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# Detection of subclinical paratuberculosis in dairy cattle in Egypt

**S.I. Anwar[1,2] and S.A. Gharieb[2,3]**

[1]Department of Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt, [2]Department of Comparative Biosciences, the University of Illinois at Urbana-Champaign, Urbana, IL, USA, [3]Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, Minia, Egypt

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***Correspondence:***
S.I. Anwar
sia5@illinois.edu

**Abstract**

Paratuberculosis (PTB) is a chronic disease affecting ruminants caused by *mycobacterium avium* subspecies *paratuberculosis* (MAP), characterized by the iceberg phenomena, as many cases are subclinical and underdiagnosed. An early diagnosis is imperative; however, no reliable single test is available, leading to delayed culling. Furthermore, using more than one test increases the rate of positively diagnosed cases. The current study aimed to detect subclinical PTB in dairy cows in El-Minia governorate, Egypt, using ELISA jointly with the detection of MAP by PCR. The positive cases were also subjected to pathological examination to determine whether lesions were present and their severity. A total of 145 cows of different breeds (Baladi, Mixed, and Holestins) and ages were tested by ELISA and PCR. Our results showed that the positive PTB cases detected by ELISA and PCR were 17.24% and 20%, respectively. Holestins have a significantly higher infection rate 31.70% than Baladi and Mixed breeds. In contrast, the native (Baladi) breed has the lowest infection rate 11.47%. Moreover, PTB is more common in cows aging (age ≥ 1.5- <2.5 is 28.2% and age ≥ 2.5- <3.5 is 22.91%). Among PCR-positive cases, 58.62% showed gross lesions, mainly thickening and folding of the intestinal mucosa and swelling of mesenteric lymph nodes. Histopathologically, 86.20% had chronic granulomatous enterocolitis and lymphadenitis. Furthermore, acid-fast bacilli were observed in 82.75%. Thus, subclinical PTB diagnosis could be more accurately confirmed when multiple diagnostic tools are used together.

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## Introduction

Paratuberculosis (PTB), or Johne's disease (JD), is a chronic enteric disease affecting large and small ruminants and caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (1,2). *Mycobacterium avium* subsp. *paratuberculosis* is the most critical member of the *Mycobacterium avium complex* (MAC) in the veterinary field, as it infects many animal species, including nonhuman primates (3). MAP has been considered a zoonotic pathogen, as it was isolated from the intestinal tract of healthy people (4). In addition, it may contribute to Crohn's disease (4-7). Johne's disease has a detrimental economic impact on the dairy and meat industry because it reduces milk and meat production, results in high culling rates, reduces fertility and incurs high treatment costs (3). Paratuberculosis is distinguished by a prolonged subclinical period characterized by little to no intermittent shedding of MAP and a small percentage of infected animals eventually developing progressive infection and exhibiting clinical signs (8-10). An adaptive T-cell-mediated cellular immune response is usually thought to cause the prolonged subclinical phase (11,12). While MAP is not completely cleared during the subclinical period, the prolonged immune response is believed to control the infection and keep it as a subclinical disease (12). However, in the later stages of the disease, it fails to do so (9). The ileum and the large intestine have primary lesions because MAP needs a high

concentration of iron in tissue macrophages for the development, which is highest in the ileocecal intestine (13). The primary gross pathology is granulomatous enteritis, which leads to thickening and corrugation of the ileocecal intestine (14). In addition, lymph nodes in the mesenteric region are enlarged and edematous. Microscopically, the disease is characterized by varying degrees of chronic granulomatous enterocolitis, regional lymphangitis, and lymphadenitis (15). Early diagnosis of MAP infection is necessary for PTB monitoring and surveillance (16,17). It is therefore recommended that farm animals be examined regularly with more than one test so that proof of MAP infection can be obtained, allowing for the implementation of control measures (18). As a result of the subclinical nature of JD, the Iceberg phenomenon, and its chronic nature, none of these tests is 100% sensitive during the entire course of the disease (18). Subclinical PTB has been reported worldwide. Briefly, it has been reported in European, such as in Ireland (19), England (20), the Netherlands (21), and Turkey (22). There have also been many reports from the American continents that have reported bovine subclinical PTB, including publications from Latin America and the Caribbean (23), Colombia (24), and Canada (25). Subclinical bovine PTB was also recorded in the Middle East and Africa. Agrawal *et al.* (26) has conducted a comprehensive review of the prevalence of PTB, which provides more information on the prevalence of PTB (26). Paratuberculosis has been reported in Sudan (27), Libya (28), Jordan (29), and Saudi Arabia (30). There has been previous research performed to assess the prevalence of JD in camels (31), sheep (32,33), and cattle (34,35) in several Egyptian governorates using ELISA, culturing, and PCR. Compared to other study areas, the disease was more prevalent in the Gharbia governorate 19.6% (34).

