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ORIGINAL ARTICLE

Pathological Characterization of Cryptosporidiosis in Naturally Infected Hybrid Converter Breeds of *Meleagris gallopavo*

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Abstract

BACKGROUND: Rearing of the hybrid converter breed of turkey (*Meleagris gallopavo*) is becoming more popular in Egyptian farms because of its high meat production; however, many pathogens affect this benefit.
OBJECTIVES: This study aimed to investigate the occurrence and pathological impact of cryptosporidiosis in hybrid converter breeds.
METHODS: Regular visits to turkey farms of hybrid converter breeds in the delta region of Egypt were conducted in 2022 and 2023. Clinical examination of the diseased birds was performed and recorded. Following the clinical examination, the diseased turkeys were euthanized and necropsied. Freshly dead birds were then necropsied. The intestinal contents of necropsied turkeys were subjected to the Wisconsin sucrose flotation technique and modified Ziehl–Neelsen staining technique to confirm the presence of *Cryptosporidium*. Tissue samples from positive cases were subjected to histopathological analysis to further characterize *Cryptosporidium*.
RESULTS: Clinical signs were nonspecific, including rales, cachexia, diarrhea, coughing, sneezing, gasping, and swelling of the infraorbital sinuses. Gross lesions included congested trachea, intestines, and the liver. Modified Ziehl–Neelsen acid-fast stained slides showed red-stained *Cryptosporidium* oocysts retrieved from the intestinal content of turkeys. Microscopic examination of the turkey trachea and intestine revealed round eosinophilic *Cryptosporidium* bodies among the detached epithelial cells in their lumen. The proventriculus showed follicular lymphocytic aggregation in the mucosa, with round eosinophilic *Cryptosporidium* bodies among the detached epithelial cells in their lumen.
CONCLUSION: To our knowledge, this is the first report to characterize the presence of *Cryptosporidium* in the liver of a hybrid converter breed in Egypt. Farmers and turkey breeders must take additional precautions to prevent and control the spread of *Cryptosporidium* in their flocks.

Keywords: *Cryptosporidium*, Hybrid converter, *Meleagris gallopavo*, Pathology, Turkey

1. Introduction

Turkey is an important source of meat, and the business involved in its production makes a considerable contribution to the agricultural economy. It is well known that the pathogens present in the gut of turkeys affect bird health and flock performance [1]. Turkey's meat production in 2021 accounted for 5.8 million tons out of 375 tons worldwide [2]. Converter birds showed rapid growth, reaching a maximum body weight of 17.06 kg at 18 weeks of age [3,4]. Enteric illnesses prevent turkeys from achieving their full potential. Numerous pathogens have been implicated, including viruses, bacteria, and protozoa; however, the exact cause has not yet been determined [5].

Cryptosporidiosis is caused by a small protozoan from the Coccidia group [6,7]. *Cryptosporidium*

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mainly colonizes the alimentary epithelium, and the respiratory, biliary, and urinary tracts [8]. *Cryptosporidium baileyi*, *Cryptosporidium meleagridis*, *Cryptosporidium galli*, and *Cryptosporidium avium* is the most common species of *Cryptosporidium* reported in birds [9–12]. In turkeys, infection is usually caused by *C. baileyi* and *C. meleagridis* [13].

Cryptosporidiosis affects various hosts. It is widespread worldwide [14–17]. Cryptosporidiosis, a significant cause of acute gastroenteritis in developing countries, was overlooked until the 1980s because of its potential zoonotic spread from human interactions with animals and birds [18]. *Cryptosporidium* species have been reported in more than 30 of domestic, wild, and captive bird [19]. Avian cryptosporidiosis, a severe illness that causes significant financial loss, can manifest in various forms, ranging from asymptomatic to severe intestinal and respiratory symptoms, leading to substantial mortality [17,20–23].

Regarding clinical signs, rales, cachexia, and diarrhea are the most prevalent nonspecific symptoms [21]. *C. meleagridis* infections can cause severe diarrhea in turkey poults. Several case reports have described severe respiratory cryptosporidiosis caused by *Cryptosporidium* spp. in commercial turkeys (probably *C. baileyi*). Lower respiratory tract infection case reports include symptoms such as rattling, coughing, sneezing, and gasping. Colonization of the trachea and bronchi occurred along with airsacculitis and pneumonia [13].

