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PREVALENCE OF BOVINE TUBERCULOSIS IN DIFFERENT CATTLE BREEDS IN DAIRY FARM IN EGYPT

Nasr, E.A[*]; Saleeb, O.R.; Abdel Rahman, M.; Shereen, A. M.; and Marwah, M.
Bacterial diagnostic products department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.
* Corresponding author: Nasr, E.A; email; Essamnasr@yahoo.com

ABSTRACT

Due to direct economic repercussions on livestock and indirect consequences for human health, knowing the prevalence rates of bovine tuberculosis is essential to define an effective control strategy. Our study was performed in 32 dairy cattle farms as we subjected 10800 dairy cattle to the Single Intradermal Comparative Tuberculin skin Test (SICTT). The skin test was interpreted according to guidelines of the World Organization for animal health (OIE). Twenty five farms (78%) have reactors to the SICTT. We also studied the relation between physiological variables with breeds, pregnancy, lactation, size of farms and the other risk factors explained in the study. Out of the 10800 tested animals, 228 (2.1%) were reactors to the skin test. The factors identified as possibly enhancing the risk of bovine tuberculosis (bTB) were herd size, age of animal, farming (housing) conditions. Other factors including breed and physiological status of the animal did not contribute to tuberculin sensitivity. The finding that large size and intensively (often poorly) managed herds were at greater risk of bovine tuberculosis and suggests that significance of bTB is increasing in Egypt parallel to an increase in the dairy operation. This surely indicates that if measures are not taken promptly, the impact of the economy and public health could be enormous. It was concluded that bTB continues to infect animals in Egypt but the prevalence was moderate, nevertheless the continued threat of economic loss in animal industry due to the persistence of bTB that should not be ignored.

Keywords: Bovine tuberculosis, *mycobacterium bovis*, Risk factor, prevalence.

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INTRODUCTION

Bovine tuberculosis caused by ***mycobacterium bovis*** is a chronic granulomatous disease infect different species of animals specially cattle and also infect human and it is also endemic to many developing countries especially the African countries. **(Abubakar *et al.*, 2011)**. Worldwide, bTB is considered one of the seven most neglected endemic zoonosis, presenting a complex epidemiological pattern and with the highest prevalence rates in cattle in african countries, parts of Asia and of the Americas. In affected countries, the disease has an important socio-economic and public health-related impact and also represent serious constraint in the trade of animals and their products **(Biet *et al*, 2005)**.

In order to develop an effective national program for bTB surveillance and control in developing countries, accurate data on bTB prevalence is needed. **(Ameni *et al.*, 2007)**. In Egypt, data on bTB epidemiology is still moderate and mostly unpublished, however bTB is estimated to be one of the most important causes of economic losses in cattle production in recent years, due to rejection of carcasses at the slaughter house and limitation on trade. In Egypt also a great proportion of the population hold livestock animals especially cattle and buffaloes **(Nasr *et al.*, 2016)**. In order to estimate the prevalence rates of bTB in Egypt in recent years in dairy farms we performed a survey covering a representative sample of cattle in dairy farms of different livestock areas within different districts.

The single comparative intradermal tuberculin skin test (SICTT) was applied as its specificity is higher than the single intradermal tuberculin test (SITT) (**De la Rua den *et al*, 2006)**. While the SITT is the standard diagnostic test used in Egypt for bovine tuberculosis program, the SICTT is the confirmatory test and can be used as screening test in herds with a history of cross reactivity. **(Nasr *et al.*, 2008).** We also assessed intrinsic determinants of the disease associated with SICTT positivity in the study area in order to define strategies suitable for bTB control and practical preventive measures in cattle in dairy farms in Egypt.

MATERIALS AND METHODS

The study population

From 32 dairy farms in different areas in Egyptian governorates, we tested 10800 animals by the SICTT reared in intensive and extensive farming type and the reactors were slaughtered for post mortem examination.

