Histomonas meleagridis in Turkeys: Dissemination Kinetics in Host Tissues After Cloacal Infection

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ABSTRACT *Histomonas meleagridis* is a flagellated protozoa causing histomoniasis, a disease of gallinaceous fowl. This disease is characterized by necrotic typhlitis, hepatitis, and high mortality, especially in turkeys. In an attempt to detect the progression of *H. meleagridis* in the turkey, birds were infected via the cloaca. Between d 0 and 19, a group of 4 turkeys was killed and autopsied every 3 d. Cecal and hepatic lesion scores were used to measure severity of infection. For each turkey, 15 tissue samples were taken. Another group of 3 infected turkeys were placed separately, and samples of cecal and intestinal stool were collected every 3 d. Samples were analyzed by PCR. For samples of cecal droppings, cecum, cecal content, rectum, proventriculus, and bursa of Fabricius, the number of birds detected as positive by PCR followed the evolution of the lesion scores. Within the liver, the parasite DNA was detected only in some severe lesions. The parasite DNA was also detected in duodenum, jejuno-ileum, spleen, heart, lungs, and brain samples. The parasite DNA was not detected in the blood, kidneys, pancreas, or muscle of the thigh. Results of the PCR were in agreement with the evolution of the clinical signs and of the cecal and liver lesions.

Key words: *Histomonas meleagridis*, protozoa, dissemination, cloacal infection, polymerase chain reaction

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INTRODUCTION

Histomoniasis (blackhead disease) is a disease of gallinaceous fowl caused by a flagellated protozoan. Most of the time, this disease induces high mortality, especially in turkeys. Traditionally, blackhead lesions are seen in the ceca and liver. The incubation period of the disease is about 8 d (McDougald, 1997). Invasion by histomonads causes thickening of the cecal wall with marked inflammation and ulceration of the cecum. Occasionally, these ulcers erode the cecal wall, leading to peritonitis. The ceca contain a yellowish green, caseous exudate or, in later stages, a dry, cheesy core. Liver lesions in turkeys are often apparent by d 10 and are highly variable in appearance. Most often the primary lesion is a circular, depressed area of necrosis up to 1 cm in diameter, which is circumscribed by a raised ring. Blackhead lesions have also been reported in unusual sites such as lungs, kidneys, spleen, pancreas, mesenteries, proventriculus, and heart (McGuire and Morehouse, 1958; Malewitz et al., 1958; Welter, 1960; Peardon and Ware, 1969). However, little is known about the actual presence of *Histomonas meleagridis*, the etiological agent, in the lesions or about the dissemination kinetics in the host tissues.

Direct identification of the parasite in the tissues was traditionally done on histological sectioning but is often limited by the small number of parasites in most of the organs. To characterize the parasitology of the infection in turkeys, a more sensitive tool has been used. It consists of a PCR that amplified a DNA target of 209 bp of the small subunit ribosomal DNA sequence (Huber et al., 2005a).

In an attempt to detect the progression of *H. meleagridis* in the turkey, birds were artificially infected and autopsied at different dates postinfection for the quantification of lesion development and PCR analysis of tissue samples.

MATERIALS AND METHODS

Choice of the Cloacal Infection Route

Although blackhead infection results mainly from ingestion of embryonated cecal worm eggs or adult cecal worms (*Heterakis gallinarum*), it has been shown that *H. meleagridis* was present on some farms in which its presence was dissociated from the presence of the nematode (Chossat, 2002) and that lateral transmission of histomoniasis can occur through a flock in the total absence of cecal worms (Hu and McDougald, 2003). Hu et al. (2004) also showed that the oral route was unimportant

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in the absence of the cecal worm, but that the phenomenon called cloacal drinking may be responsible for the rapid spread of histomoniasis through flocks under field conditions.

Experimental Inoculations

Animals were housed and treated in accordance with French law on animal experimentation (19 October 1987). In addition, the committee on animal care in research of the Ecole Nationale Vétérinaire de Lyon (France) approved the experimental design and procedure involved.

