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Prevalence and risk factors of bovine tuberculosis in dairy cattle farms in Egypt

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Abstract

A cross-sectional study was carried out from November, 2015 to April, 2018 to determine the prevalence and identify the risk factors of BTB using the comparative intradermal tuberculin (CIDT) test on 5327 dairy cattle in 16 dairy herds in mid-delta, Alexandria Road and Upper Egypt districts. Questionnaires used to collect data on herd size and management. The herd and individual animal prevalence at cut-off ≥4mm were 68.75% (95%, CI: 46 to 91.4) and1.67% (95%, CI: 1.3to 2.1) respectively. The individual prevalence was significantly associated with age, breed and different location of farms and density of cattle in yard. On herd level, history of BTB in the farm, management practices, raising of different species in the same facility and newly purchased cattle are important risk factors for BTB. The postmortem examination of positive reactors revealed 66 (85.71%) out of 77slaughtered cattle with visible lesions and 11(14.29%) with non-visible lesions. Bacteriological examination revealed 74.24% (49/66) from visible lesions and 9.09% (1/11) from non-visible lesions were *Mycobacterium bovis*. The indirect enzyme linked immunosorbent assay (IELISA) results revealed 31(40.26%) showed positive result including 29 (93.6%) visible lesions and 2(6.4%) nonvisible lesions. In conclusion, the prevalence high in herds with poor management condition, raised different species in the same facility, had previous history of BTB infection and purchased new animals. At animal level Holstein breed, 3-6 years age, kept in high density and located in mid- delta district were at higher risk to be reactor to comparative intradermal tuberculin (CIDT) test.

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Introduction

Bovine tuberculosis (BTB) is a chronic granulomatous disease of cattle caused mainly by *Mycobacterium bovis (M. bovis)*. The disease causes significant economic loss in animals, which include reduction in production, restrictions of animal movement, screening costs, culling of diseased animals, and trade restrictions (1). BTB affects cattle around the world, but some countries have been able to reduce the incidence of the disease throughout test and slaughter of the affected cattle herds. Most of Europe and Caribbean countries are free from *M. bovis*. Bovine TB is endemic in many developing countries mainly in African countries (2). In Egypt the prevalence of BTB in cattle and buffaloes during the 1980 was between 6.9% and 26.2% then it reduced to 2.6% during the 1990. Then, according to the official report of the General Organization of Veterinary services (GOVs, Egypt), the annual incidence of BTB in

cattle has been increased, with purchasing of live animals from countries where BTB is prevalent and endemic (3). Epidemiological studies as measurements of disease occurrence and risk factor analysis, transmission in cattle herds are of great importance to be studied (4). Many risk factors were responsible for occurrence of disease in animals include cattle breed, age. also host independent factors are considered more important and include herd size, management practices and cattle purchasing (5-9). Diagnosis of BTB in live animal depends on tuberculin test; the standard method is the comparative intradermal tuberculin (CIDT) test which based on delayed hypersensitivity (10). The CIDT test includes bovine and avian tuberculin used mainly to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacterium or related genera (11). ELISA appears to be the most suitable method of the antibody-detection tests and can be a complement, not an alternative, to test based on cellular immunity to detect anergic cows that may be acting as reservoirs of the organism (11-12). So the aim of this study is to estimate the prevalence of BTB disease in some cattle farms in Egypt, identifying the different risk factors play a role in transmission of this organism among cattle on these farms and serodiagnosis of bovine tuberculosis using Indirect ELISA to provide a scientific basis for the development of practical preventive measures.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee (IACUC) in Cairo University. The Reference Number of the approval letter is Vet CU20022020137. The owners of animals known about the purpose of the study before Consent obtained.

Study population and area

A total number of 5372 dairy cattle in 16 herds from a mid-delta (El-Gharbia), Alexandria Road (Alexandria and El-Beheira) and Upper Egypt (Beni-Suef and El-Fayoum) districts were tested by Comparative Intradermal Tuberculin (CIDT) test. The animals tested were dairy cattle originated from intensive dairy herds ranged from 100-1300 cattle. The majority of dairy cattle were Holstein Friesian, Simmental and Brown. Animals less than 6 months and cows in late gestation period weren't tested. Age of tested cattle was estimated by dentition, information obtained from farm records or animal owners through Questionnaire. The body condition of animals was scored based on Soares et al. (13) and classified as five body condition scores.