The pathological examination of the infected cattle is poorly studied. The current study aims to detect subclinical PTB in dairy cows in El-Minia governorate, Egypt, using ELISA jointly with the detection of MAP by PCR. Additionally, pathological examinations were performed on the positive cases to determine whether lesions were present and their severity.

## Materials and methods

### Ethical approval

All experiments followed the Guidelines of Animal Care Use and were approved by the Animal Ethics Committee of the university.

### Study population and samples collections

A total of 145 cows of different breeds, Baladi (n= 61), Mixed (n=43), and Holstein(n=41), were investigated, ranging in age from 1.5 to 4 years (mean 2 .5 years). Fecal samples were collected using disposable gloves from the rectum and then transported separately in ice-pack containers to the laboratory for analysis (36). Using anticoagulant-free vacuum tubes, blood was collected directly from the jugular vein (37). Upon receipt of the samples, they were adequately labeled, transported to the lab in ice-pack containers, and processed within 24 hours of arrival.

### Serological identification of Anti-MAP antibodies in serum samples

Serum samples were examined using a commercially available ELISA kit (mycobacterium paratuberculosis antibody test kit, IDEXX Laboratories, Inc., Westbrook, USA) to detect antibodies against MAP as directed by the manufacturer. Briefly, to neutralize cross-reactions with atypical mycobacteria, serum samples were diluted in a dilutant containing mycobacterium phlei extract. Following this, 96-well microtiter plates were loaded with 100 µL per well of the diluted sample and incubated for 45 minutes at room temperature. Following manufacturer instructions, conjugated and Tetramethylbenzidine (TMB) substrates were added. Optical density (OD) was measured at 450 nm in each well after adding the stop solution. A ratio (S/P ratio) was calculated by subtracting the mean of the sample OD from that of the negative control OD minus that of the positive control OD. S/P ratios of ≥55% were considered positive.

### Extraction of MAP-DNA from fecal samples

DNA was extracted from feces using a modified method (38). One gram of the well-homogenized fecal sample was mixed with 5 ml STAR buffer and centrifuged at 1000 × g for 1 min. One ml from the supernatant was mixed with 300 mg silibeads in Ribolyser at 6.5 ms−1 for 2 × 45 s. Then, the sample was incubated at 95°C for 10 min, followed by centrifugation at 5000 × g for 5 min, and 200 µl of supernatant was mixed with 5 µl lysozyme solution. The samples were incubated at 37°C at 550 rpm for 15 min. Then, DNA was extracted using a highly pure PCR template preparation kit (Roche, Mannheim, Germany) as directed by the manufacturer. The extracted DNA was kept at -20°C until it was subjected to PCR analysis.

### Detection of MAP-DNA using the polymerase chain reaction (PCR)

Following DNA extraction, the amplification reactions were performed by the specific primers for IS900 (IS900-F: 5′ -CCTTTCTTGAAGGGTGTTCG 3′ and IS*900*-R: 5′-CCACCAGATCGGAACGTC 3′). The PCR reactions were carried out in a 25 µl reaction volume. For a single reaction, the PCR master mix contained 1 µl of each primer (20 pmol/µl), 0.75 µl of each probe (10 pmol/µl), 12.5 µl of QuantiTect probe (Qiagen, Hilden, Germany), and 4.75 µl of RNase-free water. Finally, 5.0 µl of DNA template was added. The PCR conditions were done as follows: 94°C for 15 min, followed by 40 cycles of 95°C for 15 s, 56°C for 30 s, and 72°C for 2 min. The final extension was completed at

72°C for 10 min. On 1.5% agarose gel, ethidium bromide was used to visualize amplicons of the expected size 413 bp. After gel electrophoresis, the PCR products were examined and visualized by using the detection system, an ultraviolet (UV) transilluminator Eagle Eye II (Stratagene, La Jolla, CA, USA).

