stated that in countries where bovine milk is not pasteurized before use; bovine tuberculosis has emerged as the single major cause of extra-pulmonary human tuberculosis.

This study showed that combining of SITT and ELISA results, 30.5 and 40% of the examined suspected dairy cattle and farm workers were infected with bovine tuberculosis, respectively. Also, the presence of milk infected with *M. bovis* represented a major source of infection to human and other animals.

Conclusion

Bovine tuberculosis poses a significant risk to human and animal health. The only way to be protected from the disease is through prevention. It is important to limit the exposure of the herd to other infected cattle. Testing and eradication of the infected animals is the current method of control, though additional research is currently being explored in the areas of vaccinations and other possible preventative measures.

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