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Prevalence of *Histomonas meleagridis* in Broiler Chicken in Different Parts of Mizoram, India

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Abstract: A survey was carried out to detect the overall incidence of histomoniasis in broiler birds in different parts of Mizoram, India. Out of 4000 birds examined, 40 birds were found positive for *Histomonas meleagridis* revealing 1% infection rate. *Heterakis gallinarum* was recovered from the caeca of positive birds. Gross lesions were found in caeca and liver. Microscopic examination of stained section of liver tissue revealed numerous circular amoeboid Histomonas meleagridis together with cellular infiltration. The PCR selectively amplified 209 BP of the small subunit ribosomal DNA sequence of *Histomonas meleagridis*.

Key words: *Histomonas meleagridis*, broiler chicken, histopathology, PCR

INTRODUCTION

Histomonas meleagridis is a protozoan parasite belonging to the trichomonad group which occurs all over the world (McDougald, 2005). The parasite causes high morbidity and mortality in turkeys and the disease is manifested by a diphtheroid inflammation of the caeca and by necrosis in the liver (Sentier-cue *et al*., 2009; Poppc *et al*., 2011). Besides turkeys, the parasite may occasionally affect chicken, partridge, pheasant, quail, duck and a variety of gallinaceous birds. Mortality in diseased turkeys often goes beyond 50% (Callicceit-Cardinal *et al*., 2001). It necessitated the culling of white flocks in the European union as no prophylactic or therapeutic antihistomonal drugs are licensed for prophylaxis or therapy of histomoniasis in food producing animals since 2003 (Poppc *et al*., 2012).

The parasite is ingested in ova of Heterakis gallinarum worms or as larvae in earthworms or faeces or there is an incubation period of 15-20 days. The unprotected histomonas dies within a few hours outside the avian host and its survival on pasture is due to the protection afforded by the *Heterakis* egg. The problem is seen with highest levels in high biosecurity facilities (Lister, 2010; Aka *et al*., 2011). It has recently been demonstrated that infection spread rapidly via the cloca when birds are on contaminated litter. The disease is more common during summer months (Hauck *et al*., 2010a). Following ingestion the histomonas invades the walls of the caeca causing marked inflammation and ulcer formation. These become much enlarged and may involve the whole of the caecal mucosa. The mucosa become greatly thickened and its surface become necrotic and the caeca contain Yellowish green, caseous exudates or a dry cheesy core. Macroscopically, both the caeca are enlarged and show haemorrhagic in appearance. Eventually the organisms enter the liver via the hepatic portal system. The liver lesion is more often circular depressed area of necrosis with raised border and upto 1cm in diameter.

Scant information on the outbreak of the disease is available in India apart from sporadic cases in fowl (Kalia, 1958; Banerjee and Yadav, 2001) and one in partridge (Mir *et al*., 1996).

Direct demonstration of the parasite in tissue is based on the histopathological examination but is difficult when small number of parasites is present. Recently a more sensitive method based on PCR that amplified the target DNA of 209 bp of the small subunit r DNA sequence is used to characterize the parasite. The aim of our investigation was to present overall incidence of Histomoniasis in Mizoram, India and to establish a conventional PCR to examine its specificity as well as sensitivity in the diagnosis of histomoniasis.

MATERIALS AND METHODS

A total of 20,000 house hold broiler birds of different age groups from different parts of Mizoram, India were examined. The birds were maintained in cage system made of bamboo and saw dust used as the litter. Clinical signs and managemental practices were observed. Out of 4000 birds only 40 birds of either sex were found positive at post mortem examination. No mortality was observed. Gross lesions were recorded. Small pieces of liver from the affected part put in 10%

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formalin saline and subsequently processed for histopathological studies. Haematoxylin and Eosin was used for staining tissue as per standard staining technique. The stained slides were examined under light microscope.

The PCR was done as per Huber *et al*. (2005). The PCR DNA was extracted from approximately 250 mg of liver tissue with Hipura Tm mammalian genomic DNA miniprep purification spin kit (HIMEDIA). The tissue were thawed and 250 mg pieces removed mashed up using scissor, vortexed and then incubated overnight in an water bath with protease k. The tissues were further processed according to the manufacturer's protocol and from the sample 10 µl of eluted DNA were used for PCR amplification.

DNA amplification: Reaction mixture (50µl) for each sample contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2mM $MgCl_2$, 200µm of each deoxyribonucleotide, 100p mol of each oligonucleotide primer and target DNA. The forward primer for histomonas meleagridis was designated as HIS 5FC(5'-CCTTTAGATGCTCTGG GCTG-3') and the reverse primer was HIS 5R (5'-CAGGGACGTATTCAACGTG-3') as per Huber *et al*. (2005). The PCR was done in Master cycler Gradient Machine (Eppendrof, Germany).

RESULTS

The affected birds showed inappetence, ruffled feathers, and slight yellowish appearance of combs and wattles. The droppings of positive cases were watery and yellowish in colour with streaks of white mucus. Gross liver lesions included yellowish circumscribed depressed necrotic areas with raised border with a diameter of 2 mm to 1 cm (Fig. 1). Caecal walls were severely thickened, the lumens were distended with caseous cores and the mucosa were ulcerated. Heterakis gallinarum was found in caeca of positive birds (Fig. 2).