**Gross pathology**

Following testing and slaughtering, gross examinations were conducted on the 29 previously tested PCR-positive cows, focusing on the ileocecal area and the mesenteric lymph nodes, as these organs are MAP's most common predilection sites. In 10% formalin, representative samples of the small intestine, the ileocecal valve, the cecum, and the respective lymph nodes were collected.

**Histopathological examination and grading criteria for histopathological lesions**

The samples were processed by using the routine histopathological technique, hematoxylin and eosin (H&E). Briefly, 5 µm thick sections were cut by a microtome processed and stained with H&E (39,40). H&E-stained sections were examined by using a light microscope. Based on cellular infiltration, the lesion scores were classified, according to previously described (41,42), into 4 groups; 0, (no lesion), I (mild), II (moderate), and III, (severe)(Table 1).

Table 1: Criteria used for grading the degree of the histopathological lesions

| Grade | Type of inflammatory cells | | | |
|---|---|---|---|---|
| | Lymphocytes and plasma cells | Macrophages | Epithelioid cells | Giant cells |
| 0 (no lesion) | 0 | 0 | 0 | 0 |
| I (mild) | ++++ | ++ | + | 0 |
| II (moderate) | +++ | ++++ | ++++ | Yes/No |
| III (severe) | + | +++ | ++++ | Yes |

**Ziehl-Neelsen (ZN) method for acid-fast bacilli**

Briefly, 5µm tissue sections were deparaffinized using three changes of xylene for 5 minutes each. Then they rehydrated in absolute ethyl alcohol for 1 min each, followed by two washes in 95% ethyl alcohol for 1 min each before a single wash in distilled water for 5 min. The tissue sections were stained with TB carbol fuchsin Ziehl-Neelsen acid-fast stain (Becton Dickinson, Sparks, MD) for 1 hour. The sections were rinsed in tap water for two min, decolored in two quick washes with acid alcohol (1% hydrochloric acid in 70% ethanol), and then counterstained with methylene blue. Before being coverslipped, the sections had two quick washes in 95% ethyl alcohol, two quick washes in 100% ethanol, and two quick washes in xylene (43,44).

**Data analysis**

The Shapiro-Wilk test was used to verify normal distribution. Student's t-test was used to compare the two groups statistically. To compare multiple groups, one-way ANOVA was followed by The Turkey *post hoc* test. A chi-square test was used to compare the score of histopathological lesions. Values were considered statistically significant if $P<0.05$. Statistical analyses were conducted using Prism software (version 8.1.1, GraphPad Software Inc.). Microsoft PowerPoint was used to create all the figures and panels.

**Results**

**Identification of MAP by PCR**

Of 145 examined cattle, 29 (20%) were positive for MAP based on PCR amplification using specific primers (Figure 1). This disease has a high incidence rate in the Holstein breed, with 13 out of 41 (31.7%) cows infected. In contrast, the native breed has the lowest infection rate, with 7 out of 61 cows (11.47%) being infected. Table 2 shows the numbers and percentages of positive PCR cases. PTB can affect cows of any age, but it is more common in cows aging (age ≥ 1.5- ≤ 2.5 is 28.12%) and (age ≥ 2.5- ≤3.5 is 22.91%).

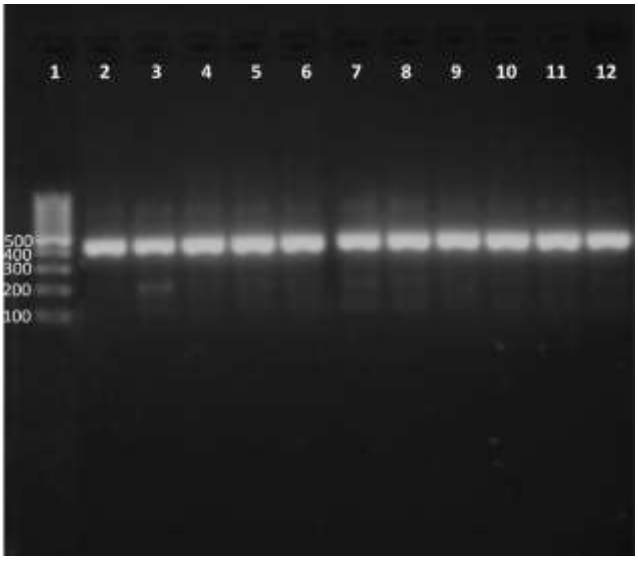


Figure 1: PCR results: a representative photograph of gel electrophoresis of IS900 PCR products from fecal samples of dairy cows using a 12 lanes gel (Lane 1 as 100 bp DNA ladder, Lane 2: Positive control; Lane 3–12: tested DNA samples PCR result of 413 bp from fecal samples.

