Comparison of the susceptibility to Histomonas meleagridis infection of two strains of turkey (Meleagris gallopavo): preliminary study

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SUMMARY

The authors have compared the susceptibility to Histomonas meleagridis infection of two turkey strains: an industrial Heavy Weight strain, (H.W.) and a traditional Light Weight strain, (L.W.), used in Bresse. The aim of the study was to know if the industrial strain is naturally more resistant to histomoniasis than the traditional strain.

Intracloacal infections were carried out experimentally using inoculums containing 10^6 and 3.10^6 Histomonas per bird according to the different groups. The repercussions on the body weight, mortality and the incidence of lesions were studied. The response to infection of the H.W. was in every way comparable to that of the L.W. with the exception of the mortality: 100% mortality was attained later (seven days) in the H.W. than in the L.W. At necropsy, lesion scores for the liver and caeca were given: there was no difference in the seriousness of the lesions or histological difference between the two strains of turkeys.

Keywords : Histomoniasis - experimental infection - response to infection - turkey.

RÉSUMÉ

Les auteurs comparent la réceptivité et la sensibilité des dindes industrielles lourdes, Heavy Weight (H.W.), et des dindes fermeières légères, Light Weight (L.W.), à une infection par Histomonas meleagridis, le but de l’étude étant de savoir si les dindes industrielles sont naturellement plus résistantes à l’histomonose que les dindes fermeières. Des infections expérimentales intracloacales sont réalisées avec des inoculums de 10^6 et 3.10^6 Histomonas par animal. Les répercussions sur la croissance pondérale, la mortalité et les incidences lésionnelles sont étudiées. La réponse des H.W. à l’infection est tout à fait comparable à celle des L.W. La seule différence observée concerne la mortalité : une mortalité de 100% est atteinte plus tardivement pour les H.W. (sept jours plus tard). A l’autopsie, les scores lésionnels sont déterminés pour le foie et les caeca ; il n’apparaît aucune différence ni dans la gravité des lésions ni dans l’examen histologique.

Mots-clés : Histomonose - infection expérimentale - réponse à l’infection - dinde.

Introduction

Histomoniasis in turkeys is a parasite-borne disease which has been documented for many years. As far back as 1934, it was the subject of an in-depth study by TYZZER [9].

It is widespread in the United States and was the subject of research pre 1970, notably on the experimental reproduction of the cycle using parasites isolated from both the caeca and liver [5] as well as work on in vitro culture [6].

Seemingly forgotten in the 1980s, these protozoans are still prevalent in both the United States and France.

Even though the United States can now add Nitarsone to feed as a preventative measure, histomoniasis and its transmission is still of interest to researcher [4]. Very severe cases with high mortality rates have been reported in industrial poultry farms [3].


In France, it is notably the production of the Bresse Turkey A.O.C (“Appellation d’origine Contrôlée” : controlled production limited to a precise geographic area) which has been affected. This is free-range farming with a government controlled quality appellation and the product specifications both favour infestation since the birds are raised in the open air, and forbid the use of preventative measures, since additives are not authorised [1]. Numerous cases have been reported and histomoniasis is now considered as a resurgent disease [10].

Today, it is industrial production units, also forbidden from using any methods of chemical prevention, which will now face serious problems if it is proved that the sensitivity and susceptibility to disease of their birds are identical to those of the free-range turkey.

The aim of this study is to state the facts regarding the sensitivity and susceptibility of an industrial strain of turkeys, an Heavy Weight strain (H.W.) and to compare the results to those obtained from a traditional turkey strain, a Light Weight strain (L.W.), used in free-range breeding units.

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2. Materials and methods

The principle of the method lies in reproducing the histomoniasis experimentally and to follow and compare the repercussions on a black free-range turkey strain, Betina, characterized by light body weight and a slow growth rate (L.W.) and a white strain used in industrial production, BUT 9, characterized by high body weight and a fast growth rate (H.W.). To do this, we studied the influence of the disease on weight gain, mortality and the incidence of lesions. Two inoculums were used for each test.