Forty-four turkeys (BUT9) were obtained at 1 d of age (Eclosion, Roussay, France). Turkeys were battery raised in a quarantine geographically isolated from experimental areas until they were 4 wk old. Feed and water were provided ad libitum.

The suspension of *H. meleagridis* used to prepare the inoculums was obtained from a strain isolated in 1998 from a farm breeding turkeys and chickens in the Dombes region of France (*H. meleagridis* HmBR-a strain, Callait et al., 2002). The strain has been maintained in the laboratory in chickens. Chickens were received in our unit when they were 1 d old and raised in a quarantine geographically isolated from experimental areas until they were 4 wk old. The strain was continuously passed by successive (every 3 to 4 mo) cloacal infections. Turkeys were used for the ultimate infection source immediately prior to the experiment.

Each inoculum was prepared by collecting the cecal contents and scraping the cecal mucosa of infected turkeys at 5 d postinfection. The collected material was suspended in a M199 medium at 39°C. The number of *H. meleagridis* per milliliter was determined using a Malassez cell with final inoculums containing 3×10^6 *H. meleagridis* per milliliter of the suspension liquid. The birds were then infected via the cloaca using a syringe fitted with a round-ended cannula with 1 mL of the suspension.

Thirty-two turkeys (BUT9) were infected, and 9 were left as negative controls. At d 2, 5, 7, 9, 12, 14, 16, and 19, a group of 4 turkeys were killed by concussion followed immediately by section of the major blood vessels in the neck. Then they were autopsied, and samples of tissue were taken using biopsy punches from the cecum, rectum, jejuno-ileum, duodenum, proventriculus, liver, periphery of liver lesions, spleen, pancreas, bursa of Fabricius, kidneys, heart, lungs, brain, and leg muscle. Samples of heparinized blood were taken from the blood vessels of the neck, and cecal contents were also taken by scraping the cecal mucosa. On d 0, 6, and 13 respectively, 4, 2, and 3 uninfected turkeys were killed and samples were processed as described previously.

At the same time 3 other turkeys (BUT9) were infected as described previously and placed separately in metabolism cages. Samples of cecal and intestinal stool were collected after their excretion on the morning of d 0, 2, 5, 7, 9, 12, 14, 16, and 19.

All the samples were kept at −20°C until analysis by PCR.

Cecal lesion scores were used to measure severity of infection. A score was attributed to each cecum. Scoring (Huber et al., 2005b) was as follows: 0 = absence of macroscopical lesions, fine cecal walls with characteristic longitudinal folds, homogenous cecal contents with a liquid-to-creamy consistency, dark colored and no caseous exudates; 1 = similar to score 0 but with either the presence of scattered petechiae on the cecal walls or a moderate thickening of the cecal walls, or both; 2 = moderate thickening of the cecal walls and modified cecal content with either caseous exudates or beginning of the formation of a caseous core or modification of the color of the cecal contents or absence of cecal content and petechiae on occasion; 3 = great thickening of the cecal walls, either greatly modified cecal content (caseous core) or absence of cecal content, and petechiae may be present; 4 = great thickening of the cecal walls with fibrinonecrotic ulceration of the cecal mucosa, either presence of a caseous core or absence of cecal content, possibility of hemorrhagic ceca, possibility of a rupture of the cecum leading to peritonitis.

Hepatic scores were only based on the circular lesions of necrosis. Scoring was as follows: 0 = absence of detectable round necrotic lesions; 1 = 1 to 5 small circular lesions (less than 5 mm in diameter); 2 = many small circular lesions (greater than 5), possible presence of large lesions (more than 5 mm in diameter) but larger number of small foci; 3 = many small and large circular lesions; 4 = multiple foci, with many confluent lesions.