Table 2: Indicates the number and the percentage of positive PCR cases among each breed

| Breed | Total examined | Positive cases (%) |
|---|---|---|
| Baladi (Native) | 61 | 7 (11.47%) |
| Mixed | 43 | 9 (20.93%) |
| Holstein | 41 | 13 (31.70) |
| Total | 145 | 29 (100%) |

**ELISA**

A total of 25 cows (17.24%) out of 145 examined cattle tested positive for MAP antibody by ELISA. The S/P ratio of the negative cases in the ELISA ranges from 34.52 to 26.17. MAP antibodies were not detected in four cows aged ≤ 2.5 years old by tested ELISA. However, a PCR test revealed that they were positive (Table 3).

Table 3: Comparison between the Positive cases of PCR, and ELISA, per age group

| Age (year) | Total | PCR Positive | ELISA Positive |
|---|---|---|---|
| ≤ 1.5 | 19 | 3 (15.78%) | 1 (5.26%) |
| > 1.5- ≤ 2.5 | 32 | 9 (28. 12%) | 8 (25%) |
| > 2.5- ≤3.5 | 48 | 11 (22.91%) | 10 (20.83%) |
| > 3.5- ≤ 4.5 | 29 | 4 (13.79%) | 4 (13.79%) |
| > 4.5 | 17 | 2 (11.76%) | 2 (11.76%) |
| Total (%) | 145 | 29 (20%) | 25 (17.24%) |

**Gross pathological findings**

Among the positive cases, 17 cows (58.62%) showed gross lesions. The apparent gross lesion was thickening, wrinkled, or corrugated intestinal mucosa, like cerebral gyri; this is observed primarily in the lower part of the jejunum and extends to the distal end of the ileum. There was a distended and thickened cecum with multiple hemorrhagic petechiae on the folded mucosa. The ileocecal valve was edematous, with thickened mucosa and transverse folds. Cutting sections of mesenteric lymph nodes showed hypertrophied and protracted mesenteric lymph nodes with a dark brown medullary region (Figure 2).

**Microscopical findings**

Of the 29 PCR-positive cases, 25 cows (86.20%) had histopathological lesions (Table 4). Generally, the detected histopathological lesions were granulomatous enteritis, ranging from mild to severe. The detailed scoring of histopathological lesions is fully indicated in table 5. In less severe cases, the ileum's lamina propria and submucosa were mildly and loosely infiltrated with mononuclear cells and lymphocytes with sparse macrophage and plasma cell populations. Giant cells were occasionally seen. In severe cases, many inflammatory cells infiltrate, mainly macrophages and epithelioid cells, with lymphocytes and plasma cells throughout the intestinal layers. In some cases, the epithelioid cells appeared as sheets. Besides, multinucleated cells are scattered throughout the intestinal mucosa. Other significant changes, including disruption of the normal architecture of the villi, villous atrophy, and fusion, were also observed. Mild cryptitis was also present. Mild to severe granulomatous lymphangitis was a prominent finding. Prominent lymphoid follicular hyperplasia was evident.