Sample size and sample Method

Sample size was estimated using the formula **(Thrusfield, 1995),** which is used to estimate prevalence in a given population. PM examination was applied and samples for bacteriological examination were collected.

Ethical approval

The abattoirs included in the research are all legal and government approval.

Comparative tuberculin skin test

All cattle older than 6 months within a herd, except clinically sick ones and cows one month before and after parturition, were subject to SICTT: two sites, 12cm apart horizontally of the mid neck of the animal were shaved and the skin thickness was measured with a caliper. Aliquots of 0.1ml of bovine tuberculin and 0.1ml avian tuberculin were injected separately into the respective shaved site. The thickness of the skin at each injection site was measured again after 72hrs. The test results were interpreted according to recommendations of **OIE (2017).** A herd with at least one positive reactor was considered as tuberculin positive. Information on farm structure and management were collected. Sanitation status was judged as poor, medium or good based on aspects such as odor, waste drainage, cleanness of floor and animals, barn ventilation and light source and animal stocking.

Postmortem examination

The selection criteria for the positive SICTT animals was based on strong response to the skin test, unproductive animals and the owner is not willing to sell the animal. Each lobe of the lung was inspected externally and then sliced into 2cm thick slices to facilitate detection of any typical TB lesions. Liver, spleen and kidney as well as other lymph nodes were sliced into thin sections and inspected for the presence of typical TB lesions.

Isolation of mycobacteria

For isolation and cultivation of mycobacteria, all bovine samples were processed using Marks' method **(Marks, 1972)** and sputum samples of labours in contact with animals were processed by Petroff's method **(Petroff, 1915).** The processed sediments were inoculated on Lowenstein-Jensen media (LJ) with or without pyruvate. The inoculated slopes of bovine and human samples were incubated at 37ºC and were observed for growth for at least 8-12 weeks. Cultures showing evidence of growth were examined for presence of mycobacteria.

Risk assessment

- Animal level risk factors
- Breeds where the animals were local, cross breeds and exotic.
- Reproductive status and milk yield.
- Sex and age.
- Nutritional status and body conditions.
- Herd level risk factors
- Size and type of herd
- Farm management

RESULTS

Table 1: Univariate analysis of risk factors for SICTT result at farm/herd level.

Risk factor	No. of tested herds	Positive	%
Type of farming			
Intensive	25	23	92
Extensive	7	5	71.4
Herd size			
<100	12	11	91.7
100-300	8	7	87.5
300-500	6	4	66.7
500-1000	3	2	66.7
>1000	3	2	66.7
Management			
Good	10	6	60
Medium	13	11	84.6
poor	9	8	88.9

Table 2: Univariate analysis of host risk factors for SICTT results at animal level.

Risk factor	No. of tested animals	positive	%
Age (years)			
<2	2050	15	0.73
2≤4	4200	84	2
4≤6	3100	89	2.9
>6	1450	40	2.8
Breed			
Local	1250	11	0.9
Cross breed	3550	70	2
Exotic	6000	147	2.5
Body conditions			
Good	3250	53	1.6
Medium	5750	160	2.8
Poor	1800	15	0.8
Lactation			
Lactating	7250	85	0.01
Non-lactating	3550	43	1.2
Pregnancy status			
Pregnant	4050	130	3.2
Non-pregnant	6750	98	1.5

Table 3: Distribution and frequency of tuberculosis lesions in six slaughtered SICTT positive cattle.

Infected organ	frequency	%
Retropharyngeal LN	65	28.5
Bronchial LN	43	18.9
Mediastinal LN	35	15.4
Lungs	75	32.9
Visceral surface of ribs	15	6.6
Mesenteric LN	34	14.9
Pre scapular LN	25	11
Supra mammary LN	0	0
Head LN	45	19.7

Table 4: Summary on housing status of respondents and milk consumption (Farm of 32 head).

