

Detection of *Histomonas meleagridis* by PCR amplification, a new diagnostic and epidemiological tool: application for studying the spread of the parasite in the turkey

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Histomonas meleagridis is a flagellated protozoan causing histomoniasis, a disease of gallinaceous fowl. This disease is characterized by necrotic typhlitis with tan-yellow sulfur droppings, hepatitis and high mortality especially in turkeys. The progressive removal from the market of all active molecules against *Histomonas* by the European Council has led to a dramatic increase in the prevalence of histomoniasis in European countries. Epidemiological data relating to this disease are still insufficient probably because tools for large scale studies are lacking. Diagnosis is mainly based on clinical signs, epidemiological information and gross appearance of lesions. It may be confirmed by direct observation of organisms. Identification should be done by a specialist because of the difficulties to differentiate it from other caecal parasites. Cultivation of organisms in artificial media may also help but this should be carried out in a laboratory mastering the cell cultivation of the parasite (Zenner et al., 2002). With the aim of developing more rapid and sensitive diagnostic alternatives, a polymerase chain reaction which amplified a 209 bp region from the small subunit ribosomal RNA gene of *H. meleagridis* was designed.

A sample of 5 µl of cultured parasites or of a dilution of tissue or caecal droppings were dropped onto a FTA Indicating Card (Whatman, Middlesex, UK) and allowed to dry. A sample disc (1.2 mm) was taken from the sample spot and washed according to manufacturer's instructions. PCR containing 1X buffer (Invitrogen, Carlsbad, CA), 1.5 mM MgCl₂, 60 µM of each dNTP (Amersham Biosciences, Buckinghamshire, UK), 5 pmol of each primer [HIS5F (5'-CCTTTAGATGCTCTGGGCTG-3') and HIS5R (5'-CAGGGACGTATTCAACGTG-3')], 0.25 units of Taq polymerase (Invitrogen), and 2 µl of DNA or a 1.2 mm FTA sample disc in a final volume of 12.5 µl, were performed. The reaction conditions were 5 min at 96°C, 40 cycles each of 1 min at 95°C, 1 min at 59°C and 1 min at 72°C, finished by 5 min at 72°C. DNA fragments were separated on 3% agarose gels. Thirty two turkeys BUT9 were infected *per cloaca* with a suspension of 3.10⁶ *Histomonas* (HmBR-a stain) per millilitre. A group of four turkeys were sacrificed and autopsied at days 0, D2, D5, D7, D9, D12, D14, D16 and D19. Caecal and hepatic lesion scores were used to measure severity of infection (Zenner et al., 2004). Samples of tissues were taken and analyzed by PCR. Three other turkeys were infected as described below. Samples of caecal stool were collected on the same days and were also analyzed by PCR.

The sensitivity of the test was evaluated using serial diluted samples of cultured *H. meleagridis* and showed positive amplification up to the concentration of 3.10⁻¹ parasites/ml. Sensitivity for droppings samples was assessed using spiked material and was found to be 3 parasites/µl of stool. For samples of caecal droppings, caecum, caecal content, rectum, proventriculus and bursa of Fabricius the number of positive birds by PCR followed the evolution of the lesion scores. Inside the liver, the parasite was detected only in some severe lesions. The parasite was also detected in duodenum, small intestine, spleen, heart, lungs and brain samples. The parasite was not detected in the blood, kidneys, pancreas and muscle of the leg (Table 1).

Table 1 Artificially infected turkeys: caecal lesion scores and number of positive turkeys detected by PCR for different kinds of samples according to the time post-infection.

	D2	D5	D7	D9	D12	D14	D16	D19
Mean lesional scores :								
Caecum	0.25	1.625	2.875	3.25	3.625	2.25	2.25	1.25
Liver	0	0	1.25	2.25	1	1	0	0
Number of positive turkeys (out of 4) :								
Caecum	3	3	2	4	3	3	3	1
Caecal content	2	4	3	4	3	3	2	2
Rectum	2	-	3	4	2	2	1	3
Jejuno-ileum	-	-	-	-	-	1	1	1
Duodenum	1	-	-	1	-	-	2	-
Proventriculus	-	-	1	1	2	1	1	-
Liver lesional free area	-	-	-	-	-	-	-	-
lesional area	-	-	1	1	1	-	-	-
Spleen	-	1	-	-	-	1	-	-
Pancreas	-	-	-	-	-	-	-	-
Bursa of Fabricius	2	1	1	1	-	-	1	-
Kidney	-	-	-	-	-	-	-	-
Blood	-	-	-	-	-	-	-	-
Heart	-	-	1	1	-	-	-	1
Lungs	-	-	-	1	-	-	1	1
Brain	-	1	-	-	-	-	-	-
Leg muscle	-	-	-	-	-	-	-	-
Number of positive turkeys (out of 3) :								
Caecal droppings	-	3	3	3	-	1	2	-
Intestinal droppings	-	1	-	-	-	-	1	-

The PCR developed in this study may be a useful tool in the detection and identification of *H. meleagridis* for rapid, routine screening. Results of the PCR appeared to be in agreement with the evolution of the clinical signs and of the caecal lesions however additional tests would be necessary for a better evaluation of the diagnostic sensitivity of this tool. The advantages of this PCR are that it does not require prior cultivation of the organisms and can be performed in less than one day. Samples of caecal droppings are easy to collect and the use of the FTA cards for DNA extraction also has an advantage in terms of safe handling and storage of the samples. In addition, large numbers of samples can be screened synchronously, as required for epidemiological studies.

References

- ZENNER, L., CHOSSAT, L. & CHAUVE, C., (2002) L'histomonose de la dinde, une maladie d'actualité ? *Bulletin des GTV*, **15**: 9-12.
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