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Surveying Three Egyptian Governorates for Bovine Tuberculosis

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Abstract

BOVINE TUBERCULOSIS, a disease caused by *Mycobacterium bovis* (*M. bovis*), represents a threat challenging animal production as it causes severe economic losses for the livestock sector. In addition, *M. bovis* infects humans through different routes: respiratory, oral, mucous membranes and injured skin causing human tuberculosis of animal origin. In Egypt, bovine tuberculosis considered as one of the most serious threats affecting cattle and other livestock. In this study, the prevalence of bovine tuberculosis in three Egyptian governorates (one in Upper Egypt and the others in Lower Egypt) was investigated. This was to figure out the status of the disease incidence and its negative impact on animal production and public health. Of 220 tuberculous-suspected cattle, 25 (11.36%) were positive by tuberculin test, (10 from Qena 11.1%, 7 from Elbeheira 11.7% and 8 from Elgharbia 11.4%). By conventional culture techniques, out of 25 TB positive cases, 16 were culture-positive (65.4%). Concerning serology, 28 of 220 serum samples of animals tested by tuberculin test, were positive with ELISA (12.7%). Lymph nodes and tissue samples (N. = 52) were collected from 25 tuberculin positive animals. Of the collected samples, 41 (78.8%) were positive by RT-PCR. Meanwhile, 35 (94.6%) of 37 tested isolates were identified as *Mycobacterium species* using *Mycobacterium tuberculosis* complex-specific primers. Our results threw a spot of light on the importance of prompt surveillance and improvement of the existing control strategies and even developing better programs to prevent the dissemination of *M. bovis* infection among animals and to protect humans, consequently.

Keywords: Bovine, Tuberculosis, Tuberculin, ELISA, Real time PCR, Egypt.

Introduction

Tuberculosis (TB) is a serious disease affecting humans and animals. It is caused by a bacterial threat of the *Mycobacterium tuberculosis* complex (MTBC). [1]

MTBC species differ in their pathogenic capability considering susceptible hosts, severity of the infection, pathogenicity and dissemination. [2]

Bovine tuberculosis (BTB) is caused by *Mycobacterium bovis* (*M. bovis*), an aerobic slow growing bacterium. Although *M. bovis* is host-adapted to cattle it is a cause of TB in other livestock and wild animals [3, 4]. The bacterium causes intercellular infection, which, results in a chronic disease in the infected animal. [5, 6, 7]

Human tuberculosis is also caused by *M. bovis*, and its implications go beyond human health posing a serious public health challenge. *M. bovis* infects Humans may potentially contract *M. bovis* via ingestion or inhalation of the organisms. In addition, mucous membranes or injured skin represent entry access for *M. bovis* infection in humans. [5, 6, 7, 8]

M. bovis has a negative economic impact on the livestock sector all over the world [9, 10]. In addition, it has been causing human TB in few reported cases and it also affects wild animals. The chronic nature of the disease in cattle has an important economic impact and inflicts significant losses in infected herds. [1, 8, 11]

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The zoonotic nature of *M. bovis* has been well-documented since over a hundred years . In contrast, MTBC transmission from humans to animals is not common. Even though, many reports of MTBC transmission from man to cattle are recently evidenced globally. This provides strong evidence of human-to-cattle transmission. [12]

The control of tuberculosis in livestock requires effective program devoted by authorities to contain the disease spread among susceptible animal hosts. This, in turn, will minimize economic losses due to animal tuberculosis and protect humans from the disease zoonotic transmission. [13, 14]

Bovine tuberculosis represents a major threat for livestock In Egypt [15]

Accordingly, this study targeted the detection of the prevalence of bovine tuberculosis in three Egyptian governorates, one in Upper Egypt (Qena) and the others in Lower Egypt (Elbeheira, and Elgharbia.) This was to estimate the current situation of the disease incidence and its negative impact on animal production, public health and the environmemt.

Material and Methods

Sample collection

A total of 220 cattle belonging to different farms in three Egyptian governorates were tested for tuberculosis employing the comparative cervical intradermal tuberculin test (90 in Qena, 60 in Elbeheira, and 70 in Elgharbia Governorate) . Positive animals were slaughtered and inspected for tuberculous lesions in lymph nodes and internal organs. Suspected lesions were collected in sterile plastic cups, labelled separately and transported to the laboratory while cold. A taken 5 ml blood sample was drawn from each animal before slaughtered for serum separation to be tested by ELISA.

