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Non-curative, but prophylactic effects of paromomycin in *Histomonas meleagridis*-infected turkeys and its effect on performance in non-infected turkeys

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ABSTRACT

Histomonosis (blackhead or infectious enterohepatitis) is a disease of gallinaceous birds, especially of turkeys, and is caused by the protozoan *Histomonas meleagridis*. Since the ban of all chemoprophylactic and chemotherapeutic products against this disease in the European Union, this parasite causes a considerable amount of economical problems in the poultry industry. Research which could ultimately lead to the discovery of new drugs against this disease is thus highly necessary. Hence, in this study, the efficacy of paromomycin against histomonosis in turkeys was investigated.

First, the prophylactic and therapeutic efficacy of this drug against *H. meleagridis* and its effect on the weight gain of turkeys was determined. Adding paromomycin to the feed (400 ppm as well as 200 ppm paromomycin) or to the drinking water (420 mg paromomycin per liter water, added prior to or on the day of challenge) significantly lowered the mortality rate and the caecal and liver lesion scores after an intracloacal infection compared to infected untreated birds. However, when paromomycin was administered to turkeys in the drinking water after the challenge, no significant differences in mortality or in lesion scores could be observed compared to the infected untreated control group. This demonstrates that paromomycin exerts a purely preventive action against histomonosis in turkeys. Additionally, the weight gain of the treated birds was positively influenced by the use of the drug, as the average weight gain of all treated groups (except for the group treated at the day of first mortality) was significantly higher than that of the untreated control group. Finally, the target site of paromomycin was detected in the SS rRNA gene of *H. meleagridis*. Consequently, the susceptibility to paromomycin can be correlated to the presence of the binding site of the drug at the 3′ end of the small subunit rRNA gene of the parasite. In conclusion, paromomycin can be used as a new prophylactic measure in the control of histomonosis in turkeys.

1. Introduction

Histomonosis or blackhead is a disease of gallinaceous birds, caused by the protozoan parasite *Histomonas meleagridis*. It is characterized by sulfur-colored droppings as an early clinical sign and by macroscopic lesions like

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thickened and ulcerated caecal walls and necrotic foci in the liver. While chickens and other poultry usually exhibit a rather mild form of the disease, turkeys, ruffed grouse and chukar partridges are much more susceptible to *H. meleagridis* (Lund, 1969; McDougald, 2005). The protozoa can be transmitted through vectors (*Heterakis gallinarum* as an intermediate host, earthworms as paratenic hosts) but recently research has confirmed that the disease can be transmitted horizontally as well (Tyzzer and Collier, 1925; Hu and McDougald, 2003; McDougald and Fuller, 2005; Hess et al., 2006). This provides a plausible explanation for the many outbreaks where no *H. gallinarum* was found.

Several chemical products like synthesized nitroheterocyclic compounds and arsenicals were used for many years to treat and prevent histomonosis (McDougald, 2005). However, due to the progressive regulatory action, all active drugs were removed from the European market, and only one prophylactic, Histostat™ (Alpharma Inc, Bridgewater, USA), is still available in the USA and other turkey-producing countries. Histomonosis is therefore a disease with a (re-)increasing importance in different poultry species, especially in turkeys. This situation has encouraged a new search for alternative products against blackhead disease.

Paromomycin is an aminoglycoside antibiotic which interferes with the amino acid translation of bacteria and protozoan organisms (Carter et al., 1962; Katiyar et al., 1995; Schroeder et al., 2000). The compound attaches to a binding site near the decoding region at the 3′ terminus of the small subunit (SS) rRNA (Edlind, 1989). This site is present in *Giardia lamblia*, *Entamoeba histolytica* and *Trichomonas vaginalis* and causes their susceptibility to paromomycin (Carter et al., 1962; Gillin and Diamond, 1981; Katiyar et al., 1995; Coelho, 1997; Nyirjesy et al., 1998).