Study design

The study design was cross-sectional and conducted from November, 2015 to April, 2018.

Samples collection

The sera were collected from 77 positive tuberculin reactor animals. Centrifugation separated the serum at 3000 round per minute for 10 minutes, the serum aspirated carefully by pipette into dry, sterile and labeled test tubes, storage at -20°C until used (14-16) and after slaughtering of 77 animals, postmortem examination was applied and tissue samples (livers, spleens, and lungs lymph nodes) and lymph nodes showing tuberculous-like lesions were collected for bacteriological examination were collected.

Comparative Intradermal Tuberculin Test (CIDT)

The CIDT test was performed through intradermal injection of bovine purified protein derivative (B-PPD) and avian PPD (A-PPD) according to the method described by Ameni et al. (10).

Indirect Enzyme Linked Immunosorbent Assay (IELISA)

The indirect enzyme Linked Immunosorbent Assay (IELISA) was applied on sera of 77 tuberculin positive cattle according to the method described by Jarad (17) and Isihak (18) using Bovine Purified Protein Derivatives (B - PPD) as coating antigen from tuberculosis department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The optical density (OD) was measured at 405 nm using tecan spectra III ELISA (Mannedorf, Swizerland) reader. Sample was considered positive if it yield a mean OD of each group equal to / or greater than the cut off value {Cut off value was calculated according to method described by Nassau et al. (19) which equal to the mean OD of negative serum plus 2 standard deviation}.

Post-mortem examination

After slaughtering of tuberculin positive reactors, post-mortem examination was done according to the method described by Corner (20) to detect the presence of suspected tuberculous lesions such as caseation, calcification, or congestion that present in any lymph nodes (head, bronchial, hepatic, mesenteric, prescapular, popliteal and internal iliac lymph nodes). Moreover, specimens were collected from the lung, liver and kidney which showed congestion or suspected tuberculous lesions.

Bacteriological examination

The organs, lymph nodes and/or tissues showing gross lesions were prepared for bacteriological examination. Samples were cultured on four tubes of Lowenstein-Jensen slants after being decontaminated with 4% H_2So_4 according to the method described by Marks (21). Obtained isolates were identified by conventional methods (rate of growth, colonial morphology, pigmentation, and biochemical properties) in department of bacterial diagnostic products (Tuberculosis department), Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (21).

Data statistical and risk factors analysis

Animal related risk factors (age, breed, body condition score and reproductive status) were recorded when the CIDT test was performed. Dairy farm owners were asked for herd characteristics (herd size, herd management system, presence of neighboring farms, keeping of different animals together, history of disease in the farm and introduction of new cattle in the herd) using questionnaire. All data analysis was carried out using the statistical software program in the statistical researches and studies institute, Cairo University. Association between the occurrence of infection and the risk factors were studied using chi square, odds ratio and confidence interval. The results were expressed as P value and odds ratio (OR) with 95% confidence interval (CI 95%).The result was considered to be significant at $P<0.05$.

Results

Results of comparative intradermal tuberculin test in different districts

The results of tested cattle by using comparative intradermal tuberculin (CIDT) test are shown in (table 1). Out of 5327 dairy cattle, 89 (1.67 %) gave positive reaction. The highest result for tuberculin skin test reactors was 22 out of 512 tested cattle (4.30%) in mid-delta, while the lowest result was 51out of 4170 tested cattle (1.22%) in Alexandria Road district. Out of 16 tested herd 11 (68.75%) were having at least one positive tuberculin reactor. The highest result was 3 out 4 tested herds (75%) in mid-delta district.

Prevalence of BTB on animal level and associated risk factors

On the basis of CIDT test the overall individual animal prevalence of BTB at ≥4mm cut-off points were 1.67% (95% CI: 1.3-2.1). The risk factors that were found to be associated with presence of BTB included location mainly in delta region (OR. 3.63, P=0.0001), age of animals: mainly 3-6 years (OR. 6.57, P=0.0001), breed mainly Holstein Friesian (OR. 5.24, P=0.0209) and density of cattle in yard (OR. 3.01, P=0.000) and not associated with body condition scoring (OR. 1.10, P=0.8376) and reproductive status of animals (OR. 1.51, P=0.9589) as shown in (table 2).

