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Original article

Toltrazuril (Baycox®) treatment against coccidiosis caused by *Eimeria* sp. in Japanese quails (*Coturnix coturnix japonica*)

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Abstract

Coccidiosis is the most predominant parasitic disease affecting Japanese quails (*Coturnix coturnix japonica*) in commercial farms. Coccidiosis as a subclinical infection is difficult to diagnose without parasitological examinations. Oocysts of two *Eimeria* species, *E. bateri* and *E. tsunodai,* were determined in the analysed quail flock. Infected birds were administered Baycox 2.5% at the dose of: group I – 7 mg toltrazuril/kg BW per day provided in drinking water (1.5 ml/0.5 l H_2O) that was available 24 h for 2 days, group II – 14 mg/kg BW (3 ml/0.5 l H_2O), and group III – 24.5 mg/kg BW (5 ml/0.5 l H_2O); in groups II and III, the solutions were available 8 h/24 h for 2 days. After the first day of the treatment, the number of excreted oocysts (OPG – oocysts per gram) increased, a steady decrease in oocyst counts began on the second day of Baycox administration and lasted until a three-day period when no oocyst were determined in faecal samples. Regardless of the dose applied, toltrazuril (Baycox) completely eliminated *E. bateri* coccidia and led to a highly significant reduction in the number of *E. tsunodai* oocysts. The results suggest that the effectiveness of toltrazuril varies depending on coccidia species and developmental stages of the parasite. From the clinical point of view, the treatment applied significantly reduces the number of coccidia oocysts in commercial flocks of Japanese quails.

Key words: coccidiosis, toltrazuril, Japanese quails, control, Baycox

Introduction

The Japanese quail (*Coturnix coturnix japonica)* is a poultry species raised for eggs and meat in both home and commercial farms. It is characterised by fast body weight gains and a short rearing period until egg production (6-7 weeks).

Coccidiosis is the most prevalent parasitic disease in quails. During propagation of an intracellular phase, coccidia damage enterocytes, causing inflammatory bowel disease of varying severity, and thus impair the digestion and absorption of nutrients. It affects the health and productivity of birds, and increases their susceptibility to secondary infections (Gesek et al.

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2014). The following coccidia species have been identified and described in Japanese quails: *E. uzura*, *E. bateri* and *E. taldykurganica* in the small intestine and *E. tsunodai* in the caecum (Tsunoda and Muraki 1971, Tsutsumi 1972, Teixeira et al. 2004, Bashtar et al. 2010, Gesek et al. 2014). In poultry, coccidiosis is treated with various chemical compounds, including amprolium, clopidol, diclazuril, decoquinate, lasalocid, monensin, narsin/nacarbazin, robenidine, roxarsone, sulfadimethoxine/ormetropin, salinomycin, semduramicin and zoalene (Tsutsumi and Tsunoda 1972, Gerhold et al. 2011).

In the breeding of chickens, turkeys and pigeons one of the most commonly used anticoccidal drug is Baycox. This medicine is not dedicated to quails, and its use in this species is considered experimental. Because of its high efficacy against different species of coccidia in other species of birds, it is important to establish an effective and safe dose of this drug for quail. The aim of this study was to evaluate the effectiveness of Baycox 2.5% (active ingredient: toltrazuril) (TOL), applied at different doses, in coccidiosis treatment in Japanese quails reared in a commercial farm.

Materials and Methods

Animals

The quails were obtained from a commercial quail flock of 10,000 birds raised for eggs. The birds were kept on litter until 21 days of age, after which they were moved to cages. They were fed a complete balanced diet containing 27% total protein (TP) and 3 000 kcal/kg metabolisable energy (ME) until the age of 21 days, 24% TP and 2900 kcal/kg ME between 21 and 42 days, and 20% TP and 2800 kcal/kg ME from 42 days of age. There was no vaccination program in the flock.

Parasitological examination

Faeces samples collected from birds aged 80 days were analysed by the flotation method with the use of Darling's solution (50% saturated sodium chloride + 50% glycerol). The test revealed the presence of two coccidia species: more abundant, oval-shaped *E. tsunodai* measuring 18.34 x 14.70 µm, and less abundant, subspherical *E. bateri* measuring 20.32 x 18.1 µm. Analyses of tissue samples from the examined quails revealed inflammatory changes in the caecum (dilated intestinal lumen, congested mucosa, blood in the intestinal contents) that scored 2 points on the scale proposed by Johnson and Reid (1970). Samples of the intestinal tissue were subjected to histopathological analysis that revealed the presence of different developmental stages of the coccidia.

