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Bacteriological and Molecular Characterization of Mycobacterium avium Subspecies Paratuberculosis in Camel



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Abstract

ARATUBERCULOSIS Is an infectious bacterial disease mostly upsets sheep, camels, goats, and cattle characterized by severe emaciation, chronic diarrhea with decreasing milk production. The World Organization for Animal Health (WOAH) has identified paratuberculosis as a noteworthy disease that needs to be reported to it. Camel milk and meat are considered an important source of protein. So that, our objective is to determine the actual frequency of Paratuberculosis in camels, using a traditional and molecular techniques, and to discover the risk factors related to camel paratuberculosis, to offer a thorough comprehension of disease's epidemiology in Egypt. In this work, 200 serum samples and 200 samples of feces were collected from adult Arabian dromedary camels (older than 5 years) from different localities between March 2023 and December 2023. After incubation for 4-20 weeks on Herrold's EggYolk Medium, bacterial culture results of fecal samples from camels were 7 positive samples from the 40 pooled fecal specimens. Identification of colonies was confirmed by using Zeihl Nelseen stain; through preparing a film from isolated colonies had red acid alcohol-fast bacilli. ELISA test revealed 62 (31%) of the 200 serum samples were positive Mycoacterium avium subsp. paratuberculosis. All tested isolates were confirmed the q- PCR. The results deduced that the culture method is the most reliable method for detecting paratuberculosis. Although the specificity and sensitivity of primers should be carefully considered, molecular diagnostics (PCR) may be a helpful technique in identifying truly positive and negative camels.

Keywords: Mycobacterium avium subspecies paratuberculosis, IS900 PCR, ELISA, HEYM.

Introduction

(the original Johne's disease name for paratuberculosis) is a chronic, infectious bacterial intestinal tract disease that mostly affects sheep, cattle (mostly dairy cattle), goats, and other ruminant animals including camels. The hallmarks of paratuberculosis include the animal's gradual atrophy and progressively worsening diarrhea. Due to reduced productivity, the agricultural sector around the world is severely impacted economically by paratuberculosis [1]. This disease has no known cure Mycobacterium treatment. avium paratuberculosis is a bacterium that causes the illness. In the camel populations of Asia, Africa, and the Middle East, Johne's illness is rapidly spreading [2]. Camels from Saudi Arabia [3], the United Arab Emirates [4], Tunisia [5], and Iran [6] have been

reported to have the disease. According to the World Health Organization's Terrestrial Animal Health Code, paratuberculosis is a disease that is listed with the WOAH and needs to be reported. The camel is a versatile animal that can be utilized for milk, meat, and wool production. Also, modern applications in the dairy industry lead to the development of camel dairy farms. Meat and milk from camels are regarded as a significant protein source. Now, booming of camel, racing in the Gulf countries made camel as a highly attractive commodity [7]. Camels are a versatile animal that are used as a source of meat and milk in Egypt, as well as for transportation and tourist rides [8]. However, camels can contract a variety of infectious pathogens that are harmful to their health [9]. Thirty Arabian camels (Camelus dromedarius) that were suffering from signs ranging from mild to severe infections [10] (that did not

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respond to antibiotics) as well as chronic or intermittent diarrhea. Eight camels were identified by PCR (26.6%), while 5 isolates of MAP were identified from the studied camel samples by culture method, yielding an isolation rate of 16.6%. ZN fecal staining revealed that the feces of five camels contain MAP (16.6%), however by ELISA, antibodies against MAP were identified in 9 serum samples of camel (30%). Ziehl-Neelsen (ZN) staining for direct recognition of MAP through rectal scraping from feces and tissue specimens for the detection of IS900 gene by polymerase chain reaction (PCR), isolation and molecular description of MAP from fecal and tissue samples, and determination of the antibodies against MAP in camels' serum were all conducted [11]. To determine whether a dromedary camel was infected with paratuberculosis, ELISA and PCR techniques were used. Subclinical paratuberculosis was identified [12, 13] by using ELISA jointly with the confirmation of MAP by PCR in El-Minia governorate, Egypt. There is a greater need than ever for trustworthy diagnosis methods and control initiatives due to the rising incidence of paratuberculosis worldwide [10]. It has been suggested that two to three diagnostic tests be used for herd screening and to improve the precision of diagnosis of MAP [14]. To give a better picture of the disease's epidemiology, it is crucial to do epidemiological research on paratuberculosis in Egypt using both conventional methods and more current molecular biology approaches. It's crucial to account for the diagnostic process's imprecise precision when measuring illness occurrence and identifying risk-preventive factors. Therefore, determining the actual frequency of paratuberculosis in camels is our goal, using traditional and molecular techniques, and to investigate the risk factors for paratuberculosis in camel in order to give a thorough grasp of the epidemiology of the infection in Egypt.

