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Incidence of *Blastocystis* sp. in chicken from Puducherry, India

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Abstract

The incidence of Blastocystis sp. *in poultry from Puducherry, India was recorded in both backyard and broiler chicken. A total of 200 intact whole intestines of both backyard (50 nos.) and broiler chicken (150 nos.) were procured from local chicken outlets as well as from the nearby villages covering 13 places. The area-wise percentage of incidence of Blastocystis was recorded. The study involved qualitative examination and different staining techniques to detect the different forms of Blastocystis viz., Giemsa staining, Gram's staining and modified acid-fast staining. Out of 200 intestinal samples comprising of both broilers and desi birds (150:50), 135 intestines were found positive for Blastocystis (broiler 112 nos and desi 23 nos), representing an overall infection 67.5 per cent (135/200) in chicken (74.66% infection in broiler and 46% infection desi birds.) Among the different staining techniques used, the vacuolar form of Blastocystis was found to be predominant compared to granular form. Modified acid fast staining was found more sensitive followed by Giemsa stain.*

Keywords: *Blastocystis, Broiler and Desi birds, Incidence, Staining, Puducherry, India*

Blastocystis sp. is an obligate, anaerobic protozoa, with a worldwide distribution, inhabiting the gastrointestinal tracts of humans and many animals and birds (Stenzel *et al.*, 1993; Stenzel and Boreham, 1996; Beghini *et al.,* 2017; Maloney *et al.,* 2021). According to Lee and Stenzel (1999), *Blastocystis* sp. infections appear to be common in birds. *Blastocystis* sp.is a polymorphic protozoan comprising of vacuolar, avacuolar, multivacuolar, amoeboid, granular, and cystic forms (Zierdt, (1991); Parija and Jeremiah, (2013) and Sayad *et al.,* 2015). Faeco-oral transmission of cyst is the most accepted pathway of transmission (Greige *et al.,* 2018). Diagnosis of *Blastocystis* sp.is a major challenge due to its polymorphic nature in wet mounts which can result in confusion with other protozoa, yeast or even fat globules. Mostly the vacuolar form is considered as the diagnostic stage, since it can be easily distinguished from other protozoa. *Blastocystis* was first thought to be a fungus (nonpathogenic yeast), but it is now recognized as a human and animal parasitic protozoa inhabiting large intestine (Yamada *et al.,* 1987; Stenzel and Boreham, 1996; Yoshikawa *et al.,* 2004). *Blastocystis* sp. is currently the most common intestinal protzoan found in human feces and is considered an emerging parasite with a worldwide distribution (Tan, 2008). Since the data on incidence of *Blastocystis* in chicken is not available from Puducherry, the present study might be a foot step for further investigations, along with other earlier observations (Arpitha, 2018; Sreekumar *et al.,* 2013). Thus, the present study was undertaken to record the incidence of *Blastocystis* infection in broiler and desi backyard chicken in order to elucidate the status of this infection in Puducherry.

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Materials and methods

Study area

The study was conducted within the city limit of Puducherry, which is located at latitude 11°56′N (Between 11°46′ and 12°30′ of North) and longitude 79°53′E (Between 79°36′ and 79°52′ of East) with an average altitude of 8.5 m high above the sea level. Puducherry covers about 293 km^2 area of land with an average population of 1,51,368 livestock and 2,35,999 poultry (20th Livestock census).

Study design

A total of 200 intact whole intestines of both broiler (150 nos) and desi (50 nos) chicken were collected from 13 local poultry stalls located in and around Puducherry viz, Mettupalayam, Kathirkamam, Nellithope, Grand bazzar, Indira Nagar, Moolakulum, Villianur, Kurumbapet, Reddiyarpalayam, Mangalam, Thirukannur, Kalapet and Andiyarpalayam (Fig. 1 and Fig. 2). The intestines of both broiler and desi backyard chicken were processed at the department of Veterinary Parasitology, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry, as per standard protocol. The intestines were dissected, faecal contents in the intestinal lumen were collected, caecal and duodenal scrapings were collected. The faecal contents were subjected to qualitative faecal examination and the caecal and duodenal scrapings were subjected to staining.