Habit of respondents	No. of respondents	%
House sharing		
Sharing	17	46.9
Non- sharing	15	53.1
Milk drinking		
Raw milk	0	0
Boiled milk	32	100
Yoghurt milk Consumption	10	31.3

Figure 1: Univariate analysis of risk factors for SICTT result at farm/herd level

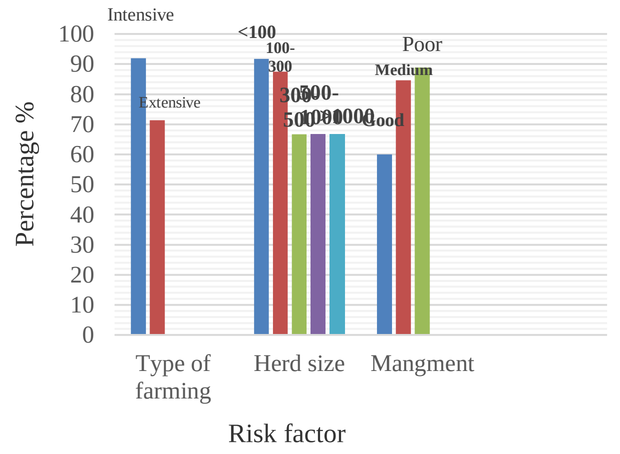


Table 5: Results PM findings and bacteriological examination of tuberculin tested cattle.

No. of tested cattle	No. of tuberculin reactors		Post mortem finding												Bacteriological examination (M.bovis)			
			VL (155)										NVL					
			Head		Respiratory		digestive		Mixed		Generalized				VL(155)		NVL(73)	
	No.	%	No	%	No	%	No	%	No.	%	No.	%	No.	%	No.	%	No.	%
10800	228	2.1	20	8.8	56	24.6	14	6.1	50	21.9	15	6.6	73	32.1	115	74.1	4	5.5

Table 6: Bovine tuberculosis prevalence at animal and herd level

Parity class	No. of animals Tested	Positive	%
Heifer	2550	18	0.7
1-2	3050	68	2.2
3-5	3750	102	2.7
>6	1450	40	2.8

Table 7: Tuberculin reactor cattle in comparison to mycobacterial isolation.

PM findings	No.	*M.bovis* isolation	
		No.	%
Head	20	15	75
Respiratory	56	41	73.2
Digestive	14	8	57.1
Mixed	50	21	42
Generalized	15	15	100
Total VL	155	115	74.2
NVL	73	4	5.5
Total animals	228	11	52.2

Figure 2: Univariate analysis of host risk factors for SICTT results at animal level.

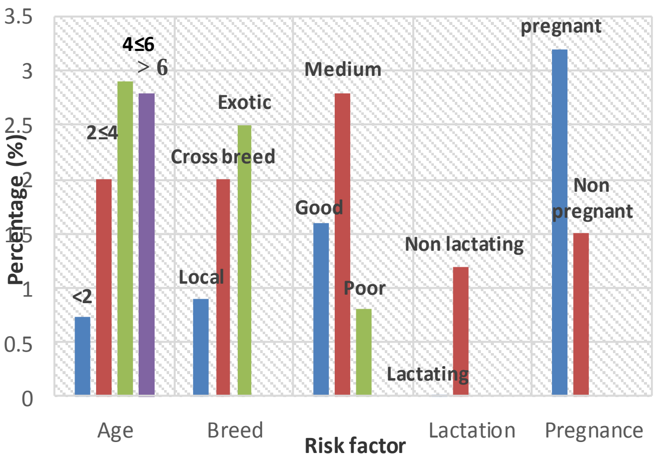


Figure 3: Distribution and frequency of tuberculosis lesions in six slaughtered SICTT positive cattle