In this study, the efficacy of paromomycin as a prophylactic or a therapeutic drug for turkeys against a *H. meleagridis* infection was determined in two *in vivo* experiments. In addition, the effect of paromomycin intake on the weight gain of turkeys was established. Finally, the presence of the binding site of paromomycin at the 3′ end of the SS rRNA of the parasite was investigated.

2. Materials and methods

2.1. Animals and parasites

Commercial turkeys, breed BIG6 (both sexes, experiment 1) or breed BUT9 (male, experiment 2) were used (Claeys, Zulte, Belgium). The birds in experiment 2 were vaccinated according to the WVPA-Belgian Branch advice. Both animal studies were approved by the Ethical Committee for Animal Experiments of the K.U.Leuven, according to international regulations. The birds were housed from day 1 on litter and had free access to food. In experiment 1, drinking water was provided *ad libitum* through a nipple drinking system. In experiment 2, this was supplied by two drinking cups per pen, connected to an internal water system, except when paromomycin was provided in the drinking water, when drinking buckets were used. The lighting scheme was 21 h of light per day.

To challenge the turkeys of experiment 1, *H. meleagridis* strain 'HNA.C2.L2' was used, which originated from breeder turkeys in France (clinical outbreak in June 2006). Microscopic analysis and a diagnostic PCR test (Bleyen et al., 2007) confirmed the identity of the protozoa. In experiment 2, the virulent strain 'PCH.C1.L2' was used, which was isolated in France in the summer of 2007. Here, the identity of the parasite was verified by microscopic analysis.

2.2. Experiment 1: evaluation of the prophylactic efficacy of paromomycin in the feed against H. meleagridis

One hundred and fifty-nine 1-day old turkeys were randomly divided into 5 groups. Three groups [IPA (32 animals), IPB (32 animals) and IPC (31 animals)] were treated orally from day 1 with respectively 100 ppm, 200 ppm and 400 ppm of paromomycin (histoBloc®, Huvepharma NV, Belgium; supplemented to the feed) while the untreated uninfected control (UUC1, 32 animals) and the infected untreated control (IUC1, 32 animals) birds received regular feed. At 21 days of age (0 days post-challenge or 0 d.p.c.) all groups were distributed into 4 replicates. In the treated groups (IPA, IPB and IPC) and the IUC1 group, 16 seeder birds (thus 4 per replicate) were challenged intracloacally with 10^5 *H. meleagridis* per animal as described by McDougald and Fuller (2005). Sixteen sentinel birds (in-contact) were not challenged, but were kept in the same pen as the seeder birds. During the experiment, all birds were examined daily and mortality was recorded. Four weeks after challenge, all surviving animals were euthanized by decapitation for post-mortem investigation of the caeca and the liver. Lesion scores were given on a scale of 0–4, where 4 is the most severe (Bleyen et al., 2009). In order to analyze the effect of paromomycin on the weight gain, the birds were weighed at day 1, at day 21 and at day 49.

2.3. Experiment 2: evaluation of the prophylactic and therapeutic efficacy of paromomycin in the drinking water against H. meleagridis

At arrival, 525 1-day old turkeys were randomly allocated to one of the twenty-eight pens for the starter period, when they all received a starter feed without any paromomycin. At 14 days of age all turkeys were randomized again and allocated to 36 pens, according to one of the six treatment groups (6 replicates per group). Two of these groups were control groups: an uninfected unmedicated control group (UUC2) and an infected unmedicated control group (IUC2). The other groups were treated with paromomycin (420 mg paromomycin per liter drinking water; Gabbrovet® 70, CEVA, Belgium). In the groups GAB −2.5 and GAB 0, paromomycin was administered to the water as a chemoprophylactic respectively as from 2.5 days before inoculation and starting on the day of inoculation. On the other hand, in the groups GAB +2.5 and GAB 1†, the drug was applied to the drinking water as a chemotherapeutic respectively as from 2.5 days post-inoculation and starting on day of first (histomonosis) mortality in the respective separate floor pen.

At 20 days of age (0 d.p.c.) approximately 10 turkeys per pen (seeders), were inoculated intracloacally with 1.6×10^5