Direct faecal examination

A pin head size quantity of faeces from large and small intestine was taken on a grease free clean glass slide and mixed with two drops of distilled water. The contents were mixed thoroughly using a tooth pick. A coverslip was placed over the contents and the slide was examined for parasites under microscope initially in low (10X) followed by high power (40X) magnifications.

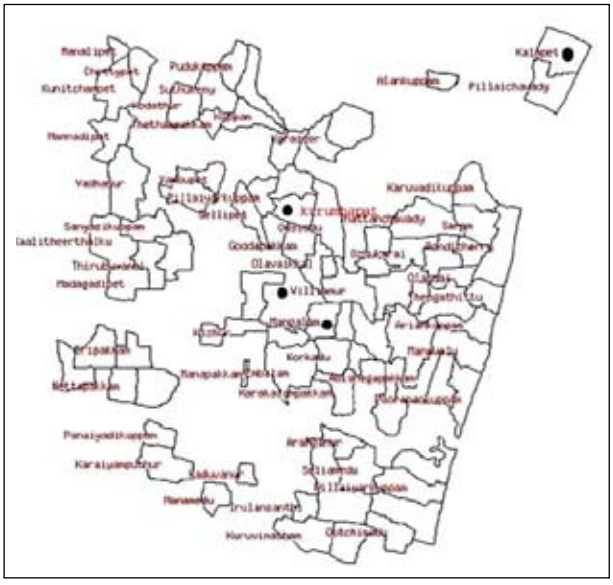

Fig. 1. Areas in Puducherry from where chicken intestines were collected for *Blastocystis* screening.

Fig. 2. Broiler chicken maintained in deep litter system beside the local meat retailer shop in Puducherry.

Faecal sedimentation method

Three grams of faeces was triturated properly in a mortar with a pestle and it was mixed with 50 mL of water. The contents were filtered through a tea strainer into a test tube and centrifuged at 2000 rpm for 2 min. The sediment was examined under microscope at low (10X) and higher power (40X) magnification after discarding the supernatant.

Faecal floatation method

Approximately, three grams of faeces was taken into a mortar and pestle and mixed with 50 mL of flotation fluid (saturated salt solution, Specific Gravity. 1.200). The contents were filtered through a tea strainer and the suspension was transferred into flotation tubes. The contents were added till a convex meniscus was formed at the brim of the tubes. A cover slip was placed over the brim of the test tube for 10 min and it was examined under microscope in low (10X) and high power (40X) magnifications.

Staining of faecal smears

Faecal smears were prepared on clean glass slides and were stained with different staining procedures *viz.*, Giemsa staining, Gram's staining, modified acid-fast staining techniques to observe the detailed morphological features of cysts of *Blastocystis* sp.

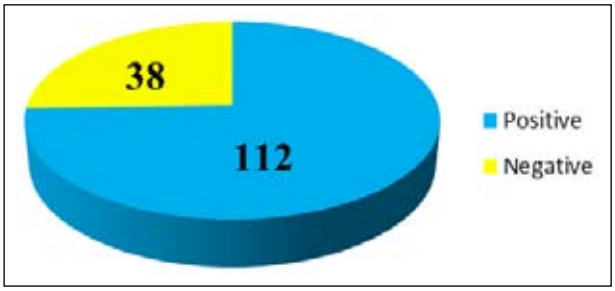

Fig. 3. Incidence of *Blastocystis* in broiler chicken from Puducherry.

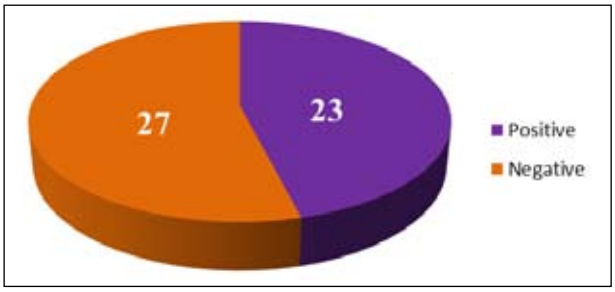

Fig. 4. Incidence of *Blastocystis* in desi chicken from Puducherry.

Giemsa staining

The faecal smears were fixed with absolute methanol for 30 sec. The working solution of Giemsa (1:5 dilution) was prepared by mixing 1 mL of stock Giemsa stain with 4 mL of neutral distilled water. The diluted staining solution was poured over the smear and allowed to act for 45min. The slides were then washed with tap water and air dried followed by examination under low and high-power magnification of the microscope.