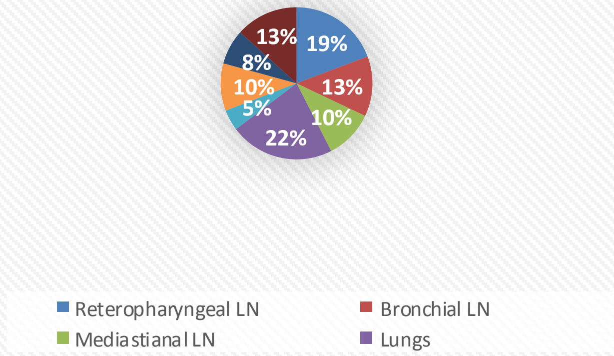
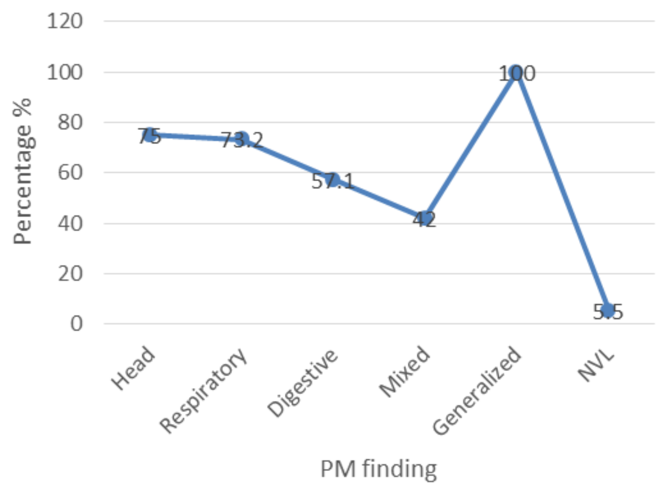


Figure (4): Tuberculin reactor cattle in comparison to mycobacterial isolation



DISCUSSION

Our study reviews risk factors that have been identified for bovine tuberculosis in cattle. The bovine tuberculosis infected herd is defined by the presence of one reactor to the SICTT. The choice of SICTT was of relevance to differentiate between animals infected with ***M. bovis*** and those responding to bovine PPD due to exposure to other mycobacteria. The prevalence of bovine tuberculosis among tested cattle was 2.1% and this agree with **Nasr *et al.*, 2008** 2.2%, **Moussa *et al.*, 2011**, 2.46%; **El Sify *et al.*,2013**, 2.24%;and **Nasr *et al.*, 2016**, 2.6% and disagree with **El-Sabban *et al.*, 1992**, 24%; **Hassanain *et al.*,2009**,23.91%; and **Ameni and Erkihun, 2007**, 11.6 %.

A high prevalence of bTB was observed in intensive farming system than in extensive one as shown in table (1) and figure (1). Also the cattle showed somewhat higher respondents to the SICTT. Management practices and hygiene condition as a risk factor was significantly associated with bTB in cattle dairy farms as shown in table (1) and figure (1).The poor type of management system also promote the spread of respiratory diseases especially bTB . Poor hygiene conditions in a farm may allow ***M. bovis*** to remain for a longer period and potentially to proliferate and these findings agree with **(Humblet *et al.,* 2009; and Kemal *et al.,* 2019)**. In small herd size ≤ 100 the positivity to SICTT was higher than any other sizes as shown in table (1) and figure (1).

This disagree with **Ameni *et al.,* 2003; Habitu *et al.,* 2019;** and **Mekonnen *et al.,* 2019;** and this may be due to the variation in number of tested animals. From results obtained in table (2) and figure (2) it was indicated that the age was an important risk factor, as the exposure increases with increase in age especially between 4-6years of age. Our results show that age was the main individual risk factor. Some authors suggest that it could be related to increased duration of exposure with age, with older cattle being more likely to have been exposed than the younger. The apparent decay at later stage in life might be due to development of an anergic state or excess mortality of infected animals. It has been re-ported that chronically infected animals with severe pathology may be unresponsive to the tuberculin test **(OIE, 2009; Mekonnen *et al.,* 2019)**. Most of cattle included in the study were exotic breed and bovine tuberculosis prevalence was higher than in local and crossbreed and this is shown in table (2) and figure (2). Several studies have shown a variation in susceptibility to bTB among cattle breeds.