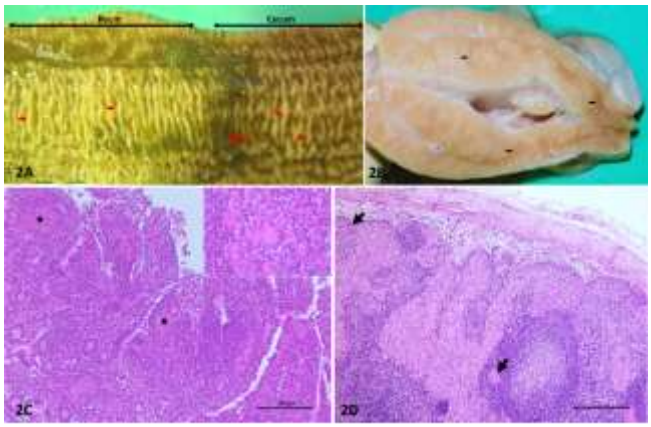

Figure 2: Pathology of PTB: 2A: gross picture of a distal portion of the ileum and the proximal portion of the cecum, close to the ileocecal junction (ILJ) of a cow severely infected with paratuberculosis; diffuse thickening of the mucosa, and transverse folding and corrugation (arrows) could be easily observed turning the mucosa into a appearance. 2B: gross picture of the mesenteric lymph node of a cow infected with paratuberculosis; the lymph node appears enlarged and hypertrophied, protracted by the cutting section, and its medullary region is dark brown (arrow). 2C: a photomicrograph of a paratuberculosis-infected cow's ileum shows the histopathological changes related to paratuberculosis in the form of transmural granulomatous enteritis: The mucosa appears desquamated and denuded; villi are moderate to marked atrophic. The submucosa shows massive infiltration of inflammatory cells, including lymphocytes, plasma cells, macrophages, epithelioid macrophages (stars), and Langhans-type multinucleated giant cells (In situ image). H&E; bar = 200µm. 2D: a photomicrograph of a paratuberculosis-infected cow's mesenteric lymph node shows granulomatous lymphadenitis in the form of epithelioid macrophage sheets admixed with a few giant cells (arrows). H&E; bar = 200µm.

**Acid-fast staining**

Acid-fast bacilli were observed in 24 cows (82.75%) either as groups or dispersed in the cytoplasm of phagocytic cells such as macrophages, epithelioid cells, and giant cells in the submucosa of the ileum and the colon also the subcapsular area of the mesenteric lymph nodes (Figure 3).

Table 4: Number and% of gross, microscopical and ZN-positive cases

| Positive cases/ PCR positive | Gross Lesion | ZN Positive cases | Histopathology Positive cases |
|---|---|---|---|
| Number of positive cases | 17/29 | 24/29 | 25/29 |
| % | 58.62 | 86.20% | 82.75% |

Table 5: Grade of histopathological findings from ilea and corresponding lymph node tissue sections in apparently healthy

| Tissue | Sample processed | Grade of lesion | | | | Total |
|---|---|---|---|---|---|---|
| | | 0 | I | II | III | |
| Small interest | Number | 1 | 14 | 7 | 3 | 25 |
| | % | 4 | 56 | 28 | 12 | 100 |
| LNs | Number | 2 | 15 | 6 | 2 | 29 |
| | % | 8 | 60 | 24 | 8 | 100 |
| cecum | Number | 4 | 16 | 3 | 2 | 29 |
| | % | 16 | 64 | 12 | 8 | 100 |

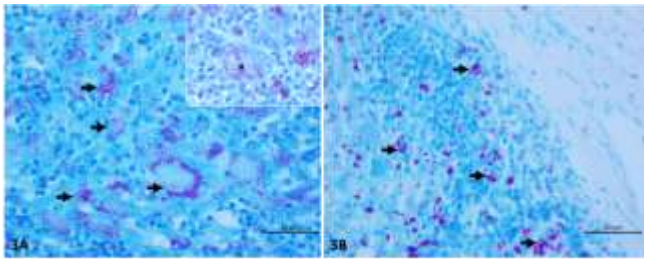

Figure 3: a photomicrograph of a paratuberculosis-infected cow's ileum (3A) and mesenteric lymph node (3B), stained with Ziehl- Nielsen stain (ZN): acid-fast bacteria (arrows) are easily detected within the cytoplasm of macrophages, epithelioid macrophages, and the multinucleated giant cells (star). ZN; bars = 50µm.

**Discussion**

Bovine PTB is an enteric disease that results in numerous financial losses due to the iceberg phenomena (45), as many cases do not show clinical cases and are still subclinically infected and underdiagnosed. Regular screening and early diagnosis, therefore, are crucial for the control. Subclinical PTB is a well-known yet difficult-to-diagnose condition (46,47). Additionally, a lack of quick and accurate tests makes effective control measures are difficult, as some cases can be underdiagnosed. As a result, an accurate and more specific diagnosis of subclinical cases needs more than one test (47,48). Therefore, in the current work, to increase the rate of positive cases, screening of JD was done on dairy cows; first, any cow that was positive for ELISA and/or PCR was subjected to culling, slaughtering and further pathological examination of the digestive tract and the associated LNs to determine whether lesions were present and their severity.