Material and Methods

Collection of the samples

A total of 200 fecal and blood samples from adult Arabian dromedary camels (C. dromedarius) (aged more than 5 years) from different localities between March 2023 and December 2023 were collected and examined in this study. The camels, which had diarrheal symptoms that persisted even after receiving antibiotics for a chronic or episodic illness, were rarely, if ever, accepted. The clinical assessment of animals was conducted in accordance with standard protocols [15]. Using sterile plastic containers and a disposable plastic sleeve, the feces samples were taken rectally and sent immediately to the animal health research laboratory in ice box. Samples were stored in a freezer at -80°C. At the same time, 10 ml of blood were drawn through the jugular vein of the same animals. After that, the serum was separated and kept at -20°C.

Pooling, decontamination and culture of fecal samples

A strategic pooling approach was used to investigate the fecal samples:-Two grams of each of the 5 separate fecal samples were combined to create the pooled fecal sample (40 pools of 200 distinct fecal samples were thus obtained). Samples decontamination protocols according to [16, 17]:-0.9% Hexadecylpyridinium Chloride solution (0.9% HPC) was used to disinfect 3 grams of each pool for duration of 24 hours. Herrold's EggYolk Medium (HEYM) was used for culture of 300 µL of the decontaminated samples, both with and without mycobactin J supplementation (Prepared Culture Media, Becton Dickinson). Slanted tubes with lightly screwed tops were incubated at 37°C. Slopes were checked for the growth of contaminating organisms after a week of inoculation. The tubes were incubated upright and the caps were sealed after two weeks. For growth, HEYM tubes were checked every week for 16 weeks and then monthly for the next six months [10, 18]. The tubes were examined for the development of opaque, smooth colonies, which, as the cultures age, become crumbly [19]. Colonial morphology was used to determine colony identity following 4-20 weeks of incubation (appearing of tiny pinhead or half-ball-shaped Map colonies that range in color from white to yellow.

Microscopic examination

Smears for Ziehl-Neelsen (ZN) staining were made from the bacterial colonies in order to provide a preliminary identification of mycobacterial isolates. The colonies were microscopic examination of positive culture revealed clumps, or clusters of three or more small, strongly acid-fast bacilli (AFB) of 0.5–1.5 m [20].

Serological identification

A commercially available ELISA kit was used to evaluate the samples of serum (ID Screen® Paratuberculosis Indirect ELISA kit (ID VET diagnostics, Montpellier, France) to determine antibodies against MAP in collected serum samples. To prevent cross-reactions, sample diluents containing Mycobacterium phlei were used to predilute the samples and test controls. The manufacturer's instructions were followed while using the reaction technique.

Molecular detection of IS900 gene using q-PCR

According to [21], in accordance with the manufacturer's instructions, the genomic DNA of the bacterial isolates was extracted using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany). The bacterial genome was detected by q-PCR with the following primers and probe:

F2: 5' AATGACGGTTACGGAGGTGGT 3', R2: 5' GCAGTAATGGTCGGCCTTACC 3'

and probe: P2 5' TCCAC GCCCG CCCAG ACAGG 3'

The PCR reaction was conducted using the Roche Light Cycler® TaqMan Master Detection kit, and the PCR mixture included 5 μL of the DNA template, 4 μL of Master Mix (5×), 1 μL of each primer (10 pmol), 1 μL of TaqMan probe, and 8 μL of water. Amplification was carried out on the LightCycler 2.0 Instrument (Roche, Life Science system) as outlined [1].

Results

Bacteriological findings:

Bacterial culture results of fecal samples from camels on HEYM were 7 from the 40 pooled fecal specimens. Identification of colonies was verified by the morphology of the colonies. (Tiny, pinhead or half-ball-shaped Map colonies that range in color from white to yellow (Fig. 1).