Types of collected samples

Specimens from the 25 slaughtered cases were distributed as follows: 25 lymph nodes, 14 lung, 8 liver and 5 spleen tissues samples distributed as shown in (Table, 1), with total 52 tissue samples.

Sample processing

Under sterile conditions, tissues taken from gross lesions of organs and lymph nodes were chopped into little parts after fat trimming. The chopped tissues were crushed in a sterile mortar containing sterile sand to form a paste-like mixture. Two millilitre of sterile distilled water 2 ml of 4% H2SO4 were added followed by incubation for 30 minutes. After adding 16 ml of sterile distilled water, the mixture was centrifuged for 20 minutes at 1000 xg (4000 rpm). The sediment was kept for bacterial isolation while the supernatant was decanted into a disinfectant (5% phenol). [16]

Isolation and biochemical identification of *M. bovis*

Smears were prepared from the sediments for Ziehl-Neelsen direct staining. A fraction from the sediment was inoculated into Lowenstein-Jensen medium (L-J medium) slants (Biolife®, Italy). After inoculated, slants were kept at 37°C for three weeks. Slants were examined for colonial growth up to eight weeks. [17]

Bacterial smears were prepared from the suspected colonies for microscopic examination after Ziehl-Neelsen staining. Acid-fastness bacilli, shape, size and arrangement were considered.

Isolates were subjected for biochemical identification by employing the niacin production, nitrate reduction, iron uptake, catalase and urease tests. [18, 19, 20]

Indirect ELISA test on bovine sera for diagnosis of BTB infection

Indirect ELISA was carried out According to Hall and Thoen [21]. A commercial kit was utilized (KPL®) and the instructions of the producer were strictly followed.

Molecular identification of *Mycobacterium tuberculosis complex* using PCR

DNA extraction from samples was done using QIAamp DNA Mini kit (Qiagen, Hilden, Germany). Real time PCR (RT-PCR) was conducted on the extracted DNA utilizing a specific commercial kit (MTplex dtec-RT-qPCR, Edifici-Quórum3, Elche, Spain). Supplier instructions were followed and negative and positive controls were run simultaneously as directed. Negative controls were template-free master mixes while positive control was represented by an MTplex positive control standard supplied with the kit..according to the manufacturer's instructions.

The thermal cycling conditions were as follows: initial denaturation at 95ºC for 5 minutes followed by 45 cycles of denaturation at 95°C for 30 seconds and 60°C for 60 seconds. The fluorogenic signal and the cycle threshold were detected using special software (StepOneTM, version 2. 2. 2., Life Technology, UK). The reactions were run in a StepOne RT-PCR System (Applied Biosystems, Thermo Fisher Scientific Inc., Ontario, Canada). Samples and controls'distinctive phases were shown in corresponding curves of amplification obtained with the amplification progression. [22]

Results

Out of 220 suspected cattle, (90 from Qena, 60 from Elbeheira and 70 from Elgharbia governorates),

25 (11.36%) were positive by tuberculin test, (10 from Qena 11.1%, 7 from Elbeheira 11.7% and 8 from Elgharbia 11.4%). (Table, 2).

Specimens from fourteen animals out of 25 (56%) showed acid fast bacilli in smear stained withthe Ziehl-Nelseen method (Table, 3).

Distribution of positive cases in different organs and in governorates was shown in (Table, 4)

By conventional culture techniques, out of 25 tested cases, 16 were positive (65.4%). (Table, 5)

Distribution of positive cases in different organs and in governorates was shown in (Table, 6)

Using ELISA technique, out of 220 collected serum samples from animals tested by tuberculin test, 28 (12.7%) serum samples were positive (Table,7).

Result of real time PCR technique done on samples and isolates were shown in (Table, 8). Analysis for the amplification blot in its linear form, the used reference dye is (FAM) and the run is for 45 cycles.