Microscopically varying amounts of histomonas trophozoites were detected in liver (Fig. 3). Mononuclear cellular infiltration often containing giant cells, necrosis of hepatocytes and infiltration of macrophages were the predominant microscopic lesions in the liver. There were multifocal necrosis and granulomatous inflammation in the caeca. The PCR selectively amplified 209 bp of the small subunit ribosomal DNA sequence of Histomonas meleagridis (Fig. 4).

The percent prevalence of histomoniasis in different parts of Mizoram, India is presented in Table 1.

DISCUSSION

No birds were found dead due to histomoniasis during investigation but caecal and hepatic lesions were observed. Histomoniasis is essentially a disease of turkeys but chicken are also occasionally found to be

Fig. 1: Gross lesions in the liver showing characteristic circular depressed necrotic lesions due to *Histomonas meleagridis*

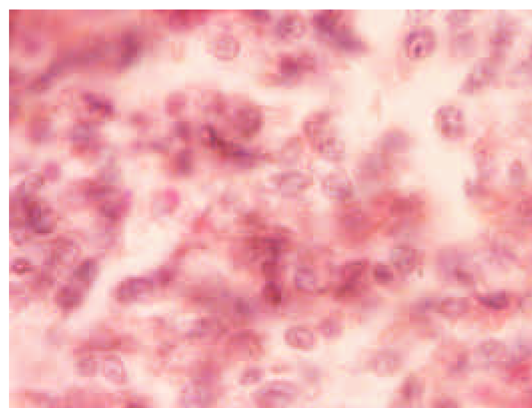

Fig. 2: Histopathological stained (H and E) section of liver showing numerous amoeboid *Histomonas meleagridis*(10X)

infected (Kaufmann, 1996). Histomoniasis is detected and described in naturally affected 400 broiler birds (Table 1). The highest incidence of infection is recorded from Aizawl District of Mizoram (3%). *Heterakis gallinarum* was recovered at post mortem examination from all the infected birds from this area suggesting vector potentiality of the ceacal worm for this protozoan. This is the first report of naturally occurring systemic histomoniasis in broiler birds from Mizoram, India. The presence of histomoniasis was linked to cleanliness of the building, wet litter and diarrhoea. In the present study *Heterakis gallinarum* was recovered from the caeca of the affected birds during post mortem examination. *Heterakis gallinarum* is a common nematode parasite of the domestic chicken and turkey. The main route of histomonas infection is by ingestion of the embryonated eggs of the caecal worm *Heterakis gallinarum*.

Fig. 3: Adult *Heterakis gallinarum* worm

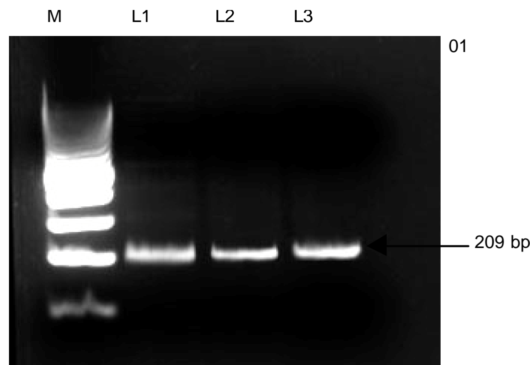

Fig. 4: PCR amplification of *Histomonas meleagridis* Agarose gel electrophoresis of the PCR products. Lane 1 Lane 2 and Lane 3: Amplified PCR products obtained from tests of DNA extracted from liver tissue samples. Lane M: 100 bp marker

Table 1: Prevalence of *Histomonas meleagridis* broiler chicken in different parts of Mizoram, India

Place	No. of birds examined	Positive No.	(%)
Durtlang	525	5	0.8
Aizawl	400	12	3*
Saiha	175	1	0.57
Kolasib	700	6	0.87
Champhai	625	4	0.64
Langtlai	565	3	0.53
Serchip	330	3	0.99
Lunglei	355	3	0.84
Mammit	325	3	0.92

*Significant level (p<.05)

The trophozoites of *H. meleagridis* possess only a low tenacity (Lotfli *et al*., 2012), thus eggs of the nematode *Heterakis gallinarum* containing stages of *Heterakis meleagridis* are regarded as a very important vector (Mc Dougald, 2005). Insects or flies, may serve as vectors (Hauck *et al*., 2010) and the existence of resistant cyst stages has been described (Zaragatzki *et al*., 2010).

As invading histomonads go deeper through the caecal mucosa, they take the hepato portal route and are carried to the liver, where they cause multi focal necrosis (Bon Durant and Wakenell, 1994). A similar observation was also noticed in the liver of affected broiler chicken in the present study.

Histopathological studies have shown that many individual and clustered histomonads are visible in lacunae near the periphery of lesions confirming the results of McDougald, 1997.

By PCR the parasite DNA was only detected in liver lesions but never in healthy parenchyma. It was also shown that PCR based detection was a more sensitive technique than detection based on histopathological examination. However, the sensitivity of PCR can be increased by adding the nested PCR. Since little molecular data are available on *H. meleagridis,* this preliminary study represents a foundation for further molecular studies on this parasite.

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