Gram's staining

The air-dried faecal smears were fixed with absolute methanol for 30 seconds. The smear was dipped in a Coplin jar containing crystal violet stain for one minute. After washing with tap water, the smear was decolorised with 95 per cent ethyl alcohol for 20 sec, followed by washing with tap water. The slides were counterstained with safranin for 20-25 seconds. Finally, the slides were washed with tap water and allowed to air dry. The slides were examined under low and high-power magnifications of the microscope for the presence of *Blastocystis.*

Modified acid fast staining

The faecal smear was fixed with methanol for 30 sec and the smear was dipped in a coplin jar containing carbol-fuchsin for five minutes. The slides were washed with water for 30 sec, and decolorised with acid- alcohol solution for 30 seconds. The slides were again washed with tap water and counterstained with Loffer's alkaline methylene blue in a coplin jar for 2 min followed by washing with water and air-drying. The slides were examined under low and high-power magnification of microscope for the presence of *Blastocystis.* The parasites were identified on the basis of morphological key by Tan (2008) and Zierdt (1991).

Results and discussion

Examination of a total of 200 chicken intestines (150 broiler and 50 desi backyard chickens) collected from 13 places in and around Puducherry revealed 67.5 per cent incidence of *Blastocystis.* Incidence of *Blastocystis* in the 150 broiler chicken intestines examined was 74.66 per cent (112/150 were positive) (Fig.3.) and in Desi

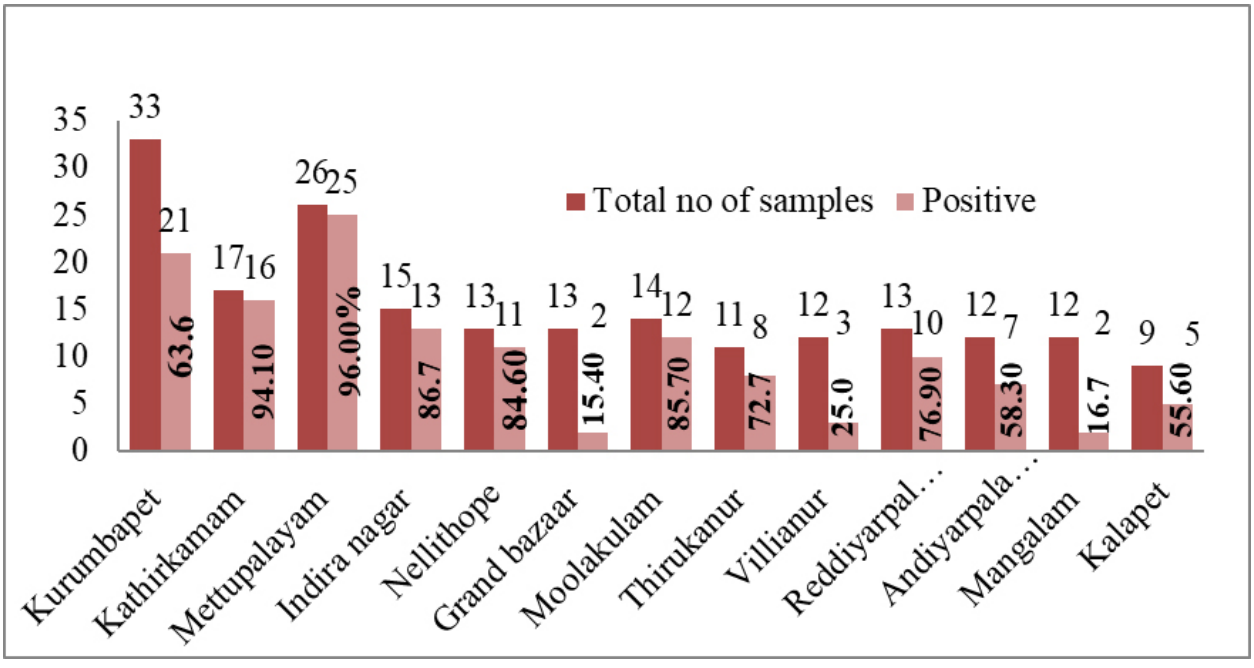

Fig. 5. Area-wise incidence of *Blastocystis* sp. in chicken from different locations in Puducherry.