Herd level prevalence and associated risk factors

Herd prevalence was 68.75 (95%CI: 34.3 to 123.01) at ≥4 mm cut-off value. The risk factors were found associated with the presence of BTB included history of bovine TB in the farm (OR. 18, P= 0.036), management condition (OR. 18, P= 0.036), Raising different species in the same facility (OR. 41.8, P=0.005), purchase of new animals (OR. 40, P=0.005) and not associated herd size (OR. 7, P=0.141) and history of BTB in the neighboring farms (OR. 3.33, P=0.346) as shown in (table 3).

Results of post mortem findings of slaughtered tuberculin reactor cattle

Out of 77slaughtered cattle, 66 (85.71%) showed visible and 11 (14.29%) had Non-visible lesions, on the same time the visible lesions showing 8 (12.12%) generalized, 26 (39.40%) pulmonary, 3 (4.54%) digestive, 11 (16.67%) mixed and 18 (27.27%) head as shown in (table 4).

Results of bacteriological examination

The results showed that, from 77 slaughtered reactor cattle 53 cultures positive were recovered (68.83%), 50 cultures positive for *M. bovis* (64.93%) and 3 (3.90%) for atypical mycobacteria as shown in (table-5). Identification characteristics of 53 mycobacterial isolates revealed that 50 isolates grew at 37ºC, gave smooth colonies without pigmentation in dark or light and 3 grew at 37 ºC, 42 ºC, gave rough colonies without pigmentation in dark or light. The 50 isolates were identified as *M. bovis* and the 3other isolates as unidentified slow growers.

Results of Indirect Enzyme Linked Immunosorbent Assay (IELISA)

IELISA results using bovine PPD as an antigen showed that 31/77 (40.26%) were positive (table-6). Out of 66 tuberculin reactor cattle with VL, 29 (43.94%) were positive and Out of 11 tuberculin reactor cattle with NVL.2 (18.18%) were positive. The highest result was found in sera of cattle had generalized lesions.

Table 1: Comparative Intradermal Tuberculin Test in dairy cattle farms in different districts

	Tested Herds		Tested animals		
	No.	TB positive No. (%)	No.	CIDT Positive test No. (%)	CIDT Positive test No. (%)
Alexandria Road	9	6 (66.67)	4170	51 (1.22)	35 (0.84)
Mid-delta	4	3 (75)	512	22 (4.33)	19 (3.71)
Upper Egypt	3	2 (66.67)	645	16 (2.48)	9 (1.39)
Total	16	11 (68.75)	5327	89 (1.67)	63 (1.18)

Table 2: Association of different risk factors to skin test positivity at ≥ 4 mm cut-off point for bovine tuberculosis

Factors	Categories	No of cattle examined	No of positive animals %	Odds ratio	CI. 95%	P value
Location						
	Alexandria Road	4170	51 (1.22)	Ref		
	Mid-delta	512	22 (4.30)	3.63	2.18-6.03	0.0001
	Upper Egypt	645	16 (2.48)	2.05	1.16-3.62	0.0130
Age						
	<3years	2038	8 (0.39)	Ref		
	3-6years	2854	72 (2.52)	6.57	3.16-13.66	0.0001
	>6years	435	9 (2.07)	5.36	2.06-13.97	0.0006
Breed						
	Simmental	552	2 (0.36)	Ref		
	Holstein Friesian	4546	85 (1.87)	5.24	1.29-21.35	0.0209
	Brown	229	2 (0.87)	2.42	0.34-17.31	0.3777
BCS						
	Good	3088	46 (1.49)	Ref		
	Medium	1934	38 (1.96)	1.33	0.86-2.04	0.2027
	Poor	305	5 (1.64)	1.10	0.43-2.79	0.8376
Reproductive status						
	Pregnant	3572	67(1.88)			
	Non pregnant	1755	22(1.25)			
The density of cattle in yard						
	<30	2356	21(0.89)	Ref		
	30 - 50	1190	21(1.76)	2.00	1.09-3.67	0.020
	> 50	1781	47(2.64)	3.01	1.79-5.06	0.000

No: Number BCS: Body condition scoring p-values presented at 95% confidence interval and p~0.05 considered statistically significant.