The seropositive rate of MAP infection among the tested cows was 17.2%. Our results have strongly come in accordance with a previous study by Pourmahdi (38) which stated that the percentages of MAP antibodies in sheep and cattle were 19.5 and 19.7%, respectively. According to a report published by Salem (49), Ismailia, El Monefeia, and Cairo have the highest rates of JD among clinically suspected Egyptian cattle and those in contact with them. In two Egyptian areas, no positive cases could be found (El Giza and El Gharbeiah). 16.7% of the native Egyptian cattle studied had the illness, compared to 85.7% of the Egyptian-raised Holstein cows. In the present study, there is a high incidence rate of this disease in the Holstein breed, with 13 out of 41 (31.7%) cows being infected. In contrast, the native breed has the lowest infection rate, with 7 out of 61 cows (11.47%) being infected. The better resistance of native breeds to *M. avium ssp. paratuberculosis* infection or the higher exposure rate for imported cattle from endemic areas can both be used to explain these findings. At the same time, the PCR's positivity for detecting the MAP IS*900* gene was 20%. Several investigations identified MAP-infected animals by detecting the highly specific IS900 gene, which can identify 10-100 MAP per gram of feces or milk. Compared to serology, PCR is more specific and sensitive (50,51). Cows with MAP infection can shed the bacteria in milk and feces early in the disease, two years before clinical symptoms start; however, a detectable immune response could be fully established at a late infection (38).

PTB can affect cows of any age; however, it is more common in cows 1.5 to 3.5 years old. Paratuberculosis is less common in cows under 1.5 years old, which could be attributed to the prolonged chronic nature of the disease. As one of the few native taurine breeds that remain in the original domestication area of Bos taurus, the Nile Valley (Upper Egypt), the Egyptian Baladi cattle is one of the few remaining native taurine breeds (52). As well as its evolutionary relevance, the breed has great potential for integrating into traditional family farming systems and contributing to local, sustainable rural development. There is no doubt that native dairy cows are more resistant to disease

and able to withstand intensive farming conditions than a high-yielding, highly selected dairy breed like the Holstein (53).

Among PCR-positive cases, 58.62% showed gross lesions, mainly thickening, and folding of the small intestine and swelling of mesenteric lymph nodes. The gross lesions closely resembled those described previously by Buergelt (41) and Cheng (54). In the current work, the main finding was the thickening and folding of the small intestine, particularly the last portion of the ileum, ileocecal valve and the first part of the cecum. An intense transmural inflammation of the ileum and the cecum was blamed for the cerebroid look in the intestinal mucosa because of the intestinal wall's enhanced thickening (55). The massive infiltration of the inflammatory cells in the submucosa of the intestine is the main cause of the profuse watery diarrhea associated with JD, as cellular infiltrations act as a barrier and decrease water and nutrient absorption. Moreover, swelling and enlargement of mesenteric lymph nodes were detected. Previously, it has been reported that the thickening of the intestinal mucosa and cordness of lymphatic vessels hinder the absorbance of water and nutrient from the intestine, resulting in profuse watery diarrhea and loss of electrolytes and essential nutrients (56). As a result, the affected animals appear emaciated and cachectic (thin cow syndrome).

Histopathologically, 86.20% had chronic granulomatous enterocolitis and lymphadenitis, with most cases being mild or moderate. The histopathological grading system and findings were consistent with the majority of previously published data (14,57). The scores of histopathological lesions in the vast majority of cases in the current study are mild to moderate. The data mentioned above confirm the high rate of subclinical JD. The main microscopic lesions observed in the intestine were transmural granulomatous enteritis and lymphadenitis, a prominent mononuclear cell infiltration including lymphocytes, plasma cells, macrophages, and epithelioid macrophages with degenerative and necrotic changes in the intestinal villi. Langhans giant cells were also observed mainly in severe cases. It has been previously reported that the more giant cells present-the more influential the inflammation (14). A considerable eosinophilic infiltration was observed in some cases (58,59). Mycobacteria remove iron from macrophage ferritin, which is crucial for the growth of mycobacterium, using iron-chelating proteins called exochelins, iron-reductases and possibly siderophores. Moreover, iron availability is most significant in tissue macrophages of the ileocecal junction. Therefore, the area of the ileocecal junction, which includes the last portion of the ileum and the proximal portion of the cecum, is the most affected part (60,61). Acid-fast bacilli were observed in 82.75% of positive cases. It could be used as a reliable diagnostic tool.