These findings agreed with **Vordermeier *et al.,* (2012)** and **Bermingham *et al.,* (2009).** The probable reason could be the fact that genetically improved cattle suffer more severely from poor housing, under-and malnutrition and kept under intensive and semi-intensive production systems subsequently become more susceptible to infection **(Nega *et al.,* 2012; Romha *et al.,* (2014)** . Several studies reported a correlation between body condition and bTB. In our study, as shown in table (2) and figure (2), animals in reasonable (satisfactory) and poor body condition showed more positive skin test results than animals in good body condition. Few studies have examined the effect of reproductive status on susceptibility to bTB infection, possibly because of the difficulty in separating out gestation and lactation effects. The dairy cow experiences large hormonal shifts and stresses throughout production and it seems likely that these will affect both her response to diagnostic tests that measure immunological factors and susceptibility to infection.

In contrast to susceptibility to bTB infection, cows in the weeks before and after calving tend to have weaker reactions to the tuberculin skin test, but the IFN-γ is less affected. Also the presence of other livestock with cattle (sharing) increases the prevalence of bTB. Mixed herd with multiple species particularly dairy cattle with goats , sheep and other species managed under intensive management system play an important role in recirculation of bTB in cattle dairy farms and these results agree with **(Tschopp *et al.* 2011 and Napp *et al* .,2013)** as shown in table (4)**.** Results of post-mortem findings for the slaughtered tuberculin reactor cattle was shown in table (5) The number of non –visible lesion (NVL) reactors 4(5.5%), may be attributed to the non-specific reaction to the SICTT which may be due to sensitization by other mycobacteria other than ***M. bovis*** or the closely related microorganisms especially of the genus Nocardia or a combination of liver fluke infestation with saprophytic mycobacteria **(Cortina and Vera, 1986*)***.

Moreover, **(Huitema 1994; Nasr *et al.,* 2016*)*** assumed that non-specific reactors may be slaughtered at stage of the disease where the tuberculous lesions are invisible or the lesions may be found in parts of carcass such as bone or skin. The isolation rate of ***M.bovis*** for visible reactors was 74.1%, these results agree with **(Moussa *et al.,* 2011,** 68.97%**;** and **Nasr *et al.,* 2016 80**%, while the isolation rate of ***M. bovis*** for non-visible reactors was 5.5%. Concerning the parity class as shown in table(6), it was detected that the reaction or response to SICTT increase with the increase in number of parities as it reached highest percent in parity >6 as more births weakens the immune system of the cattle.

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CONCLUSION

High internal and external biosecurity is required to control herd transmission that tends to be the main focus in infected herds. Proper cleaning and disinfection of cattle farms are expected to reduce bovine TB. History of bovine TB in Neighboring farms as risk factor was significantly associated with Bovine TB in cattle dairy farms, the presence of neighboring bTB infected farms considered one of the most important routes of bovine TB herd breakdowns. Slaughter surveillance is one of important components to control bovine TB. Annual herd testing using CID tuberculin testis required. Contacts with other species as a risk factor was significantly associated with Bovine TB in cattle dairy farms. In many regions of Egypt, mixed herd with multiple species particularly dairy cattle with goats, sheep and other species managed under intensive management system and play an important role in recirculation of BTB in cattle dairy farms. We are in urgent need to effective control measures to reduce bovine tuberculosis prevalence and prevent its spread to human population. It was a good habit that milk was boiled before consumption in all farms examined.

Declaration of Competing interest

On behalf of all authors, I hereby declare that no conflict of interest may interfere with the publication of the manuscript.

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