DNA Extraction

A dilution of tissue or of droppings [3 μL of droppings or 30 mg of tissue ground into 50 μL of TE^{-1} buffer (10 m*M* Tris-HCl, 0.1 m*M* EDTA, pH 8.0) or 5 μL of heparized blood] was dropped onto a FTA Indicating Card (Whatman, Middlesex, UK) and allowed to dry for 1 h at room temperature. A sample disc was taken from the sample spot using a 1.2-mm punch and placed in a PCR amplification tube. Samples were washed according to manufacturer's instructions, 3 times in 100 μL of FTA Purification Reagent and twice in 100 μL of TE^{-1} buffer. Discs were dried at 56°C for 10 min.

DNA Amplification

The PCR were performed as described by Huber et al. (2005a). The PCR containing 1× buffer (Invitrogen, Carlsbad, CA) 1.5 m*M* $MgCl_2$, 60 μ*M* of each dNTP (Amersham Biosciences, Buckinghamshire, UK), 5 pmol of each primer [HIS5F (5′-CCTTTAGATGCTCTGGGCT G-3′) and HIS5R (5′-CAGGGACGTATTCAACGTG-3′)], 0.25 units of Taq polymerase (Invitrogen), and 2μL of DNA or a 1.2-mm FTA sample disc in a final volume of 12.5 μL, were performed in a TGradient thermocycler (Biometra, Goettingen, Germany). The reaction conditions were 5 min at 96°C, 40 cycles each of 1 min at 95°C,

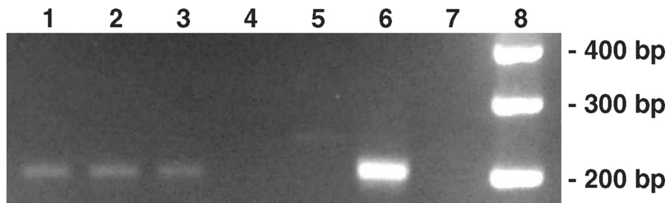

Figure 1. Sensitivity of the PCR for the detection of *Histomonas meleagridis* in droppings samples. A 3-mL sample of cecal dropping were spiked to obtain concentrations of 300 *H. meleagridis*/μL (lane 1), 30 parasites/μL (lane 2), 3 parasites/μL (lane 3), 3×10^{-1} parasites/μL (lane 4), 3×10^{-2} parasites/μL (lane 5), positive control for *H. meleagridis* in lane 6, negative feces control in lane 7 and a 100-bp DNA ladder in lane 8.

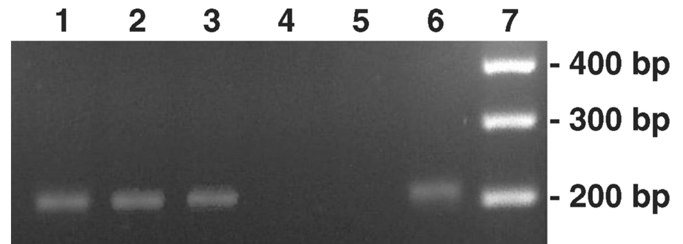

Figure 2. Sensitivity of the PCR for the detection of *Histomonas meleagridis* in blood samples. A 5-mL sample of heparinized blood were spiked to obtain concentrations of 200 *H. meleagridis*/μL (lane 1), 20 parasites/μL (lane 2), 2 parasites/μL (lane 3), 2×10^{-1} parasites/μL (lane 4), 2×10^{-2} parasites/μL (lane 5), positive control for *H. meleagridis* in lane 6, and a 100-bp DNA ladder in lane 7.

1 min at 59°C, and 1 min at 72°C, finished by the addition of a final extension period of 5 min at 72°C. The DNA fragments were separated on 3% (wt/vol) agarose gels and visualized by ultraviolet illumination of ethidium bromide-stained DNA.

A positive control made up of 5 ng of total DNA extracted from *H. meleagridis*, cultured on Stepkowski media (Stepkowski and Klimont, 1979), was included in all experiments. A negative extraction (a negative FTA sample disc treated as described later) control was included to check for cross-contamination during extraction and for quality control of extraction chemicals, and a negative PCR control was included in each run to quality-control the PCR chemicals.