## Conclusion

Overall, the Prevalence of MAP infection is high among the examined dairy cattle in Egypt. The main pathological findings are mild to moderate granulomatous enterocolitis and lymphadenitis. The association of diagnostic methodologies such as PCR and ELISA gives high accuracy in confirming the diagnosis of paratuberculosis. As well as Ziehl-Neelsen staining, histopathology could provide reliable diagnostic tools for subclinical cases. Further studies are needed to design nationwide surveys in other governorates to establish control programs.

## Conflict of interest

The authors declare no conflict of interest. The funders had no role in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the manuscript.

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# الكشف عن مرض نظير السل تحت الإكلينيكي في أبقار الألبان في مصر


**شحاتة إبراهيم أنور[١،٢] و سلوى عادل غريب[٣،٢]**

[١]قسم الباثولوجيا، كلية الطب البيطري، جامعة بنى سويف، بنى سويف، جمهورية مصر العربية، [٢]قسم العلوم البيولوجية المقارنة، جامعة إلينوي في أوربانا شامبين، أوربانا، إلينوي، الولايات المتحدة الأمريكية، [٣]قسم الأحياء المجهرية الزراعية، كلية الزراعة، جامعة المنيا، المنيا، جمهورية مصر العربية



**الخلاصة**

نظير السل هو مرض مزمن يصيب المجترات ويسبب المرض جرثومة العصيات نظيرة السل، ويتميز المرض بظاهرة الجبل الجليدي، حيث أن العديد من الحالات تحت السريرية وغير مشخصة. التشخيص المبكر أمر حتمي. ومع ذلك، لا يوجد اختبار واحد موثوق متاح، مما يؤدي إلى تأخير استبعاد الحيوانات المصابة من القطيع. علاوة على ذلك، فإن استخدام أكثر من اختبار يزيد من معدل تشخيص الحالات الإيجابية. هدفت الدراسة الحالية إلى اكتشاف مرض نظير السل تحت الإكلينيكي في أبقار الألبان في محافظة المنيا، مصر، باستخدام اختبار الاليزا بالتزامن مع استخدام تقنية تفاعل البلمرة المتسلسل كما تم إخضاع الحالات الإيجابية للفحص المرضي لتحديد ما إذا كانت الآفات موجودة ومدى خطورتها. تم اختبار إجمالي ١٤٥ بقرة من سلالات مختلفة (بلدي، مختلط، هولستين) وأعمار مختلفة بواسطة اختبار الاليزا بالتزامن مع استخدام تقنية تفاعل البلمرة المتسلسل .أظهرت نتائجنا أن الحالات الإيجابية التي تم اكتشافها بواسطة اختبار الاليزا بالتزامن مع استخدام تقنية تفاعل البلمرة المتسلسل كانت ١٧,٢٤ و ٢٠٪ على التوالي. وكانت نسبة الإصابة في أبقار الهوليشتين أعلى بكثير ٣١,٧٠٪ من السلالات البلدي والمختلطة. في المقابل، فإن السلالة المحلية (البلدي) سجلت أقل نسبة إصابة ١١,٤٧٪. علاوة على ذلك، يعتبر مرض نظير السل أكثر شيوعا في الأبقار ذات العمر الكبير. من بين الحالات الإيجابية لتقنية تفاعل البلمرة المتسلسل، أظهرت ٥٨,٦٢٪ إصابات مرضية ترى بالعين المجردة، وكانت بشمل رئيسي عبارة عن سماكة وانطواء الغشاء المخاطي المبطن للأمعاء وانتفاخ الغدد الليمفاوية المساريقية. من الناحية النسيجية، كان ٨٦,٢٠٪ مصابين بالتهاب الأمعاء والقولون الحبيبي المزمن والتهاب العقد اللمفية. علاوة على ذلك، لوحظ وجود عصيات صامدة للأحماض في ٨٢,٧٥٪. وبالتالي، يمكن تأكيد تشخيص نظير السل تحت الإكلينيكي بشكل أكثر دقة عند استخدام أدوات تشخيص متعددة معا.