Although there have been reports of clinical outbreaks, the importance of *Cryptosporidium* spp. in commercially reared turkeys is unclear [13]. The macroscopic lesions observed in the bursa of Fabricius included atrophy of the bursa and the presence of caseous exudate in the lumen. The tracheal mucosal surface was covered with a thick layer of mucoid exudate, and the air sacs were opalescent. Lesions in the gut are restricted to mild congestion of the mucous membrane [21].

The epithelia of the excised organs were abundantly covered with *cryptosporidia*, which had an oval shape and diameter of ~2–4 μm. This was noticeable at the level of the bursa of Fabricius, which is the most affected organ [21]. The trachea exhibited hyperplasia, hypertrophy, and disappearance of cilia from the lining epithelium [21]. The brush border of the mucosa of the middle and lower small intestines is lined with many parasites. Villi in the afflicted areas were atrophied, crypts swelled up, and many lymphocytes, heterophils, macrophages, and plasma cells gathered in the lamina propria [13]. Intestinal samples showed destruction of microvilli, villous atrophy, and villi fusion [21].

The primary approaches for diagnosing *Cryptosporidium* infection include microscopic detection of oocysts and serological or molecular techniques to distinguish between various *Cryptosporidium* species. The consumption of food and water contaminated by oocysts or direct contact with infected hosts makes humans and animals very vulnerable to infection by various *Cryptosporidium* species [20,21].

As little is known about the pathogens affecting the hybrid converter turkey breed, we undertook this study to ascertain whether *Cryptosporidium* is present in the hybrid converter turkey breed, a new breed that was brought to Egypt, as well as to study the effects of this pathogen on tissues.

2. Materials and methods

2.1. Sampling

From 2022 to 2023, regular visits to four turkey farms of the hybrid converter breed in the Al Sharqia Governorate were conducted. Each farm consisted of 200 12-week-old birds reared in a semi-intensive system with no history of protozoan treatment. Clinical examination of the diseased birds was performed and recorded. Following clinical examination, five turkeys from each farm ($N = 20$) were euthanized and necropsied. Fifteen fresh dead birds were necropsied. Representative samples were collected from the ailing and dead turkeys. Clinical signs and postmortem findings were recorded. The clinical signs and gross lesions were imaged using a Canon EOS 2000D digital camera. Representative tissue samples were taken from the lungs, trachea, liver, kidneys, spleen, heart, and intestine of all necropsied birds, labeled with particular interest to respiratory and intestinal organs, and subjected to different diagnostic techniques. Part of the tissue samples with gross lesions was labeled and fixed separately in 10% neutral buffered formalin at room temperature for 48 h for histopathological analysis. Intestinal contents were collected and stored in a refrigerator until processing using the modified Wisconsin sucrose flotation technique. This study was approved by the ethical committee of the Faculty of Veterinary Medicine, Mansoura University, Code: Ph. D/100 2021.

2.2. Wisconsin sucrose flotation technique [24]

In a plastic cup, 3–5 g of intestinal content was mixed with 50 ml of tap water and filtered into another cup through a sieve and double layers of gauze. The suspension was centrifuged at 3000 rpm for 10 min. After discarding the supernatant, the

sediment was mixed with 14 ml Sheather's sugar solution and then centrifuged at 3000 rpm for 10 min. A convex meniscus was then formed by filling the tubes with Sheather's solution.

Cryptosporidium oocysts were retrieved by placing a cover slip over the convex meniscus of each tube for 1 h to allow the oocysts to float and adhere to the coverslips. The coverslips were lifted and washed thoroughly with 10 ml distilled water in 50 tubes and centrifuged at 3000 rpm for 10 min. After discarding the supernatant, the sediment was mixed with 150 μl distilled water. The mixture was thinly smeared on a glass slide and stained with modified Ziehl–Neelsen (MZN) stain.

2.3. *Staining* Cryptosporidium *oocysts by modified Ziehl–Neelsen staining technique [25]*

The thin smear slides were dried, fixed with absolute methanol, and air-dried. The fixed slides were stained with cold carbol fuchsin for 10 min and washed with slow-running tap water. The slides were decolorized with a 5% acid alcohol solution for 30–60 s, then washed with slow-running tap water. The slides were counterstained with methylene blue for 30 s, washed with slow-running tap water, and left to dry. The slides were examined using an oil immersion lens (× 100) in a zigzag manner.