Evaluation of the Sensitivity of the PCR

The sensitivity of the PCR for tissues and dropping samples was assessed using spiked materials. Tissues and cecal droppings of an uninfected turkey were inoculated with a known number of *H. meleagridis* obtained by serial dilutions to have concentrations ranging from 300 to 3×10^{-2} parasites/mg of hepatic or cecal samples, from 300 to 3×10^{-2} parasites/μL of cecal dropping samples, and from 200 to 2×10^{-2} parasites/μL of heparinized blood samples. Thirty milligrams of cecal or hepatic tissue samples or 3 μL of stool was ground into 50 μL of TE^{-1} buffer and dropped onto a FTA card. Spiked blood was dropped directly onto the card. These samples were subjected to DNA extraction as described previously.

RESULTS

Sensitivity of the Assay

The sensitivity evaluated using spiked material was found to be 3 parasites/μL of cecal stool (Figure 1), 2 parasites/μL of blood (Figure 2), 3×10^{-1} parasites/mg of cecal tissue, and 3 parasites/mg of hepatic tissue.

Dissemination of the Parasite in the Artificially Infected Turkeys

All 4 autopsied turkeys presented a cecal and hepatic lesion score of 0 at d 0. At d 2 only one turkey had a cecal score of 1. First visible cecal lesions appeared at d 5. Mean cecal lesion scores increased from d 5 to 12 followed by a progressive lowering of the intensity of the cecal lesions from d 12 to 19 (Table 1, Figure 3). The first hepatic lesions appeared at d 7, mean hepatic lesion score was maximal at d 9 and decreased at d 12 and 14, with no more hepatic lesions detected at d 16 and 19. The negative controls revealed no lesions.

The parasite DNA was never detected by PCR in the uninfected turkeys from the control group on d 0, 6, and 10.

In the cecum and in the cecal content, the parasite DNA was present from d 2 to the end of the experiment at d 19 (Table 1). In the rectum, the parasite DNA was also detected from d 2 to 19 (except at d 5) and in the proventriculus from d 7 to 16. Inside the liver, the parasite DNA was detected in some lesions between d 7 and 12 only in individuals having high hepatic lesion scores. In the bursa of Fabricius, the parasite DNA was present from d 2 and remained detectable until d 9, although it was found in 1 sample at d 16.

The parasite DNA was also found sporadically in the duodenum, the jejuno-ileum, the spleen, the heart, the lungs, and the brain. The parasite DNA was never detected in the blood, the kidneys, the pancreas, or the muscle of the leg.

The parasite DNA was not detected in the droppings before the infection. The droppings remained negative until d 2. Between d 5 and 9, all 3 turkeys monitored produced positive cecal droppings for *H. meleagridis*. At d 12 the parasite DNA was not detected, at d 14 only 1 turkey was positive, and at d 16 2 were positive. At d 19 the parasite DNA was no longer detected. In intestinal droppings it was detected occasionally.

DISCUSSION

The infection level obtained was of medium intensity: no bird died because of the infection during the experiment, but severe (score = 4) cecal and hepatic lesions were observed. Some turkeys seemed to recover during the experiment with a decreasing of the lesional scores after d 12. Whereas some gross lesions have been reported in organs not commonly affected (Peardon and Ware, 1969),

Table 1. Artificially infected turkeys with *Histomonas meleagridis:* positive samples detected by PCR and cecal and hepatic lesional scores