Table 3: Evaluation of the association of risk factors to herd tuberculin test results

Herd factor	Categories	No of herd examined	No of positive herd%	Odds ratio	CI. 95%	p-value
		Herd size		7	0.73-2.77	0.141
	<200	8	4 (50)			
	>200	8	7 (87.50)			
History of BTB in the farm				18	1.57-3.89	0.036
	Yes	10	9(90)			
	No	6	2(33.33)			
History of bovine TB in the neighboring farms				3.33	0.76-2	0.346
	Yes	6	5(83.33)			
	No	10	6(60)			
Management condition				18	1.57-3.89	0.036
	Good	6	2(33.33)			
	Poor	10	9(90)			
Raising different species in the same facility				41.82	2.29-4.61	0.005
	Yes	9	9(100)			
	No	7	2(28.57)			
Purchase of new animals				40.00	2.79-6.31	0.005
	Yes	11	10(90.91)			
	No	5	1(20)			

No: Number p-values presented at 95% confidence interval and p~0.05 considered statistically significant.

Table 4: Results of postmortem findings of slaughter tuberculin positive cattle

Total No. of slaughtered reactor cattle	PM findings													
	VL (66)												NVL(11)	
	Generalized		pulmonary		Digestive		Mixed		Head		Total VL			
77	No	%	No	%	No	%	No	%	No	%	No.	%	No.	%
	8	12,12	26	39.40	3	4.54	11	16.67	18	27.27	66	85.71	11	14.29

No: Number PM: Post Mortem VL: Visible Lesion NVL: Non-visible Lesion %: Percentage according to the total No. of slaughtered reactors.

Table 5: Bacteriological examination results by using culture method

PM finding	Sites of lesions	No. of slaughter animals		*M. bovis*		Atypical mycobacteria		Total mycobacteria isolates	
		No.	%	No.	%	No.	%	No.	%
I. Visible lesions	Generalized	8	10.38	8	100	0	0	8	100
	Pulmonary	26	33.76	21	80.77	0	0	21	80.77
	Digestive	3	3.90	0	0	1	33.33	1	33.33
	Mixed	11	14.29	6	54.54	1	9.09	7	63.64
	Head	18	23.38	14	77.78	0	0	14	77.78
Sub total		66	85.71	49	74.24	2	3	51	77.27
II. Non visible lesions	NVL	11	14.29	1	9.09	1	9.09	2	18.18
Total	Total VL,NVL	77		50	64.93	3	3.90	53	68.83

No.: number. VL: Visible Lesion NVL: Non-visible Lesion Total No.: total number in VL& NVL.
% of *M. bovis* culture according to total No. of each site which taken.

Table 6: ELISA results (using Bovine PPD) in comparison to PM findings and bacteriological examination

Site	No. of tuberculin positive animals		Bacteriological examination		ELISA	
A-visible Lesions	No	%	No	%	No	%
1-Generalized	8	10.38	8	100	7	87.50
2-Pulmonary	26	33.76	21	80.77	12	46.15
3-Digestive	3	3.90	1	33.33	0	0
4-Mixed	11	14.29	7	63.64	4	36.36
5-Head	18	23.38	14	77.78	6	33.33
Total VL	66	85.71	51	77.27	29	43.94
B- NVL	11	14.29	2	18.18	2	18.18
Total	77	100	53	68.83	31	40.26

VL: Visible Lesion NVL: Non Visible Lesion, No: number, B.PPD: Bovine PPD.

Discussion

Bovine TB caused by *Mycobacterium bovis* is considered one of the most important diseases facing farming industry, cattle owners, government, abattoir workers and veterinary profession in Egypt. On the basis of the CIDT test the individual animal level prevalence of bovine TB was 1.67% (95% CI: 0.7-2.8). These results agreed with that reported by other researchers in Egypt 2.4%, 1.6%, and 1.8% (22-24). In contrast, several authors have reported either low prevalence rates 0.9%, 0.13%, 0.96%, and 0.30% (25-28) or high values 11.6%, 11.3%, and 4.3% (5,7,29). The herd-level prevalence of bovine TB was 68.75%; these results agree with 74.1%, 67%, and 65.8% (8,30,31) and higher than prevalence reported 15%, 40.9%, and 40% (5,29,32). The variation in BTB prevalence may be due to the difference in location, cattle management practices, cattle breeds largely involved in the study, history of BTB in that specific area and purchasing of an infected animal.