2.1. ANIMALS

Twenty-four L.W. (males and females) and twenty-four H.W. (males and females) were received in our unit; they were 1 day old. They were raised on wood-chips under heat-producing lamps in a location isolated from infection until they were 4 weeks old. They were fed with a specially manufactured additive-free poult feed. This was distributed in rationed doses sufficient to cover their dietary needs. Water was available on demand from automatic water troughs.

2.2. INOCULUMS

The suspension of *Histomonas* 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. It was maintained in the laboratory by successive per cloacal infections of chickens; turkeys were used for the ultimate infection before the experiment.

Each inoculum was prepared by collecting the caecal contents and scraping the caecal mucosa of the infected turkeys. The collected material was suspended in a M199 medium at 39°C. The number of *Histomonas* per millilitre was determined using a Malassez cell; the 2 test inoculums contained respectively 10^6 and 3.10^6 *Histomonas* per millilitre of the suspension liquid. The doses have been chosen in order to obtain a severe infection. The birds were then infected via the cloaca using a syringe fitted with a round-ended cannula.

2.3. EXPERIMENTAL PROCEDURE

The birds were separated into 6 groups of 8 birds: 2 control groups: L.W. non infected, H.W. non infected, and 4 infected groups: L.W. 10^6; L.W. 3.10^6; H.W. 10^6; H.W. 3.10^6.

To avoid a digestive overload, each bird was put on a hydric diet 5 hours before it was infected.

On Day 0, the birds from the groups to be infected were moved to a new location and put into experimentation boxes, while those of the control groups remained in their original infection-protected location. All the boxes were identical. The birds were infected via the cloaca and suspended by their feet, head downwards for 30 minutes. The control birds were subjected to the same stress producing manipulation.

The birds were weighed on Day 1 and then on Days 4, 7, 11, 18 and 26 (i.e. once a week). The behaviour of the birds in each group was monitored and the average of the weekly weights was calculated for the totality of the live birds.

The birds which died during the experiment were autopsied and the surviving animals were killed according to the directive of the Council of Europe, 86/609/EEC, article 1c and autopsied at the end of the experiment.

Macroscopic, caecal and hepatic lesions were looked for on each bird. Lesion scores were defined (Table I). Samples were taken for histological examination from the liver and one caecum of each bird. Where a lesion had been spotted macroscopically, the sample was taken from this area. The fragments were about 1 cm thick. They were fixed in a 10% buffered formol-physiological water solution and processed using classic histological techniques: blocking in paraffin, 4 µm sections cut and then stained with Hemalun-Eosin-Safran. The slides were screened for the presence of parasites and the histological lesions were described.

The slaughtered birds were screened for the presence of parasites. The caecal matter was examined microscopically and when this proved negative, this was then cultured.

### Table I. — Definition of the lesion scores for description of the lesions due to histomoniasis.

<table>
<thead>
<tr>
<th>Liver</th>
<th>Score 0</th>
<th>No discernible lesions</th>
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<tbody>
<tr>
<td></td>
<td>Score 1</td>
<td>Small number of diffuse, poorly demarcated lesions</td>
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<td></td>
<td>Score 2</td>
<td>Numerous, well demarcated typical lesions</td>
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<tr>
<td>Ceca</td>
<td>Score 0</td>
<td>No discernible lesions</td>
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<tr>
<td></td>
<td>Score 1</td>
<td>Thickened caecal wall, normal caecal contents, no haemorrhaging</td>
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<td></td>
<td>Score 2</td>
<td>Thickened caecal wall, caecal contents + or – modified, haemorrhaging mucosa</td>
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<td></td>
<td>Score 3</td>
<td>Very thick caecal wall, modified caecal contents (caseous &quot;plug&quot;), necrotic mucosa</td>
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3. RESULTS

3.1. MORBIDITY AND MORTALITY

The clinical symptoms of histomoniasis were observed during the experiment: sulphur yellow diarrhoea, exhaustion, wing droop, sensitivity to cold, curled up posture, loss of appetite. It was always the farmhouse birds which displayed the earliest signs of histomoniasis, usually 1 or 2 days before the industrial birds.

