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A study on prevalence of cryptosporidiosis in commercial broiler chicken

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Abstract

Cryptosporidiosis is one of the main protozoan infections in birds. It manifests as either a respiratory or a digestive illness, and it affects a very large number of avian species. The present study was undertaken to study the prevalence of *Cryptosporidium* infection in broilers by Sheather's sugar floatation and modified Ziehl-Neelsen acid fast staining technique in different broiler chicken farms in and around Thalaivasal region of Salem District. A total of 100 faecal samples and 25 tracheal swabs were collected from apparently healthy and diarrhoeic birds. These samples were screened for detection of oocysts of cryptosporidiosis by Sheather's sugar floatation and modified Ziehl-Neelsen acid fast staining techniques. None of the samples screened by these methods were found positive for cryptosporidiosis.

Keywords: Cryptosporidiosis, modified Ziehl-Neelsen staining, Sheather's sugar floatation

Introduction

Cryptosporidium is one of the most common and important parasites in poultry. It is an intracellular protozoan parasite and ubiquitous in nature. It manifests as either a respiratory or a digestive disease, and it affects a very large number of avian species (Nakamura, *et al.*, 2015) [7]. The cryptosporidiosis in chickens is caused by *Cryptosporidium baileyi* and *C. meleagridis* (Fayer, 2010, McDougald, 2013) [3, 6] and rarely *C. galli* (Ryan, *et al.*, 2003), *C. parvum* (Berrilli, *et al.*, 2012) [2] and *C. baileyi* can cause mild to severe gastroenteritis or respiratory infections (O'Donoghue, 1995, Ramirez, *et al.*, 2004) [8, 10], whereas *C. meleagridis* mainly causes gastroenteritis (Fayer, *et al.*, 1997) [4]. Sometimes birds can be infected with *C. parvum* or *C. galli* without showing any signs of infection (Ryan, *et al.*, 2003) [11]. Hence, the present study was undertaken to study the prevalence of *Cryptosporidium* infection in broilers by sugar floatation and modified Ziehl-Neelsen acid fast staining technique in different broiler chicken farms in and around Thalaivasal region of Salem District.

Materials and Methods

A total of 100 faecal samples and 25 tracheal swabs were collected from different broilers farms in and around Thalaivasal region of Salem district. The samples were labelled with details of sample number, age, place and date of collection. In this investigation, each broiler farm was sampled once only and based on the faecal consistency; the samples were categorized and placed into different groups.

Collection of faecal samples

The faecal samples were collected from the litter materials by wearing a disposable hand gloves and transferred immediately into a sterile plastic zip lock cover. All the collected samples were brought to the laboratory, where the samples were screened after being processed by floatation and acid fast technique for the presence of oocysts.

Collection of data

A questionnaire was prepared and data pertaining to strain, age, flock strength, strain, system of rearing, morbidity, mortality and management, etc., were collected in order to determine the risk factors associated with prevalence of cryptosporidiosis.

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Detection of Cryptosporidiosis in Broilers

Modified Ziehl-Neelsen acid fast staining technique

Materials

Chemicals and ZN Acid fast stains-Kit used in this study were obtained from Hi-media (India).

Staining solution

Carbol Fuchsin	-100 ml
Acid fast Decolourizer	-100 ml
Methylene Blue	-100 ml

Methods

The procedure recommended by OIE (2008) was followed in this study to process the faecal samples by direct faecal smear examination for identification of oocysts of *Cryptosporidium* sp.

- A thin and transparent smears were prepared from fresh faecal samples using tooth pick, air dried and fixed in methanol for three min
- After fixing, the smears were stained with one percent cold carbol fuchsin solution for 20 min, and then rinsed in tap water
- Then the smears were decolourized with one percent acid methanol for 15 sec
- The decolourized smears were then rinsed with tap water
- Thereafter the smears were counterstained with 0.4 percent methylene blue for 60 sec
- After counterstaining, the smears were washed in tap water, air-dried and examined under microscope under high and oil immersion

Sheather's sugar floatation method

Materials

Composition of Sheather's sugar solution (Specific gravity-1.2-1.25)

Granulated sugar (Sucrose)	-454 g
Tap water	-355 ml
Formaldehyde	-06 ml

- Tap water (355 ml) was taken in a 1 litre glass beaker and heated on a induction stove to near boiling
- Then the granulated sugar (454 g) was added little by little and stirred well until the sugar fully got dissolved
- After that, the solution was allowed to cool to room temperature
- Then formaldehyde (6 ml) was added to prevent microbial growth

Methods

- Faecal samples (approximately 2-3 g) were taken in a cup, to which five ml of distilled water was added and emulsified using glass rod until homogenous suspension was obtained
- The suspension was strained through tea strainer, filtrate was transferred to centrifuge tube and centrifuged at 2000 rpm for three min
- After centrifugation, the supernatant was discarded, 2 ml of Sheather's solution was added to the sediment and vigorously shaken to make it becomes homogenous suspension

- Then 3/4th of the floatation tube was filled with suspension and remaining 1/4th was filled with the same solution by adding drop by drop until convex surface was formed
- After 20 min, the convex surface of the Sheather's solution was touched with cover slip, placed on to a glass slide and examined under high and oil immersion objectives for the presence of oocysts.

Results

A total of 100 faecal samples with the consistency of diarrhoeic and non-diarrhoeic faecal droppings and 25 tracheal swabs collected from different age group of birds and screened by direct faecal smear staining and Sheather's sugar floatation to determine the prevalence of *Cryptosporidium*. In the present study, the oocysts of *Cryptosporidium* could not be detected in all the faecal samples and tracheal swabs screened by floatation and modified Ziehl-Neelsen acid fast staining technique.

Discussion

In the present study, the oocysts of *Cryptosporidium* could not be detected in faecal samples and tracheal swabs screened by modified Ziehl-Neelsen acid fast staining technique and faecal samples alone screened by Sheather's sugar floatation method. The findings of the present study do not corroborate with the findings of Wang *et al.* (2014)^[12] who detected the oocysts of *Cryptosporidium* in 10 percent of the samples out of 385 screened from broilers by Sheather's sugar floatation method in seven regions of Zhejiang Province, China. Similarly, Kadhim *et al.* (2018)^[5] also observed 33.21 percent of the faecal samples out of 280 screened by Sheather's sugar solution method and modified Ziehl-Neelsen staining technique in slaughtered broiler chicken carcasses in local markets in some areas of Baghdad city.

The failure to detect the oocysts of *Cryptosporidium* in this study may be due to low number of oocysts in faecal samples and tracheal swabs screened by modified Ziehl-Neelsen acid fast staining technique. Further, the absence of infection in broilers could be the reason for the birds which is not exposed to cryptosporidial infection in the study area. The differences between different studies may be due to various factors such as seasonality, the age of chickens, and the density of birds (Wang *et al.*, 2014)^[12]. Moreover, the various environmental factors and host species may also account for the difference in the prevalence of cryptosporidial infections (Amer *et al.*, 2013)^[1].

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Conflict of Interest

There is no conflict of interest among authors for this study.

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