The relation between risk factors and BTB in cattle revealed that Age as a risk factor was significantly associated with BTB in cattle dairy farms. There was a significant increase in risk with increasing age up to 6 years then decrease. These results agreed with (33,34). The decrease in

infection at later stage may be due to development of an anergic state or due to excess mortality of infected animals. Breed as a risk factor was significantly associated with BTB in cattle dairy farms. Holstein cattle showed higher prevalence than Simmental and Brown. These results agreed with some previous studies (35-37). It may be due to most of tested animals in this study from Holstein breed. The prevalence at animal level was significantly associated with different location of farms. It might be due to climatic condition differences in the farms, stress on cattle when kept in poor management and overcrowded environment (38) which may be explain for the high prevalence of BTB in the mid-delta area. An increase in tuberculin positive reactors was observed in cattle kept in high density yards not in large herd size agrees with findings in previous studies (35,39) this may be due to high stocking density and poor ventilation facilitate cattle to cattle transmission.

History of BTB in the farm as a risk factor was significantly associated with BTB in cattle dairy farms. This observation agrees with the results of other studies carried out in many countries which concluded that BTB occur in repeated way in the same areas. These results agreed with that reported elsewhere (6,40,41). It is may be due to the source of infection has not been removed and act as permanent factors for re-emergence of BTB in these areas. Purchasing of new animals has a significant association with BTB. Movements of animals are regarded as one of the most common factors contribute to the spread of BTB and considered as major risk factors in many BTB herd breakdown studies. These results agreed with that reported elsewhere (7,39,42,43). These animals may be infected with BTB due to their contact with many animals during their stay at different dairy farms or at animal markets and may contribute to increasing infection with BTB on the farm.

Management practices and hygiene condition also was significantly associated with BTB in cattle dairy farms and these findings are in agreement with (38,44). Herds kept in farms with poor management have a higher risk of being BTB positive than herds kept in farms with good hygiene as in mid-delta district in Egypt where there are poor hygiene and muddy soil. Poor hygiene condition in a farm may allow *M. bovis* to remain for a longer period and potentially to proliferate through storage of manure or slurry indoor (45,46). The raising of different species in the same facility as a risk factor was significantly associated with BTB in cattle dairy farms as in previous reports (8,47). 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 act as important factor in recirculation of BTB in cattle dairy farms. On the other hand, the finding that body condition and reproductive status weren't risk factors associated with BTB agrees with previous studies (48). Postmortem findings in (Table-4) were comparable with (49-51) and disagree with (53,48).The number of non -visible lesion (NVL) reactors 11 (14.29%), may be attributed to the non-specific reaction to the tuberculin test which may be due to sensitization by other mycobacterium rather than *M. bovis* or closely related microorganisms especially Nocardia or a combination of liver fluke infestation with saprophytic mycobacteria (51). Moreover non-specific reactors may be slaughtered at a stage of invisible lesions of the disease or the lesions may be found in parts of carcass such as bone or skin (51,52).

Bacteriological examination showed that 53 (68.83%) cultures positive was recovered. The isolation rate of *M. bovis* for visible reactors was 75.75% (50/66). The isolation rate of *M. bovis* for non-visible reactors was 1 (9.09%). The total recovery rate of *M. bovis* for both visible and non-visible reactors was 50 (64.93%), these results agreed with (22,24,53,54). and the number of atypical mycobacteria isolates was 3 (3.9%) which agree with (26,54).

The ELISA result showed that 31/77 (40.29%) were positive, 29 (43.94%) from serum of animals with visible lesions showed positive results including 7 with generalized lesions 12 with pulmonary lesions, 4 with mixed lesions and 6 of head lymph nodes and out of 11 reactors with NVL 2 (18.18 %) were ELISA positive .these results were comparable with (51). The result of ELISA test in this study was lower than that of tuberculin test and bacteriological examination which may be due to humeral immune response more dominant in the late stage of infection but cell mediated immune response as in case of tuberculin skin test may appear as early as three weeks post infection (55) So diagnosis of BTB is primarily based on the detection of Cell Mediated Immune responses in live animals (56) and antibody response seen only when animals were in advanced stages of infection or in anergic state (57). So ELISA acts as a complementary to tuberculin test, but it doesn't act alone as diagnostic test for BTB.