Evaluation of the effectiveness of Baycox treatment

The effectiveness of Baycox treatment was evaluated in 80 randomly selected quails aged 12 weeks that were kept in an animal research laboratory under conditions identical to those at the farm. The birds were divided into four groups of 20 individuals per cage: control group (C) without treatment, group I – administered 7 mg toltrazuril/kg BW per day in drinking water (1.5 ml/0.5 l H_2O) that was available 24 h for 2 days, group II – administered 14 mg/kg BW (3 ml/0.5 l H_2O), and group III – 24.5 mg/kg BW (5 ml/0.5 l H_2O); in groups II and III, fresh solutions were available 8 h/24 h for 2 days.

Faeces samples were collected daily from cages over a period of 14 days. The collected material was stirred with a rod, samples of 10 g were weighed, and four specimens of 1 g each were collected randomly and analysed by Fülleborn's flotation method. The specimens were mixed with Darling's solution to obtain a volume of 10 ml, and they were centrifuged at 2500 rpm for 5 min.; 10 µl of the supernatant was placed on a slide and analysed under a light microscope at 400x magnification. The average number of oocysts per g of faeces (OPG) was determined.

Oocysts were observed and measured under a light microscope connected to a digital camera and a computer with Olympus image analysis software. The oocysts were identified based on their shape and size. The percentage shares of the identified coccidia species, sporulated oocysts (S), unsporulated oocysts (US) and damaged oocysts (D) were determined in five fields of view.

The results were analysed by Newman-Keuls test ($p \leq 0.05$) and processed in the Statistica v. 9 for Windows application.

Results

Before the administration of TOL, the average OPG in all experimental groups was determined at 7.4 x 10^6 (detailed results are given in Table 1). Two types of oocysts were identified based on microscopic observations: subspherical *E. bateri* and oval *E. tsunodai,* which accounted for 20% and 80% of all oocysts, respectively. The examined samples contained 20% sporulated oocysts (S), 65-75% unsporulated oocysts (US) and 5-15% damaged oocysts (D). The first

Table 1. Average number of oocysts per g of faeces (OPG) (x 10^6) and percent content of sporulated oocysts (S), unsporulated oocysts (US) and damaged oocysts (D) subject to toltrazuril dose and experimental period.

Experimental period / day		Group / toltrazuril dose (ml/l)															
		I/1.5				II/3				III/5				C/-			
		Average OPG x10^6	% oocysts			Average OPG x10^6	% oocysts			Average OPG x10^6	% oocysts			Average OPG x10^6	% oocysts		
			S	US	D		S	US	D		S	US	D		S	US	D
Before treatment	0	7.4(100%)	20	75	5	4.3(100%)	20	70	10	9.2(100%)	20	75	5	8.8(100%)	20	65	15
After treatment	1	8.8	20	60	20	4.4	20	60	20	1.6	20	75	5	9.1	20	70	10
	2	7.2	20	55	25	9.8	20	60	20	5.2	25	65	10	7.2	25	70	5
	3	1.2*	–	40	60	2.4*	–	–	100	2*	5	80	15	7.2	25	65	10
	4	0.4	–	50	50	0.2	–	–	100	0.6	–	–	100	7.6	20	70	10
	5	0.2	–	–	100	–	–	–	–	–	–	–	–	9	25	70	5
	6	–	–	–	–	–	–	–	–	–	–	–	–	9.4	25	65	10
	7	–	–	–	–	–	–	–	–	–	–	–	–	9.2	20	70	10
	8	–	–	–	–	0.2	–	–	100	0.4	–	–	100	12	25	70	5
	9	0.4	–	–	100	0.8	–	–	100	2.2	–	–	100	10.6	25	70	5
	10	0.2	–	–	100	0.8	–	–	100	1.2	–	–	100	12.2	20	65	15
	11	0.2	–	–	100	0.4	–	–	100	0.8	–	50	50	13.2	20	60	20
	12	0.4	–	40	60	0.6	–	–	100	1.2	–	60	40	10.4	25	70	5
	13	0.2	–	100	–	0.6	–	–	100	1.4	–	100	–	8.2	25	65	10
	14	0.2	–	100	–	0.8	–	90	10	2.0	–	95	5	8.2	20	60	20

S – sporulated, US – unsporulated, D – damaged; ($p \leq 0.05$)

analysis of faecal samples after the administration of TOL revealed an increase in OPG values in the control and experimental groups regardless of the dose applied. During that test, the presence of subspherical oocysts (*E. bateri*) was not confirmed in faeces samples collected from the experimental group birds, which remained free of the above parasite until the end of the experiment. On the second day of the experiment, oocyst counts increased only in group II (significant increase). On day 3, the number of oocysts decreased in all experimental groups, and the observed drop was significant in groups II and III. In the control group, OPG ranged from 7.2 to 13.2 x 10^6 throughout the experiment, and the percent composition of different oocyst groups (S – 20-25%, US – 60-75%, D – 5-20%) and coccidia species (*E. bateri* – 20% and *E. tsunodai* – 80%) was maintained.