Microscopic findings:

By using ZN stain, the film prepared from isolated colonies possessed the red acid alcohol-fast ZN staining that characterizes bacillary mycobacterium (Fig.2).

Serological findings:

The ELISA test was performed on all serum samples in this investigation to determine whether any had antibodies against MAP. Sixty-two (31%) of the 200 serum samples that were analyzed tested positive for the ELISA test.

Molecular findings:

All tested isolates were positive for MAP by q-PCR (Fig.3).

Discussion

Paratuberculosis is one of the most severe intestinal illnesses that affect both ruminant and non-ruminant animals, findings in significant financial losses.

Typical clinical indications of paratuberculosis, such as severe emaciation, dehydration, and edematous swelling of the ventral body locations, were found during a clinical examination of the affected dromedary camel, prolonged intermitted progressive watery diarrhea, and deprivation of appetite (pica), which may be caused by a gastrointestinal parasite infestation or deficiencies of trace elements, as reported in earlier studies [1, 9, 22].

In accordance with the OIE report [23], samples were collected from suspected camels for this study between the age of two and five for our investigation. Therefore, the first step in managing MAP is to diagnose its etiology early. Although the bacterial culture approach is specific and sensitive, it is costly, time-consuming, and labor-intensive. To find acid-

fast bacilli in the isolated colonies, we employed ZN stain which resulted in positivity of 7 (17.5 %) out of 40 pooled fecal samples of camels. However, the sensitivity and specificity of ZN staining are limited since it is hard to differentiate between MAP and other acid-fast bacilli. All things considered, ZN staining is the fastest, simplest, and least expensive method and it can be used for the first identification of MAP [13, 15]. The potential viability of this method for camel MAP diagnosis was recently confirmed by the identification of anti-MAP antibodies in camels using bovine ELISA [24]. Determination of the antibodies against MAP in camels serum in our study revealed that 62 (31%) out of 200 samples examined were positive for ELISA. ELISA has a low sensitivity but a high specificity [25]. Furthermore, ELISA is the preferred test because to its affordability, ease of use, and speed [26]. The inability of ELISA to detect every animal exposed to the bacterium was confirmed by the PCR retesting. Our results for molecular characterization of IS900 gene specific for MAP confirmed culture and ZN staining (7 out of 40 pooled fecal isolates were positive for IS900 gene). Although PCR and ELISA may be essential for tracking the disease in camels, they cannot be used as a definitive method to eradicate MAP from camel herds. [27] used an intriguing approach by reviewing the effectiveness of MAP control and survey initiatives over the past 20 years in eliminating the MAP infection. Fecal culture and longitudinal surveys using PCR and/or ELISA were ineffective in eradicating MAP infection [28]. ELISA was able to identify seroconverted animals prior to the shedding starting in the majority of cases. Nevertheless, some ELISA-positive animals may continue to have negative fecal cultures, while others may begin to shed prior to the seroconversion. The findings of this investigation also demonstrated the sensitivity of the conjugated antibodies to detect camel's antibodies, as documented [12], as well as the cross reactivity of the MAP antigen in the ELISA kit with the camel anti-MAP antibodies. Instead of being a sensitive test for the early detection of the infection, ELISA is nevertheless a helpful screening tool in control programs [1]. Additionally, we targeted the IS900 gene with RT-PCR molecular analysis. The existence of the microorganisms was verified by our q-PCR results. Our findings are consistent with earlier reports that found Ct values for IS900 analysis of MAP to range from 17 to 32 [10, 21, 29]. Our study's findings imply that the paratuberculosis pathogen, which causes serious pathological alterations with a noticeable clinical appearance, can infect dromedary camels.

Conclusion

Despite its drawbacks, we deduced from the results that the culture method is the gold standard for diagnosing MAP. It may be possible to distinguish between truly positive and negative

camels using molecular diagnostics (PCR). The most sensitive technique for identifying positive clinical cases in camels is currently ZN staining. Sensitivity may be reduced in subclinical situations, though. ELISA and PCR have demonstrated their viability as reliable diagnostic tools for screening for Johne's illness in camels, and the study has established the spread of MAP infection in camel herds. Ruminant ELISA was found to be helpful for screening for MAP infection in camels.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

TABLE 1. Results of recognition of antibodies against MAP in the serum of camel by ELISA

| No. of serum examined samples | Positive | negative | |
|-------------------------------|----------|-----------|--|
| 200 | 62 (31%) | 138 (69%) | |