The percentage of deaths observed are given in Figure 1. With regards the 2 prepared doses of the infection, 10^6 and 3.10^6, we noticed that the L.W. died before the H.W. : the L.W. began to die on Day 7 regardless of the infecting dosage, whereas the H.W. who had received the 3.10^6 dose only began to die on Day 9 and on Day 10 for those birds who had received the 10^6 dose. 100% mortality was achieved earlier for the L.W. than for the H.W. For the L.W. this was Day 10 for those birds infected with the 3.10^6 dose and Day 11 with the 10^6 dose. For the H.W. 100% mortality occurred on Day 17 for the 3.10^6 dose and Day 18 for the infection with the 10^6 dose. There was no mortality observed in the control groups.

3.2. WEIGHT

In view of the 100% mortality rate observed, we were unable to perform all the planned weighings of the birds. Only two weighings were done on Day -1 and Day 4 of the L.W., and three for the H.W. on Days 1, 4 and 11 (Fig 2).

The weighing on Day 4 showed no significant difference for the L.W. turkeys, between the L.W. non infected and L.W. 10^6 and L.W. 3.10^6 respectively (variance analysis: p= 0.134 and p= 0.09) and for the H.W. non infected and H.W. 10^6 and H.W. 3.10^6 respectively (variance analysis: p= 0.73 and p= 0.60). It’s probably in relation with the very beginning of the infection.

3.3. MACROSCOPIC LESIONS

The lesion scores for the liver and caeca of each animal autopsied are given in Table II. When autopsying the dead birds, there was no difference in the seriousness of the lesions, whether hepatic or caecal, for either of the 2 doses or for either strain of turkey.

3.4. HISTOLOGICAL LESIONS

The birds in the 4 infected groups all died naturally of histomoniasis. The histological samples taken therefore correspond to the terminal phase of the disease. However, we were unable to autopsy all of the birds immediately after their death and some of the bodies remained at room temperature for several hours. This led to post-mortem necrosis which added to the lesions caused by the histomoniasis.

All the slides examined, whether they came from the farmhouse turkeys or the industrial turkeys and whatever the test inoculum used, showed the massive and constant presence of Histomonas in the livers and caeca. This was associated with inflammatory lesions (cellular infiltration with numerous monocytes, heterophils, eosinophils, giant cells) and with necrosis affecting the mucosa, submucosa and the caecal submucosa. This extended, in varying degrees, into the hepatic parenchyma.

We were unable to evidence any histological difference between the L.W. and H.W.

The histological examination of the 4 birds in each control group was found to be normal, except for some moderate lymphocyte invasion of the caeca.

4. Discussion

The H.W. were shown to be susceptible to infection by Histomonas meleagridis and all the birds infected either with the 10^6 dose or the 3.10^6 dose were suffering from histomoniasis and 100% were died at Day 18 or Day 17 post-infection respectively. There was a slight difference observed for the mortality of the L.W. which was, on average, earlier at Day 10 with the 3.10^6 dose or Day 11 with the 10^6 dose. This could be explained by the greater corporal mass of the industrial birds when compared with farmhouse birds at the same age.

All of the H.W. turkeys displayed the caecal and hepatic lesions typical of histomoniasis. Histological examination of all the birds confirmed the massive presence of the parasites, in both the caeca and the livers, associated with previously well-documented inflammatory and necrotic lesions [8, 7].

These results were in every way identical to those obser-
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...it seems there is no difference between the two strains. Another study conducted with Histomonas meleagridis on 2 chicken strains (White Leghorn and New Hampshire) and Heterakis gallinarum pointed out only a slightly difference in caecal involvement [2].

The current absence of any chemical means of prevention in industrial production units correlated with the susceptibility of the H.W. make this protozoan the cause of a disease which should be a matter of deep concern.

Acknowledgements

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References


Table II. — Lesion scores for the livers and caeca, as a function of time for each bird autopsied.

<table>
<thead>
<tr>
<th>Table II. — Lesion scores for the livers and caeca, as a function of time for each bird autopsied.</th>
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<tbody>
<tr>
<td>Group 1</td>
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L.W. = Light Weight ; H.W. = Heavy Weight
F = Liver ; C = Caeca
(0) (1) (2) (3) = Lesion scores determined in the table I.