Conclusions

The prevalence of BTB at the individual animal level was associated with age, breed, location and density of cattle in dairy farms. Holstein Friesian breeds, with 3-6 years age, and located at mid-delta district were at higher four-fold risk positivity to comparative intradermal tuberculin compared to <3 years old cattle located at Alexandria road being. The herd prevalence of BTB recorded in the present study was higher than other studies. Herds with poor management condition and newly purchased cattle were more likely to be infected with BTB. Purchasing cattle have a significant association with BTB and regarded as one of the most common factors contribute to the spread of BTB and considered as major risk factor. ELISA could be useful as a complementary for tuberculin test in some cases as in the late stages of the disease (anergic cattle).

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Conflict of interest

There is no any conflict of interest regarding the publication of this manuscript.

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معدل الانتشار وعوامل الخطورة لمرض السل البقري في مزارع الماشية الحلابة في مصر

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الخلاصة

تم إجراء دراسة مقطعية في الفترة من نوفمبر٢٠١٥ إلى أبريل ٢٠١٨ لتحديد معدل الانتشار وعوامل الخطر المرتبطة بمرض السل البقري باستخدام اختبار التوبركولين المقارن داخل الجلد على ٥٣٢٧ من الأبقار الحلابة والموجودة في ١٦ قطيعًا في مناطق وسط الدلتا، طريق الإسكندرية الصحراوي وشمال الصعيد. تم استخدم استبيانات لجمع البيانات عن حجم القطيع ونظم التربية. كان معدل الانتشـــار في القطيع

والحيوان عند قيمة قطع ≤٤ مم ٦٨,٧٥٪ (٩٥٪، مجال الثقة: ٤٦ إلى ٩١,٤) و ١,٦٧٪ (٩٥٪، مجال الثقة: ١,٣ إلى ٢,١) على التوالي. ارتبط الانتشار على مستوى الحيوانات بشكل كبير بالعمر والسلالة والمواقع الجغرافية المختلفة للمزارع وكثافة الماشية في كل فناء. على مستوى القطيع، يعتبر وجود تاريخ مسبق للإصابة بمرض السل البقري في المزرعة وطرق التربية وتربية الأنواع المختلفة من الحيوانات في نفس المكان والماشية التي تم شراؤها حديثًا من اهم عوامل الخطر للإصابة بمرض السل البقري. وبإجراء الفحص الظاهري للحيوانات الإيجابية لاختبار التيوبركلين بالمجازر تبين وجود إصابة سلية ظاهرية في عدد٦٦ من أصل ٧٧ من الماشية المذبوحة بنسبة ٨٥,٧١٪، بينما لم يشاهد إصابة سلية ظاهرية في ١١حيوان بنسبة ١٤,٢٩٪. وبإجراء عملية العزل البكتريولوجي للحالات الإيجابية المذبوحة كلها كانت نسبة العزل لميكروب الميكوبكتريا البقرية ٤٩ من أصل ٦٦ (٧٤,٢٤ ٪) للحالات التي بها إصابات سلية ظاهرية وواحد من أصل ١١حيوان بنسبة ٩,٠٩% للحالات التي لا يوجد بها إصابات سلية ظاهرية. أظهرت نتائج اختبار الاليزا غير المباشر وجود ٣١ (٤٠,٢٦%) عينه إيجابية من بينها ٢٩ (٩٣,٦٪) بإصابات سلية ظاهرية و ٢ (٦,٤٪) من التي لا يوجد بها إصابات سلية ظاهرية. وتبين من هذه الدراسة ارتفاع معدل الانتشار في القطعان التي تطبق طرق تربيه سيئة، تربي أنواع مختلفة من الحيوانات في نفس المكان، وكان له تاريخ إصابة سابق بمرض السل البقري واشترى حيوانات جديدة. على مستوى الحيوانات، كانت سلالة هولشتاين، التي تتراوح أعمارها بين ٣ و ٦ سنوات، والتي تم تربيتها بكثافات عالية وتقع في منطقة وسط الدلتا أكثر عرضة لتكون إيجابية لاختبار التوبركولين المقارن داخل الجلد.