Item	Days after infection								
	0	2	5	7	9	12	14	16	19
Dissemination in tissues[1]									
Cecum	− − − −	− + + +	− + + +	− + + −	+ + + +	+ − + +	+ + + −	+ + + −	− + − −
Cecal content	− − − −	− + − +	+ + + +	− + + +	+ + + +	+ − + +	+ + + −	− − + +	+ − + −
Rectum	− − − −	− + − +	− − − −	+ − + +	+ + + +	+ − + −	+ + − −	+ − − −	+ − + +
Jejuno-ileum	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	+ − − −	− − − +	− − + −
Duodenum	− − − −	− + − −	− − − −	− − − −	+ − − −	− − − −	− − − −	− − + +	− − − −
Proventriculus	− − − −	− − − −	− − − −	− − − +	− + − −	− + − +	− − − +	− − − +	− − − −
Liver (lesion-free area)	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −
Liver (lesional area)	− − − −	− − − −	− − − −	− + − −	+ − − −	− − − +	− − − −	− − − −	− − − −
Spleen	− − − −	− − − −	− − + −	− − − −	− − − −	− − − −	+ − − −	− − − −	− − − −
Pancreas	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −
Bursa of Fabricius	− − − −	− + − +	+ − − −	− − + −	+ − − −	− − − −	− − − −	− − − +	− − − −
Kidney	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −
Blood	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −
Heart	− − − −	− − − −	− − − −	− − + −	− − + −	− − − −	− − − −	− − − −	− + − −
Lungs	− − − −	− − − −	− − − −	− − − −	− − − +	− − − −	− − − −	− − − +	− − − +
Brain	− − − −	− − − −	+ − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −
Leg muscle	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −	− − − −
Lesion scores[2]									
Ceca 1	0 0 0 0	0 0 0 1	2 0 2 2	3 3 3 2	3 3 2 3	3 3 4 4	1 3 3 1	1 3 2 1	2 2 0 0
Ceca 2	0 0 0 0	0 0 0 1	2 1 2 2	3 3 4 2	3 4 4 4	4 3 4 4	3 3 3 1	2 3 3 3	3 2 1 1
Liver	0 0 0 0	0 0 0 0	0 0 0 0	2 2 1 0	3 0 2 4	1 1 1 1	1 2 1 0	0 0 0 0	0 0 0 0
Mean ceca	0	0.25	1.625	2.875	3.25	3.625	2.25	2.25	1.25
Mean liver	0	0	0	1.25	2.25	1	1	0	0
Excretion in droppings[3]									
Cecal droppings	− − −	− − −	+ + +	+ + +	+ + +	− − −	− + −	− + +	− − −
Intestinal droppings	− − −	− − −	+ − −	− − −	− − −	− − −	− − −	− + −	− − −

[1]Thirty-two turkeys (BUT9) were infected with 3×10^6 histomonads. Groups of 4 turkeys were killed every 3 d. Samples of tissue were taken using biopsy punches and were analyzed by PCR.

[2]Lesion scores for liver and ceca were graded on a scale of 0 to 4, where 0 = normal and 4 = great thickening of the cecal walls with fibrinonecrotic ulceration of the cecal mucosa, or liver presenting multiple foci, with many confluent lesions.

[3]Three turkeys (BUT9) infected with 3×10^6 histomonads and placed separately in metabolism cages. Samples of cecal and intestinal stool collected were analyzed by PCR.