Out of 52 tissue samples of 25 tuberculin positive animals, 41 (78.8%) tissue samples were positive. While 35 (94.6%) of tested isolates were confirmed as *mycobacterium species*. by using the primers of *mycobacterium tuberculosis complex.*

Discussion

Mycobacterium tuberculosis is a pathogenic bacterium causing tuberculosis. The disease is a chronic granulomatous affection in man and animals [23]. In developing countries (Africa, Asia, Latin America and most countries in the Middle East.), tuberculosis is still endemic and largely uncontrolled [24]

Pathogenic *Mycobacterium species* are more than one collected in a group called *Mycobacterium tuberculosis* complex that include *Mycobacterium bovis,* a species that infects cattle and wildlife. Although *M. bovis* causies bovine tuberculosis (BTB)it can also cause a small proportion of tuberculosis cases in humans. [3]

Therefore, accurate detection of infected animals is critical for the effective control of BTB, especially during the early phases of the disease and to protect human zoonotic infection. Moreover, rapid diagnosis of BTB is crucial to identify and remove infected cases where any delay may exaggerate the transmission of *M. bovis* within herds with consequent increased public health. Several diagnostic tests are used for surveillance of BTB. All the applied tests have several limitations that must be taken into account when dealing with the control of the disease. [25]

Unfortunately, only about 25% of cattle and buffalo populations in Egypt are tuberculin-tested annually which represents a major obstacle against BTB eradication programs in Egypt. **[26]**

Accordingly, this study targeted surveying three major Egyptian governorates for BTB among cattle populations. This was to figure out the current situation of BTB incidence among the tested animals and herds. The selected governorates represented upper, middle and lower Egypt as a trial to get a real image as much as we can.

The comparative intradermal tuberculin test results revealed that 11.36% (25/ 220) tested positively for BTB. This is alarming when compared with the results recorded by Cadmus *et al*. [27], in which 10.5% of animals tested positively using single intradermal tuberculin test.

By bacteriological examination of samples collected from positive animals after being slaughtered resulted in isolation of *M. bovis* from 65.4% of the samples (17/ 25). Comparatively, this percentage is lower than that obtained by ELNakwr *et al.* [28], who detected higher percentage of *M. bovis* (72.22%) in slaughtered cattle. However, incidence reported in this study is higher than that recorded by Elsayed *et al.* [29], who confirmed that 20/36 (55.6%) cattle were positive for *M. bovis* isolation. When compared with the most recent study [29], prevalence of *M. bovis* in our study is as alarming as the result of tuberculin test.

It is of significance to denote that although tuberculin test positive reactors percentages were almost similar in the three surveyed governorates, the *M. bovis* isolation rates differed (63.6%, 71.4% and 62.5%) in Qena, Elbeheira and Elgharbia respectively. The higher isolation rate of *M. bovis* from Elbeheira could be attributed to the animal husbandry system as this governorate has huge animal population in flocks rather than individual rearing followed in the other two governorates.

A real time PCR (RT-PCR) assay was applied on DNA extracted from all the collected samples and bacterial cultures to confirm the results of bacteriological examination and the tuberculin test. It is of sensitivity similar to or better than that of the culture method, but in short time **[30, 31]**. In the present study, PCR technique was a rapid and accurate method for diagnosis of TB within 3 days, while bacteriological culture took several weeks.

In this study. 41 out of 52 tissue samples collected confirmed the existence of mycobacteria on the genus level using RT-PCR, with percentage of 78.8%. Comparatively, this percentage is lower than that obtained by ELNakwr *et al.* [28], who detected higher percentage (83.3%), and by Elsayed *et al.* [29], who detected genus *Mycobacterium* in all tested DNA extracted from 54 tissue samples and 25

isolates with (100%),using the real time PCR method employed in this study.

In conclusion, the present study highlighted the prevalence of bovine tuberculosis among cattle in three governorates in Egypt. This poses significant socioeconomic consequences to livestock farmers and the public. Our results threw a spot of light on the importance of prompt surveillance and improvement of the existing control strategies and even developing better programs to prevent the dissemination of *M. bovis* infection among animals and to protect humans, consequently.

Conclusion

Despite the official control program, bovine tuberculosis is still endemic in high incidence rates at the governorates investigated in this study. This is alarming as the infected cattle represent sentinels for the organism and are potential disseminators for the disease among livestock and humans. The control program is to be re-evaluated and molecular diagnostic methods are to be added to the program.