2.4. *Histopathological examination*

The 10% buffered formalin-fixed tissue specimens were trimmed, fit into cassettes, and labeled. Tissue samples were routinely dehydrated in ascending grades of alcohol (70, 95, and 100%), cleared with xylene, and embedded in melted paraffin. Serial sections (4 μm) were cut using a microtome and placed on glass slides. When staining, the slides were dewaxed with xylene, rehydrated with descending grades of alcohol, washed, placed in hematoxylin for 5 min, washed with tap water, and counterstained with eosin for 8 min. Finally, the slides were washed in water, dehydrated with ethanol, cleared with xylene, and mounted using a quick mount. Stained slides were examined under a light microscope [26].

3. Result

3.1. *Clinical signs and gross lesions*

Clinical signs were nonspecific, including rales, cachexia, diarrhea, coughing, sneezing, gasping, and swelling of the infraorbital sinuses. Gross lesions included congested lungs, intestines, the heart, and the liver (Fig. 1).

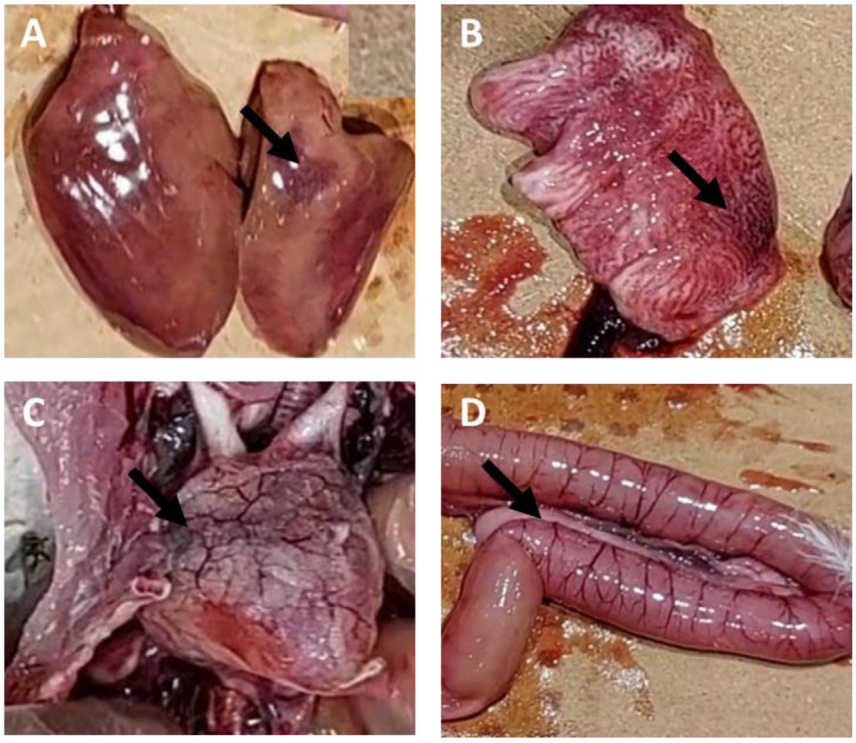

Fig. 1. (A–D) Macroscopic pictures showing gross findings in poults infected with Cryptosporidium. *Pale liver with focal congested areas (A), congested lung (B), enlarged congested heart and pericardium (C), and congested swollen intestine (D).*

3.2. Cryptosporidium *examination*

Microscopic examination of MZN-stained slides of intestinal contents revealed red oocysts of *Cryptosporidium* against a blue background (Fig. 2).

3.3. *Histopathological examination*

Microscopic examination of formalin-fixed samples collected from *Cryptosporidium*-infected birds is shown in Fig. 3. The trachea showed round eosinophilic bodies (*Cryptosporidium*) among detached epithelial cells in the lumen. The intestine showed sloughing of villi in the presence of round eosinophilic bodies (*Cryptosporidium* oocysts) among the detached epithelial cells in their lumen. The liver showed marked dilation of the bile duct, proliferation, and desquamation of the biliary epithelium, with round eosinophilic bodies (*Cryptosporidium* oocysts) among detached epithelial cells in the lumen. The proventriculus showed follicular lymphocytic aggregation in the mucosa, with developmental stages of *Cryptosporidia* attached to epithelial cells.

4. Discussion

In Egypt, hybrid converter turkey breeds are gaining popularity, but understanding their interactions with pathogens such as *Cryptosporidium* is crucial for understanding their impact on birds, animals, and humans. In the current study, *cryptosporidia* were detected in the trachea, intestine, liver, and proventriculus of turkey hybrid converter breeds in Egypt, whereas Guechtouli *et al*. [21] detected *cryptosporidia* in the bursa, trachea, cloaca, intestine, and proventriculus.

