SHORT COMMUNICATION

PROTOZOAN INFECTION IN SCAVENGING CHICKENS FROM PENANG ISLAND AND BOTA, PERAK, MALAYSIA

FARAH HAZIQAH M.T.* AND NIK AHMAD IRWAN IZZAUDDIN N.H.

School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia
* *Corresponding author*: farahhaziqah@usm.my

ABSTRACT. Chickens are the most abundant birds in the world, providing protein in the form of meat and eggs. Meat from scavenging chickens or 'ayam kampung' has a strong flavour and is juicier than that of commercial chickens. Most of the rural villagers still keep the chickens in small flocks, allowing to range freely around the house or the backyard, require little attention and feed mainly on kitchen wastes. Due to their free-range and scavenging habits, protozoan infections are commonly high because they have an increased opportunity to encounter the oocysts and intermediate hosts such as mosquitoes and flies. Out of 240 scavenging chickens examined, two protozoan parasites have been recovered, namely *Eimeria* sp. (27.1%) and *Leucocytozoon* sp. (1.3%), the latter of which was the blood parasite. None of the chickens examined showed any symptoms of coccidiosis or blood parasite infection because *Eimeria* species and *Leucocytozoon* spp. vary in their pathogenicity. Thus, future studies on the genetic variants is required in order to reveal the level of varieties and taxonomy of these protozoan parasites.

Keywords: Eimeria sp., *Leucocytozoon* sp., scavenging chicken, prevalence, protozoan.

INTRODUCTION

Protozoans are unicellular organisms in which the body consists of the cytoplasm with at least one nucleus. Protozoan parasites are responsible for causing severe infections both in humans and animals worldwide. The infection is mainly transmitted through a faecal-oral route (for example, contaminated food or water) or by arthropod vectors through blood transfusion by vectors which are ticks or mosquitoes, namely *Mansonia* spp., *Aedes* spp., *Culex* spp. and *Armigeres* spp. (Permin and Hansen, 1998; Salih *et al.,* 2015). Protozoan are divided into five major groups; flagellata, amebida, ciliophora, sporozoa and cnidosporidia. Most of the protozoan parasites of avians are flagellates (trypanosomes) and sporozoans (*Eimeria* and *Plasmodium*).

The abundance of these protozoan parasites is known to be pathogenic and may contribute significantly to the low productivity in poultry especially in broiler chickens. Higher prevalence of protozoans in scavenging chickens is expected as the birds are reared for longer duration and are allowed for scavenging for most of the days, and thus are more frequently exposed to infective stages or arthropod vectors.

Therefore, this study was conducted to determine the prevalence as well as the symptoms associated with the occurrence of these protozoans in scavenging chickens or also known as 'ayam kampung' in Malaysia.

MATERIALS AND METHOD

The study was carried out on Penang Island (latitudes 5° 8′ N to 5° 35′ N and longitudes 100° 8′ E to 100° 32′ E) at the north-west coast of Peninsular Malaysia and Bota (latitudes 4° 19′ 08″ N and longitudes 100° 56′ 23″ E) in the state of Perak, Malaysia.

The study was conducted on 240 scavenging chickens randomly obtained from various villages around Bota and Penang. They are generally small in size with body weights ranging from 1.3 to 2.4 kg. All the chickens were bought directly from the owners or the villagers.

The chicken was placed in a cage and fed with commercial feeds such as pellet, corns and wheat. As samples could not be taken directly from the cloaca, the chickens were observed in the act of defaecation and fresh faeces were immediately collected from ground. The samples were stored in a plastic container, labelled and brought back to the laboratory for examination. Faecal samples collected were subjected to the flotation method and a simple qualitative method for detection of coccidia oocysts in the faeces (Dryden *et al.,* 2005).

Blood was obtained from the large vein under the wing (brachial vein) by venous puncture. Thin blood film was prepared, fixed in absolute methanol and stained with 10% Giemsa for 45 minutes following the protocol by Benjamin (2005). The slides were examined using the oil immersion lens under the microscope.

Identification was based on the descriptions provided by Soulsby (1968), and Permin and Hansen (1998). The samples were also confirmed by researchers from Veterinary Research Institute Ipoh, Perak, Malaysia.