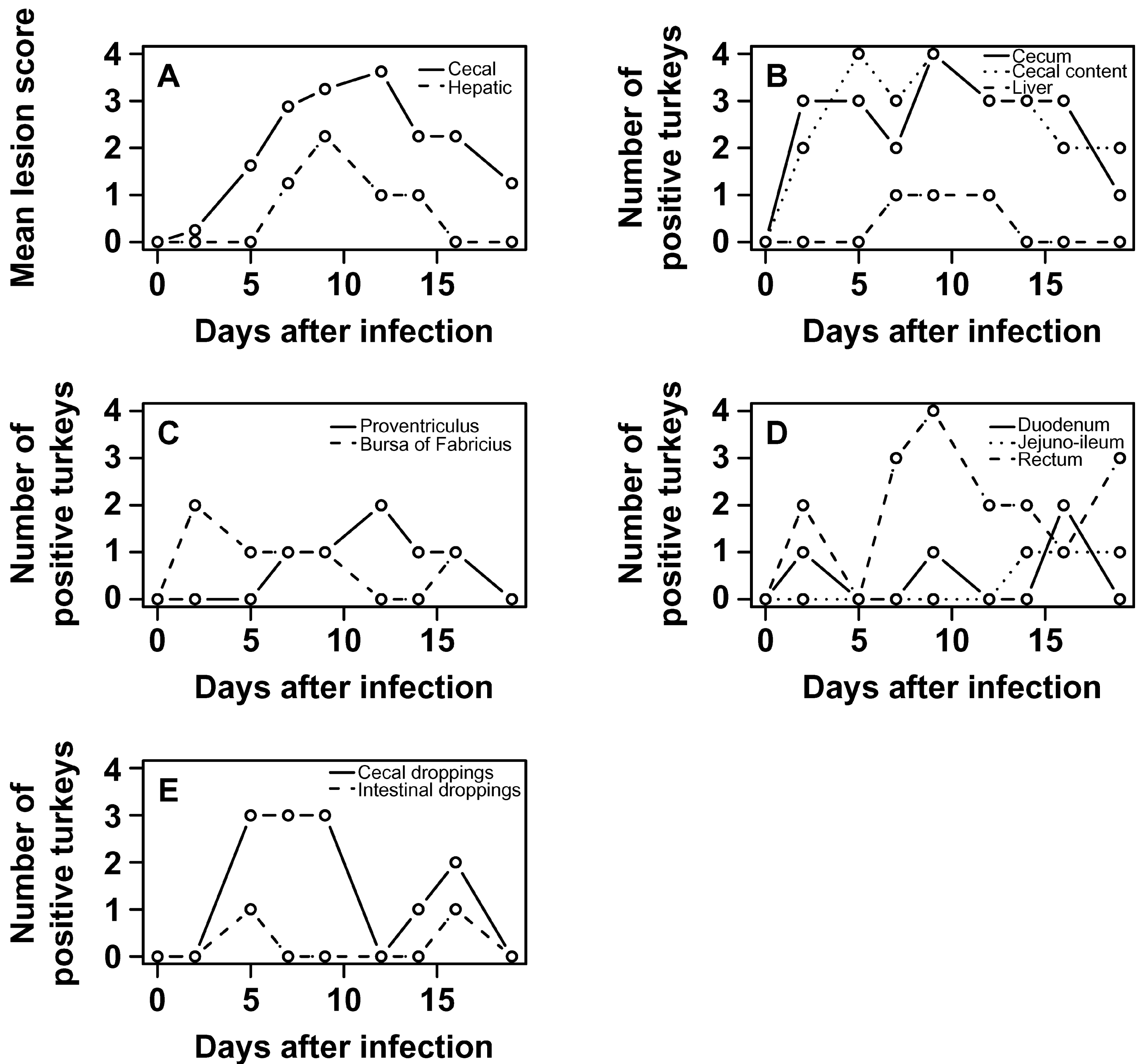

Figure 3. Kinetics of infection in turkeys after cloacal inoculation with 3×10^6 *Histomonas meleagridis*: mean cecal and hepatic lesion scores graded on a scale of 0 to 4 (A) and number of positive turkeys (out of 4) detected by PCR in cecum, cecal content, and liver (B), proventriculus and bursa of Fabricius (C), duodenum, jejuno-ileum, and rectum (D), cecal and intestinal droppings (out of 3; E).

no other macroscopic lesions were detected in our study. The results of the PCR appeared to be in agreement with the evolution of the cecal and hepatic lesions for samples of cecal droppings, cecum, cecal content, rectum, proventriculus, and bursa of Fabricius. The parasite DNA was also detected in the duodenum, jejuno-ileum, spleen, heart, lungs, and brain samples, but it was not detected in the blood, kidneys, pancreas, or muscle of the thigh.