Avian cryptosporidiosis symptoms can range in severity from asymptomatic illness to severe intestinal and/or respiratory manifestations, with substantial mortality [17,20–23]. In the current study, the infection was subclinical, with few nonspecific symptoms, including rales, cachexia, diarrhea, and sneezing, in line with Guechtouli *et al*. [21]. Gharagozlou *et al*. [27] detected *cryptosporidia* associated with diarrhea in the intestine and bursa of Fabricius native turkeys in Iran. *Cryptosporidium* infections can occur in healthy turkeys. However, cryptosporidia were not detected in the bursa of Fabricius.

Gross lesions found in the intestines align with those reported by Guechtouli *et al*. [21] and Tacconi *et al*. [28], who found mild congestion in the mucous membrane. While the lungs were congested, these findings were similar to those reported by McDougald [13]. However, lesions of the heart and liver are rare and may result from concurrent infections with other pathogens. In the current study, microscopic examination of intestinal content revealed the presence of *Cryptosporidium* oocysts in intestinal debris. Microscopic examination of intestinal contents of diseased turkeys by Wisconsin sucrose flotation and MZN staining techniques is considered an acceptable method for diagnosing cryptosporidiosis, in agreement with Lamido *et al*. [29].

Histopathological examination revealed round eosinophilic bodies (*Cryptosporidium*) in the turkey trachea, intestine, and proventriculus among the detached epithelial cells in their lumen. These findings agree with those of McDougald [13] and Guechtouli *et al*. [21]. In contrast to the findings of Guechtouli *et al*. [21], tracheal hyperplasia is unclear. Interestingly, no previous reports have documented histopathological findings in the livers of hybrid converter breeds. Therefore, this study

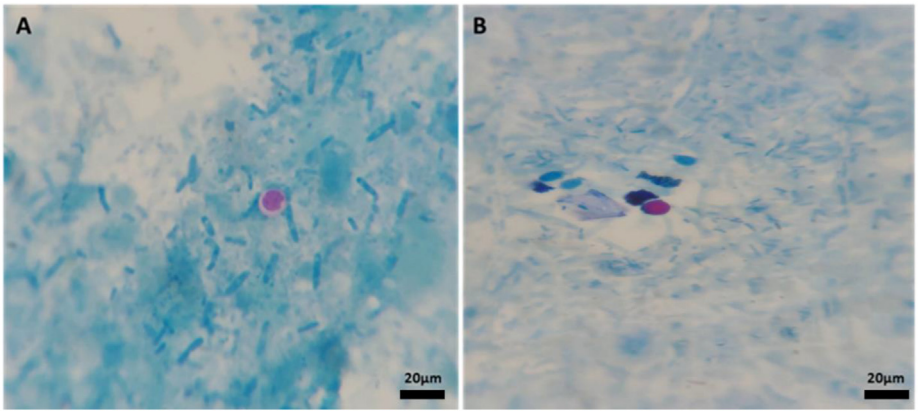

Fig. 2. (A, B) Modified Ziehl–Neelsen acid-fast stained slides showing red-stained Cryptosporidium *oocysts retrieved from intestinal contents of turkeys. Magnification power: × 1000 bar 20.*