TABLE 1. Samples collected from slaughtered cattle to isolates mycobacteria

Governorate	Types and numbers of collected samples				Total
	Lymph node	lunge	liver	Spleen	
Qena	10	7	3	2	24
Elbeheira	7	3	2	1	12
Elgharbia	8	4	3	2	16
Total	25	14	8	5	52

TABLE 2. No. of positive tuberculin cases

Governorate	No. of tested animals	No. of tuberculin +ve cases	Percent of tuberculin +ve cases
Qena	90	10	11.1%
Elbeheira	60	7	11.7%
Elgharbia	70	8	11.4%
Total	220	25	11.36%

TABLE 3. No. and percent. of +ve by direct smear

Governorate	No. of tested animals	No. of +ve by direct smear	Percent. Of +ve by direct smear
Qena	10	6	60%
Elbeheira	7	3	42.8%
Elgharbia	8	5	62.5%
Total	25	14	56%

TABLE 4. Distribution of +ve result of direct smear in different organs

Governorate	Distribution of +ve result of direct smear in different organs								Total	
	Lymph node		Lung		Liver		Spleen			
	No.	%	No.	%	No.	%	No.	%	No.	%
Qena	6	60	4	57.1	2	66.67	1	50	13	54.16
Elbeheira	3	42.8	2	66.67	2	100	1	100	8	66.67
Elgharbia	5	62.5	3	75	2	66.67	1	50	11	68.75
Total	14	53.8%	9	64.28	6	75	3	60	32	61.5

TABLE 5. No. & percentages. of +ve by culture

Governorate	No. of tested caese	No. of +ve by culture	Percent. of +ve by direct smear
Qena	10	7	63.6%
Elbeheira	7	4	71.4%
Elgharbia	8	5	62.5%
Total	25	16	65.4%

TABLE 6. Distribution of +ve result of culture in different organs

Governorate	Distribution of +ve result of culture in different organs								Total	
	Lymph node		Lunge		Liver		spleen			
	No.	%	No.	%	No.	%	No.	%	No.	%
Qena	7	70	5	71.4	3	100	2	100	17	70.8
Elbeheira	4	57.1	2	66.67	2	100	1	100	9	75
Elgharbia	5	62.5	3	75	2	66.67	1	50	11	68.75
Total	16	65.4	10	71.4	7	87.5	4	80	37	71.15

TABLE 7. No. of positive ELISA

Governorate	No. of +ve samples by ELISA	Percent of +ve samples by ELISA
Qena	12	13.3%
Elbeheira	7	11.7%
Elgharbia	9	12.85%
Total	28	12.7%

TABLE 8. Result of real time PCR technique

Type of tested samples	No. of tested samples	No. and perc. of +ve for RT-PCR	
Tissue samples	52	41	78.8%
Isolates	37	35	94.6%

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التقصي عن مرض السل البقري في ثلاث محافظات مصرية

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الملخص

يمثل مرض السل البقري تحدياً كبيراً للثروة الحيوانية لما يسببه من خسائر اقتصادية في القطاع الحيواني. إضافة إلى ذلك فإن البكتيريا المسببة وهي ميكوباكتيريام بوفيس تنتقل للإنسان بطرق مختلفة مسببة السل الآدمي من أصل حيواني. ويعتبر السل البقري من أهم المشكلات التي تهدد صحة الأبقار والحيوانات المزرعية الأخرى. وعليه، فإن هذه الدراسة استهدفت التقصي عن مدى انتشار السل البقري في ثلاث محافظات مصرية (واحدة في الجنوب وإثنان في الشمال). وكان ذلك لمعرفة نسبة الإصابة الحالية وانعكاساتها السلبية على الإنتاج الحيواني والصحة العامة. أظهر 25 حيوان (10 من قنا، 7 من البحيرة و 8 من الغربية) ، من 220 من قطعان أبقار مختلفة، نتائج إيجابية مع اختبار التيوبركلين وبنسبة 11,36%. وأسفر 16 حيوان من ال 25 الإيجابية عن عزل بكتيريا السل البقري. وباختبار الإلايزا، كانت 28 عينة مصل من بين 220 مأخوذة من الحالات سالفة الذكر إيجابية لإصابة بالسل. وباستخدام تفاعل البوليميريز التسلسلي اللحظي نتائج إيجابية مع 41 عينة نسيج مأخوذة من ال 25 حيوان الإيجابي مع اختبار التيوبركلين. وعليه فإن هذه الدراسة أظهرت أهمية تقوية برنامج التقصي والذبح المتبع في مصر بل والحاجة إلى برامج جديدة تتماشى مع الوضع الراهن.

الكلمات الدالة: السل، البقر، التيوبركلين، الإلايزا، اختبار البي سي آر، مصر.