RESULTS AND DISCUSSION

Coccidiosis is caused by protozoans from the genus *Eimeria*. There are nine *Eimeria* species frequently recorded in chickens. These species have its own characteristics and differ in their pathogenicity (Shirley *et al.,* 2005). *E. brunetti*, *E. maxima*, *E. necatrix*, and *E. tenella* are highly pathogenic, *E. acervulina*, *E. mitis* and *E. mivati* are rather less pathogenic, and *E. praecox* and *E. hagani* are regarded as the least pathogenic (Morris *et al.,* 2007). The oocysts of *Eimeria* are expelled with the faeces and sporulate in a few days. Infection is initiated when the sporulated oocysts are ingested by the chickens. The sporozoites are then released and subsequently enter the intestinal epithelial cells and grow. According to McDougald (1998), *Eimeria* infections may also lead to mortality particularly in young chicks.

In this study, a high prevalence of *Eimeria* infection was recorded (27.1%, 65/240, Figure 1) as compared to a study in Kelantan with only 7.4%, identified as *E. maxima* and *E. mitis* (Wan Norulhuda *et al.,* 2017). The high prevalence of *Eimeria* species in this study could be due to favourable environment that encourages oocyst sporulation as well as their scavenging habits in which they are more likely to contact with

the infective sporulated oocysts in the faeces (which are the main source of infection) (Ashenafi *et al.,* 2004). The most easily recognised clinical sign of severe coccidiosis is the presence of bloody droppings as well as dehydration. However, none of the chickens examined showed any symptoms of coccidiosis as different *Eimeria* species vary in their pathogenicity (Morris *et al.,* 2007).

Leucocytozoon sp. (Figure 2) was the only blood parasite found in this study with very low prevalence (1.3%, 3/240) similar with a study by Gimba *et al.* (2014) in which low average infection rates of *L. sabrazesi* (0.7%) and *L. caulleryi* (0.5%) in the Galliformes from Selangor, Malaysia. *Leucocytozoon* spp. was first reported by Kuppusamy (1936) in pigeons. This protozoan infects the blood and tissue cell of internal organs (Ruff, 1999). Infections arise when the intermediate host such as black flies and midges (*Culicoides*) are present. Infections are more likely connected to variations in appearance of intermediate hosts. To date, only two species of *Leucocytozoon* have been detected in poultry in Malaysia namely, *L. sabrazesi* and *L. caulleryi* (Colley *et al.,* 1971; Amin Babjee *et al.,* 1985; Gimba *et al.,* 2014).

According to Omar (1968), infected chickens had greenish diarrhoea, ruffled feathers, anorexic, with pale combs and wattle. Infection with *Leucocytozoon* parasites could also result in decreased the egg production and even death (Valkiunas, 2005). However, none of the infected chickens in this study showed any definite sign of disease because *Leucocytozoon* spp. may differs in pathogenecity depending on species (Levine, 1985).

Identification of blood protozoan in this study was only based on the morphological characteristics. According to Zhao *et al.* (2016), PCR method was more sensitive than blood smear in detecting *Leucocytozoon* infection. It is suggested that further studies on the molecular approaches is required as it need to be supported with genetic data in order to reveal the

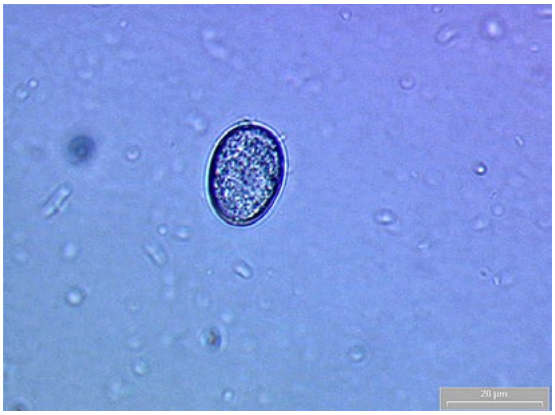

Figure 1. Oocysts of *Eimeria* sp.

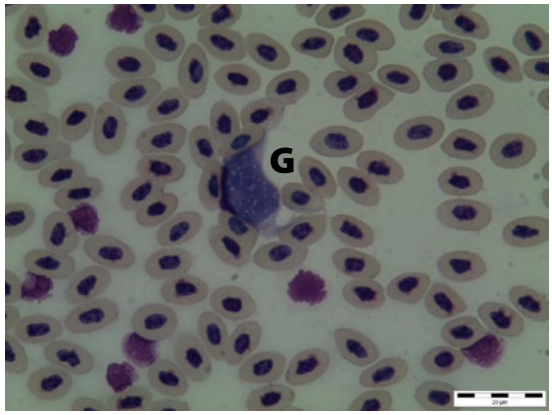

Figure 2. *Leucocytozoon* infection is largely based on the observation of gametocytes in the blood smear of an infected chicken. G = gametocytes.

prevalence, level of varieties and taxonomy of this parasite.