Complete oocyst damage (100%) was observed in group II on day 3, in group III on day 4 and in group I on day 5. No oocysts were found in the experimental groups in the course of three successive days. Only oval oocysts (*E. tsunodai*) reappeared in group II and III birds on day 8 and in group I on day 9, and they were damaged in all cases. Complete oocyst damage was observed in groups I and III over a period of three days and in group II over a period of six days.

Unsporulated oocysts reappeared on day 11 in group III (US – 50%), on day 12 in group I (US – 40%) and on day 14 – in group II (US – 90%). On the last day of the experiment, faeces samples from the experimental group birds contained 90-100% US oocysts, and samples from the control group quails – 60% US oocysts. Beginning on day 8 in groups II and III and on day 9 in group I, a significant drop in OPG values was observed relative to the control (8.2 – 13.2 x 10^6), and OPG was determined at 0.2 – 0.4 x 10^6 in group I, 0.2 – 0.8 x 10^6 in group II and 0.4 – 2.2 x 10^6 in group III.

Discussion

Birds, among them quails, kept on litter come into contact with faeces and are susceptible to infections caused by oocysts, including coccidia. Various developmental stages of coccidia are observed in the intestines of quails that are naturally infected with *Eimeria* sp. In this experiment, coccidiosis was a subclinical disease. Unlike in other studies (Mazurkiewicz et al. 1967), an apparent deterioration in bird health and diarrhoea were not reported. Feed intake was similar in all groups, and it was within the norm established

for quails at the onset of the laying season. Coccidiosis was diagnosed by routine/preventive parasitological examinations of faeces samples. Oocyst counts in the control group birds indicate that infected birds compensate for the presence and harmful effects of coccidia in the gastrointestinal tract, which can lower immunity, increase susceptibility to other pathogens, decrease egg production and insignificantly increase feed intake. The above can be attributed to the slow regeneration of intestinal epithelial cells and the exfoliation of damaged cells.

In our study, subspherical oocysts (*E. bateri*) were completely eliminated and the number of excreted oval oocysts (*E. tsunodai*) was reduced in all the experimental groups receiving different doses of TOL. According to the manufacturer, TOL, a coccidiocidal drug, is characterised by a broad spectrum of activity against many coccidia species invading chickens and turkeys. Mehlhorn et al. (1984) demonstrated that TOL effectively destroys *Eimeria* sp. coccidia at all developmental stages. It disrupts nuclear division and mitochondrial activity responsible for respiration in parasitic cells. In macrogametes, TOL damages wall-forming bodies of type II. Advanced vacuolization caused by swelling of the endoplasmic reticulum is observed at all developmental stages inside a cell. In the present study, oocysts were not observed in quail faeces for only three days, which suggests that the susceptibility of oocysts to TOL varies throughout their development (Kandeel 2011).

Similar TOL administration guidelines are observed in chickens (Greuel and Ruhrmann 1986, Mathis et al. 2004), rabbits (Balicka-Ramisz 1997) and pigeons (Michalczyk et al. 2011). According to the literature and the product leaflet, TOL is generally administered over a period of two days. In practice, selected *Eimeria* species infecting quails demonstrate low levels of sensitivity or are resistant to TOL (Vertommen et al. 1990), which relatively quickly leads to new infection episodes despite the introduction a strict sanitary regime to minimize the risk of disease caused by excreted oocysts.

The transfer of birds from a litter-based housing system to cages does not eliminate the risk of coccidiosis. Single oocysts may survive in the intestinal epithelial cells of treated birds, and housing conditions and intensified egg production contribute to repeated proliferation of coccidia. New generation of coccidia (US) were observed already on day 11 in group III (highest TOL dose), on day 12 in group I and on day 14 in group II. OPG values in experimental groups were significantly lower than those found in the control group, which indicates that TOL is a highly effective coccidiocidal drug regardless of the administered dose.

Bird flocks should be regularly screened for parasites, and a successive course of treatment should be prescribed when faecal tests deliver positive results. Further work is required to evaluate the effectiveness of TOL in the treatment of coccidiosis caused by different coccidia species, in particular in quails where the disease has no recognizable clinical symptoms.

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