TABLE 2. Results of different diagnostic tools for MAP on tested pooled camel fecal samples:

| Number of samples | pooled fecal | Culture method | | ZN | | PCR | |
|-------------------|--------------|----------------|----------|------------|----------|------------|----------|
| 40 | | positive | negative | positive | negative | positive | negative |
| | | 7 (17.5 %) | 33 (82%) | 7 (17.5 %) | 33 (82%) | 7 (17.5 %) | 33 (82%) |

The percentage calculated according to the total No. of the pooled samples (40) examined



Fig.1. Morphological appearance of MAP positive culture: appearing of tiny, pinhead or half-ball-shaped paratuberculosis colonies that range in color from white to yellow.

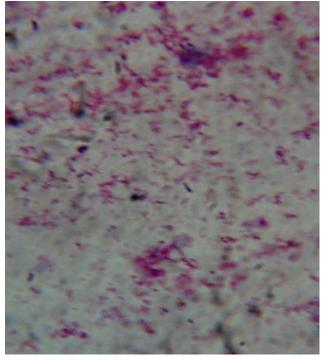


Fig.2. Microscopic appearance of Ziehl-Neelsen stained positive culture which illustrates the clumps of acid-fast bacilli bacteria.

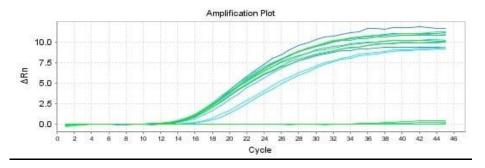


Fig.3. As shown in q-PCR, the curve of amplification of specific *MAP*. The control negative was represented by straight line (no amplification).

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التوصيف البكتريولوجي والجزيئي لبكتريا نظير السل في الإبل

 1 أسماء السيد جمال الدين 1 ، رانيا صالح محمود 1 ، عبد الحليم الشوادفي 2 و عطيه عبد الله الجداوي

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الملخص

نظير السل مرض بكتيري مُعدٍ يُصيب غالبًا الأغنام والإبل والماعز والأبقار، ويتميز بهزال شديد وإسهال مزمن وانخفاض في إنتاج الحليب. وقد صنفت المنظمة العالمية لصحة الحيوان (WOAH) نظير السل كمرض جدير بالملاحظة يجب الإبلاغ عنه. يُعدَّ حليب الإبل ولحومها مصدرًا مهمًا للبروتين. لذا، يتمثل هدفنا في تحديد معدل الإصابة الفعلي بنظير السل في الإبل، باستخدام تقنيات تقليدية وجزيئية، واكتشاف عوامل الخطر المرتبطة به، وذلك لتقديم فهم شامل لوبائيات المرض في هذا العمل، تم جمع 200 عينة مصل و 200 عينة براز من الإبل العربية البالغة (أكبر من 5 سنوات) من مواقع مختلفة بين مارس 2023 وديسمبر 2023. بعد الحضانة لمدة 4-20 أسبوعًا على المستنبت الخاص بالبكتريا، كانت نتائج زراعة البكتيريا لعينات البراز من الإبل 7 عينات إيجابية من 40 عينة براز مجمعة. تم تأكيد تحديد المستعمرات باستخدام صبغة XN؟ من خلال إعداد فيلم من المستعمرات المعزولة التي تحتوي على عصيات حمضية حمراء مقاومة باستخدام صبغة XN؟ من خلال إعداد فيلم من المستعمرات المعزولة التي تحتوي على عصيات حمضية حمراء مقاومة باراتيبركيولوزس. تم تأكيد جميع العزلات المختبرة بواسطة اختبار تفاعل انزيم البلمرة المتسلسل (Q-PCR). استنتجت باراتيبركيولوزس. تم تأكيد جميع العزلات المختبرة بواسطة اختبار تفاعل انزيم البلمرة المتسلسل الإبل الإيجابية والسلبية. ولسلبية البادئات بعناية، إلا أن التشخيص الجزيئي (PCR) قد يكون تقنية مفيدة في تحديد الإبل الإيجابية والسلبية.

الكلمات الدالة: الميكوباكتريوم باراتيوبركيولوزس ، تفاعل انزيم البلمرة المتسلسل، جين IS900، اختبار الاليزا، المستنبت الخاص لعزل الباراتيوبركيولوزس (HEYM).