Kinetics of the Dissemination

Chronologically, *H. meleagridis* DNA was first detected at d 2 in the lower digestive tract (bursa of Fabricius, ceca, and rectum). In poultry, material is naturally absorbed by cloacal way and rapidly transported by vigorous sucking movements of the vent into the bursa of Fabricius (Sorvari and Sorvari, 1977). The bursa of Fabricius has an important function as a central lymphoid organ responsible for the development of humoral immunity in the avian species and may also have an immune function comparable with that found in peripheral lymphoid organs (Sorvari and Sorvari, 1977; Scott, 2004). The bursa of Fabricius allows the poultry's immune system to come into contact with environmental antigens that are conveyed among the lymphoid cells of the bursal lymphoid follicles (Hu et al., 2004).

Material experimentally inoculated per cloaca is also taken rapidly into the ceca by retrograde peristalsis (Browne, 1922). The ceca fill not from material passing down from the small intestine but by the retrograde peristalsis from the large intestine (Souliem and Gogny, 1994) aided by contraction of the ileo-ceco-colic sphincter and the contraction of the narrow cecal neck region (Hill, 1971). The parasites first multiply in the ceca after a 5-d lag period followed by a rapid growth and plateau (Hirsch, 1979). Concomitantly, the parasites invade the cecal wall, leading, after 5 d, to macroscopic cecal lesions. The parasite invades the cecal tissues of the bird and migrates through the tissues by putting out pseudopodia, which force their way between the cells of the host (Lee et al., 1969).

As invading histomonads penetrate deeper through the cecal mucosa, they gain access to the hepatic portal system and are carried to the liver, where they cause multifocal necrosis. (BonDurant and Wakenell, 1994). In our study, the first macroscopical lesions of the liver appeared at d 7. By PCR the parasite DNA was only detected in some lesions between d 7 and 12 but never in the healthy liver parenchyma. Histological studies have shown that many individual and clustered histomonads are visible in lacunae near the periphery of lesions corroborating these results (McGuire and Morehouse, 1958; McDougald, 1997).

It has been hypothesized that there is a direct transfer of the parasite from the liver lesions to the wall of the proventriculus (Welter, 1960). However, we have detected the parasite in the proventriculus of turkeys without any hepatic lesions. This could be explained by a retrograde progress of the parasite along the digestive tract, by an oral infection, or through the bloodstream. Peardon and Ware (1969) thought that the observed lesions of the lungs were due to the fluids carrying histomonads during the experimental infection process that entered the trachea at the time of administration and progressed directly to the interior of the lungs. Our turkeys were infected per cloaca, so this explanation is not suitable. A possible explanation is that the parasites were carried by the blood. This route could also explain the presence of the parasite DNA in some other organs such the heart and brain. However, in our experiment, the parasite DNA was never detected in the blood, perhaps because of too fleeting a parasitemia or because it was under the detection threshold of the PCR.

Excretion of the Parasite

After infection, parasite DNA was detected by PCR in cecal droppings from d 5 for the 3 infected turkeys and before the appearance of overt signs that occur most commonly 11 d postinfection (McDougald, 1997). Turkeys infected at the same time, in the same conditions, and autopsied at the same dates (see previous explanations) showed the first notable cecal lesions also at d 5. The parasite DNA was detected by PCR up to d 16 in cecal droppings. Complete clinical recovery of the turkeys was observed after d 19 with a progressive lowering of the intensity of the cecal lesions from d 12 to 19 on autopsied turkeys. Results of the PCR on droppings are in agreement with the evolution of the clinical signs and of the cecal lesions except at d 12 when no positive droppings were detected. This could be explained by the elimination of caseous cores that has occurred for most of the turkeys between d 7 and 14, probably leading to a kind of washing of the ceca, and so the PCR become temporarily negative.

In conclusion, this study describes the dissemination pattern of *H. meleagridis* parasites after cloacal infection of turkeys. Parasites DNA were detected by PCR in various organs including unusual sites with a different chronology and without automatically leading to macroscopic lesions. These results, together with pathological and immunological data, would lead to a better understanding of the infection by *H. meleagridis*.