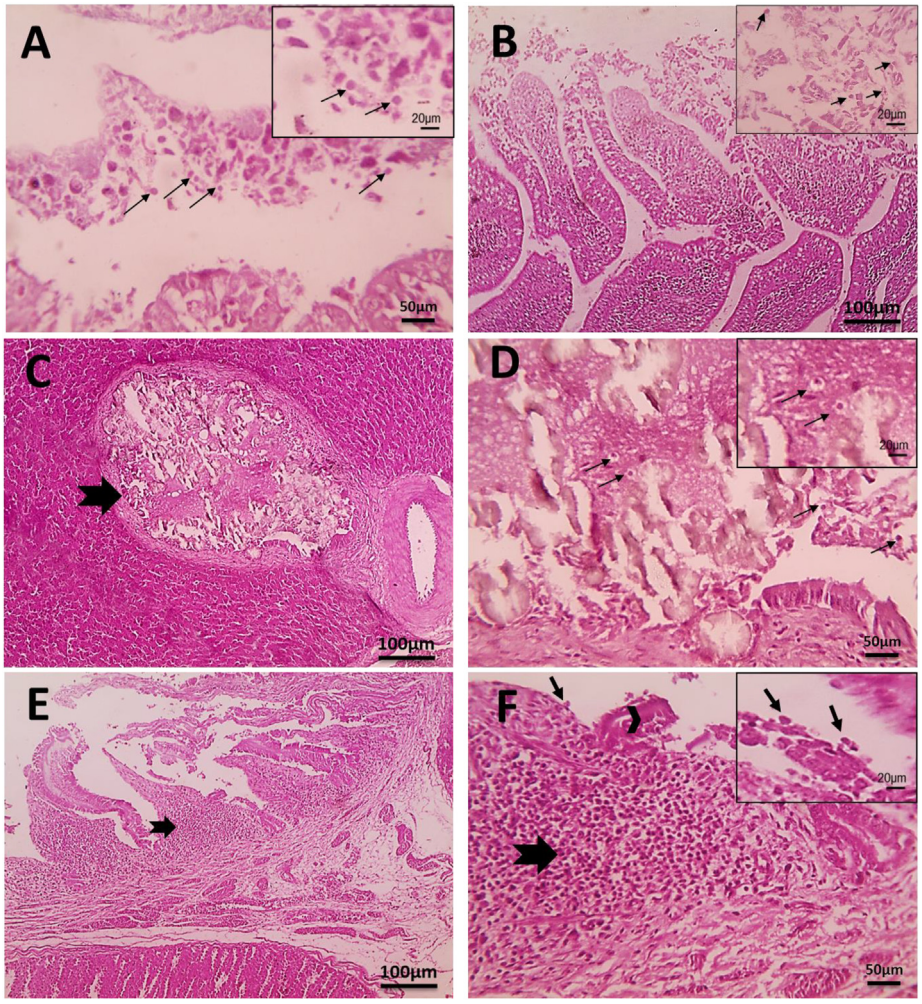

Fig. 3. (A–E) Photomicrographs of histopathological lesions in hematoxylin and eosin-stained tissue samples collected from Cryptosporidium*-infected birds. (A) Trachea, showing the presence of round eosinophilic* Cryptosporidium *bodies among detached epithelial cells in their lumen (thin black arrows), (B) intestine, showing the presence of round eosinophilic* Cryptosporidium *bodies among detached epithelial cells in their lumen (thin black arrows), (C, D) liver, showing dilated bile ducts (thick black arrow) with presence of round eosinophilic* Cryptosporidium *bodies among detached epithelial cells in their lumen (thin black arrows), (E, F) proventriculus, showing follicular lymphocytic aggregation in mucosa (thick black arrows) with the presence of developmental stages of* Cryptosporidia *attached to epithelial cells (thin black arrows). Magnifications × 100 bar 100, × 400 bar 50, × 1000 bar 20.*

aimed to fill this knowledge gap and offer valuable insights into the liver health of the hybrid converter breed in Egypt.

The hybrid converter breed is a popular choice for poultry farmers in Egypt owing to its high productivity and adaptability. However, little is known about the histopathological changes that may occur due to *Cryptosporidium* infections in these birds. Understanding the health of the hybrid converter breed is essential for implementing appropriate management practices and ensuring the long-term sustainability of poultry production in Egypt. Through this study, we hope to provide valuable insights into the health of these birds and contribute to their overall health and productivity.

5. Conclusion

The results of our study shed light on the presence of *Cryptosporidium* in hybrid converter breeds of turkeys and provide valuable insights into the potential health risks associated with its presence. Histopathological investigation of *Cryptosporidium* in hybrid converter breeds is crucial, as little is known about the diseases of these breeds. To our knowledge, this is the first report to characterize the presence of *Cryptosporidium* in the liver of a hybrid converter breed in Egypt. Farmers and turkey breeders must take extra precautions to prevent and control the spread of *Cryptosporidium* in their flocks, such as implementing strict biosecurity measures and maintaining proper hygiene.

Declarations

Ethics approval

This study was approved by the ethical committee of the Faculty of Veterinary Medicine, Mansoura University, Code: Ph. D100/ 2021.

Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

Conflict of interest

There is no conflict of interest among authors.

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Authors' contributions

Conceptualization, S.Sh., A.E. and W.A.; methodology, S.Sh., W.A. and A.E.; software, S.Sh.; validation, S.Sh.; formal analysis, S.Sh., and A.E.; investigation, S.Sh., W.A. and A.E.; resources, S.Sh. and S.S.; data curation, S.Sh., S.S., and W.A.; supervision, A.E., S.S. and W.A.; project administration, S.Sh. All authors discussed the results and contributed to the final manuscript.

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