CONCLUSION

The precise knowledge on these protozoans might lead to a better understanding of the mechanisms involved in poultry parasitism and may lead to higher productivity. This information is vital, especially to the poultry farmers as well as the veterinary officers so as to facilitate the isolation and identification of parasitic problems in backyard poultry production systems.

REFERENCES

1. Amin-Babjee S.M., Lee C.C. and Krishnasamy M. (1985). A preliminary survey of parasites of Malaysian Red Jungle fowl (*Gallus gallus spadiceus*). *Kajian Veterinar* **17(2)**: 141-146.
2. Ashenafi H., Tadesse S., Medhin G. and Tibbo M. (2004). Study on coccidiosis of scavenging indigenous chickens in central Ethiopia. *Tropical Animal Health and Production* **36**: 693-701.
3. Benjamin M.M. (2005). *Outline of veterinary clinical pathology. III*. New Delhi: Kalyani Publishers.
4. Colley F.C., Mohammed A.A.R. and Ismail O. (1971). Blood parasites of domestic fowl in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **2:** 84-85.
5. Dryden M.W., Payne P. A., Ridley R. and Smith V. (2005). Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. *Veterinary Therapeutics* **6(1)**: 15-28.
6. Gimba F.I., Zakaria A., Mugok L.B., Siong H.C., Jaafar N., Moktar M.A., Rahman A.R.A., Amzah A., Abu J., Sani R.A., Amin-babjee S.M. and Sharma R.S. (2014). Haemoparasites of domestic poultry and wild birds in Selangor, Malaysia. *Malaysian Journal of Veterinary Research* **5(1)**: 43-51
7. Johan N., Jangi M. S. and Wan K.L. (2011). Isolation and characterisation of an *Eimeria tenella* population from the local jungle fowl. *Sains Malaysiana* **40(6)**: 605-611.
8. Kuppusamy A.R. (1936). Leucocytozoa and microfilariae of fowls and *Haemoproteus columbae* of pigeons in Province Wellesley. *Indian Veterinary Journal* **13**: 25.
9. Levine N.D. (1985). *Veterinary protozoology*. First edition. Iowa State University Press, Ames, IA. pp. 283-289.
10. McDougald L.R. (1998). Intestinal protozoa important to poultry. *Poultry Science* **77**: 1156-1158.
11. Morris G.M., Woods W.G., Richards D.G. and Gasser R.B. (2007). The application of a polymerase chain reaction (PCR)-based capillary electrophoretic technique provides detailed insights into *Eimeria* populations in intensive poultry establishments. *Molecular and Cellular Probe* **21**: 288–294.
12. Omar A.R. and Lim S.Y. (1968). Diseases of Poultry 1961-1966. *Kajian Veterinar* **1(4)**: 224-235.
13. Permin A. and Hansen J.W. (1998). Epidermiology, Diagnosis and Control of Poultry Parasites. In: *FAO Animal Health Manual*. Food and Agriculture Organization of the United Nations. pp. 15-115.
14. Ruff M.D. (1999). Important parasites in poultry production systems. *Veterinary Parasitology* **84**: 337-347.
15. Salih D.A., El Hussein A.M. and Singla L.D. (2015). Diagnostic approaches for tick borne haemoparasitic diseases in livestock. *Journal of Veterinary Medicine and Animal Health* **(2)**: 45-56.
16. Shirley M.W., Smith A.L. and Tomley F.M. (2005). The biology of avian *Eimeria* with an emphasis on their control by vaccination. *Advanced Parasitology* **60**: 285-330.
17. Valkiunas G. (2005). *Avian malaria parasites and other haemosporidia*. Boca Raton, Florida, USA: CRC Press.
18. Wan Norulhuda W.A.W., Nur Syakila M.Z., Nik Kamarudin T., Norlida O. and Saipul Baharia R. (2017). Coccidiosis in village chicken: a preliminary survey in Pasir Puteh district, Kelantan, West Malaysia. *Malaysian Journal of Veterinary Research* **8(20):** 28-32.
19. Zhao W., Pang Q., Xu R., Liu J., Jian Li S.L., Su X. (2016). Monitoring the prevalence of *Leucocytozoon sabrazesi* in Southern China and testing tricyclic compounds against gametocytes. *Plos One* **11(8)**: e0161869. https://doi.org/10.1371/journal.pone.0161869

ACKNOWLEDGEMENT. The authors would like to thank Universiti Sains Malaysia (304/PBIOLOGI/6315156) for funding this study and the staff in the Department of Parasitology and Haematology from Institute of Veterinary Research (VRI) for providing tremendous support and assistance on this study.