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REFERENCES

BonDurant, R. H., and P. S. Wakenell. 1994. *Histomanas meleagridis* and relatives. Pages 189–206 in Parasitic Protozoa. Vol. 9. 2nd ed. J. P. Kreier and J. R. Baker, ed. Acad. Press, San Diego, CA.

Browne, T. G. 1922. Some observations on the digestive system of the fowl. J. Comp. Pathol. Therap. 35:12–32.

Callait, M.-P., C. Granier, C. Chauve, and L. Zenner. 2002. In vitro activity of therapeutic drugs against *Histomonas meleagridis* (Smith, 1895). Poult. Sci. 81:1122–1127.

Chossat, L. 2002. L'histomonose en production AOC "Dinde Fermière de Bresse." Essai de prevention par phytothérapie. Veterinary Thesis. Univ. Claude Bernard, Lyon, France.

Hill, K. 1971. The physiology of digestion. Pages 25–47 in Physiology and Biochemistry of the Domestic Fowl. D. J. Bell and B. M. Freeman, ed. Acad. Press, New York, NY.

Hirsch, R. P. 1979. Dynamic of protozoan population density, plasma glutamic oxalacetic transaminase and plasma bilirubin concentrations during histomoniasis in turkeys. Int. J. Parasitol. 9:395–399.

Hu, J., L. Fuller, and L. R. McDougald. 2004. Infection of turkeys with *Histomonas meleagridis* by the cloacal drop method. Avian Dis. 48:746–750.

Hu, J., and L. R. McDougald. 2003. Direct lateral transmission of *Histomonas meleagridis* in turkeys. Avian Dis. 47:489–492.

Huber, K., C. Chauve, and L. Zenner. 2005a. Detection of *Histomonas meleagridis* in turkeys cecal droppings by PCR amplification of the small subunit ribosomal DNA sequence. Vet. Parasitol. 131:311–316.

Huber, K., M.-C. Reynaud, C. Chauve, and L. Zenner. 2005b. Détection d'*Histomonas meleagridis* par PCR, un nouvel outil de diagnostic et de suivi épidémiologique: Application dans l'étude de la dissémination du parasite chez la dinde. Pages 403–407 Proc. Sixièmes Journées Rech. Avicole. St. Malo, France.

Lee, D. L., P. L. Long, B. J. Millard, and J. Bradley. 1969. The fine structure and method of feeding of the tissue parasitizing stages of *Histomonas meleagridis.* Parasitology 59:171–184.

Malewitz, T. D., R. A. Runnells, and M. L. Calhoun. 1958. The pathology of experimentally produced histomoniasis in turkeys. Am. J. Vet. Res. 19:181–185.

McDougald, L. R. 1997. Other protozoan diseases of the intestinal tract. Pages 890–899 in Diseases of Poultry. 10th ed. B. W. Calnek, ed. Iowa State Univ. Press, Ames, IA.

McGuire, W. C., and N. F. Morehouse. 1958. Blood-induced blackhead. J. Parasitol. 44:292–295.

Peardon, D. L., and J. E. Ware. 1969. Atypical foci of histomoniasis lesions in a study of direct oral transmission. Avian Dis. 13:340–344.

Scott, T. M. 2004. Our current understanding of humoral immunity of poultry. Poult. Sci. 83:574–579.

Sorvari, R., and T. E. Sorvari. 1977. Bursa Fabricii as a peripheral lymphoid organ—Transport of various materials from the anal lip to the bursal lymphoid follicles with reference to its immunological importance. Immunology 32:499–505.

Souliem, O., and M. Gogny. 1994. Particularités de la physiologie digestive des volailles. Rev. Med. Vet. (Toulouse) 145:525–537.

Stepkowski, S., and S. Klimont. 1979. Obserwacje nad hodowla in vitro *Histomonas meleagridis* (Smith, 1895). Med. Weter. 35:502–505.

Welter, C. J. 1960. The effect of various stresses upon histomoniasis in chickens and turkeys. Poult. Sci. 39:361–366.