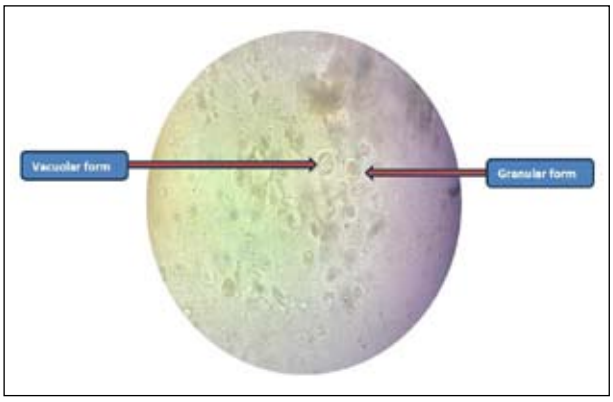

Fig 6. Vacuolar and granular forms of *Blastocystis* in a faecal sample of broiler chicken (Floatation technique)-40x

birds was 46 per cent (23/50) (Fig.4.). In the survey of 13 locations in and around Puducherry, *Blastocystis* infection was ranging from 15.5-96.0 per cent. The average level of incidence was 63.95 per cent with the highest incidence (96%) in Mettupalayam area and lowest incidence (15.4%) in Grand Bazaar (Fig. 5). The variations of *Blastocystis* spp due to in environmental conditions, sanitary measures, different transmission routes and host specificity. Incidence of *Blastocystis* sp., has been reported from intestinal tract of wide range of animals. Though reports on incidence of *Blastocystis* in non-human hosts is very less, the infection has been reported in food animals like small ruminants, poultry and pigs in Chennai (Arpitha *et al.,* 2018). Sreekumar *et al.,* (2013) reported the incidence of *Blastocystis* in 20 chicken out of 24 (83.33%), three turkeys out of four (75%) and one piglet examined was positive for *Blastocystis* where samples were collected during necropsy in Chennai and diagnosed by staining of intestinal scraping with Giemsa stain. The present study recorded a comparatively lower incidence of *Blastocystis* infection (67.5%) in chicken compared to Sreekumar *et al.,* (2013). The overall incidence of *Blastocystis* observed in the present study in desi chicken was 46 per cent which was less compared to the findings of Haziqah *et al.,* (2014) who reported 80-100 per cent incidence rate in village chicken, jungle fowl and white silkie chicken in Malaysia. This might be due to variations in geo climatic conditions, adaption of better management practices and host immunity. However, the exact nature of transmission of *Blastocystis* is yet to be established. Besides, the exact reason for high incidence of infection of *Blastocystis* in broiler birds, observed in present study could not be ascertained. It might be probably due to contaminated food and water but this requires further investigation

No difference was observed among the desi and broiler birds in terms of different forms of *Blastocystis*, however, in both broiler and desi birds the vacuolar and granular forms were observed (Fig. 6). Other two forms (amoeboid and cyst) were not frequently encountered. Among all the staining techniques used, the vacuolar form of *Blastocystis* was found to be predominant. Modified acid-fast staining was found more sensitive followed by Giemsa staining and Gram's staining (Table. 1). Efficacy of different staining protocols used for staining of faecal samples to diagnose *Blastocystis,* in the present study was in agreement with the opinion of Sreekumar *et al.* (2013) and Arpitha (2018). Gram's stain was able to detect *Blastocystis* sp. infection only in in fresh samples. The current study also revealed that flotation technique was very sensitive for quick detection of *Blastocystis* than sedimentation and direct smear technique and it was a less time-consuming procedure.

Conclusion

The present study was carried out to find out the incidence of *Blastocystis* sp. in both broiler and desi birds from Puducherry. Overall incidence of *Blastocystis* sp. infection in chicken was 67.5 per cent with 74.66 per cent incidence in broiler and 46 per cent in desi birds. The faecal samples were stained with Giemsa, Gram's and modified acid-fast staining for detection of *Blastocystis* sp. among which the modified acid-fast staining was found better followed by Giemsa. Gram's stain was able to detect *Blastocystis* sp. infection only in in fresh samples.

Conflict of interest

The authors declare that they have no conflict of interest

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