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Use of afoxolaner for the treatment of lice (*Goniodes pavonis*) in different genera (*Chrysolophus* spp, *Lophura* spp, *Phasianus* spp, and *Syrmaticus* spp) and species of pheasants and West Mexican Chachalacas (*Ortalis poliocephala*)

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ABSTRACT

Lice are ectoparasites capable of affecting birds, and can result in direct and indirect damage to their host. Afoxolaner is an isoxazoline that has been shown to be effective against these ectoparasites without known adverse effects. The objective of this research was to evaluate the effect of afoxolaner on lice in pheasants and plain chachalacas. A total of 29 pheasants of different genera and species (*Chrysolophus pictus, C. amherstiae, Lophura swinghoii, L. nycthemera, Phasianus colchicus, and Syrmaticus reevesii*) and 18 West Mexican Chachalacas (*Ortalis poliocephala*) naturally infested with *Goniodes pavonis* were used. The birds were allocated to one of two groups: group 1 treated with 2.50 mg/kg of afoxolaner, and group 2 given no treatment. Ectoparasites were collected using the adhesive tape technique and identified. Afoxolaner was administered later as a single dose to group 1, and the clinical assessment to detect ectoparasites was repeated 28 days post-treatment. On day 28 post-treatment, group 1 was found to be negative for the presence of lice. The body weights were compared at the beginning and end of the clinical assessment in both groups and a significant difference in weight of treated birds was found. The mean body weight decreased by 0.017 g in control group, whereas it increased by 0.016 g in treated group. Oral administration of afoxolaner is an effective option for the treatment of *Goniodes pavonis* infestations in zoo birds.

1. Introduction

Ectoparasites displaying a seasonal pattern especially during humid periods of the year are common in birds (Rushton and Ngongi, 1998), and are responsible for growth delays, stress, pruritus, decreased egg-production, damage to the feather structure, and color alterations. Lice (Insecta: Phthiraptera) are among the most frequent ectoparasites invading nests both during egg-laying and after hatching (Salifou et al., 2013). Lice feed on cutaneous crusts, feathers, sebaceous secretions, and/or blood, and thus severe infestations can cause anaemia (Price et al., 2003). Nonetheless, scarce information exists on the species of lice parasitising zoo birds in Mexico. As the study conducted by Yarto et al. (2019) who reported that lice infestations in peacocks were due to *Goniodes pavonis*. It is reported that there are approximately 4000 species of lice that affect birds in the world (Price et al., 2003; Wall and Shearer, 2001). In Mexico, 206 species of chewing lice associated with birds distributed in 31 states have been registered (Sánchez-Montes et al., 2018). In the case of Chachalacas (*Ortalis poliocephala*) there are no reports of the species of lice that can affect them. Six species of chewing lice (Phtiraptera: Amblycera, Ischnocera) were described for the pheasants; *Goniocotes chrysocephalus*, *Goniodes colchici*, *Amyrsidea perdicis, Lipeurus maculosus*, *Goniocotes chrysocephalus* and *Oxylipeurus colchicus* (Ashraf et al., 2015; Goldová et al., 2006; González-Acuña et al., 2009). Different ectoparasiticides have been used for mite and lice infestations in birds, however, although they have shown efficacy, their use can have adverse effects on animals or could even lead to

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toxicity for both humans and animals (Barretto et al., 2017; Marangi et al., 2009; Sivajothi y Reddy, 2016). Isoxazolines belong to a new class of ectoparasiticides introduced in 2013, whose mechanism of action is through the inhibition of glutamate and gamma-amino butyric acid (GABA) and depends on chloride channels at a different site to fiproles. They have been used in dogs, cats, and exotic animals for the treatment of a number of insect and mite infestations (Sojka, 2018). However, no previous reports on the use of isoxazolines in pheasants and chachalacas exist. Therefore, the objective of this research was to evaluate the effect of afoxolaner on lice (*Goniodes pavonis*) in pheasants and chachalacas in Zoológico Benito Juárez, Morelia, Michoacán, Mexico.

2. Materials and methods

2.1. Study area

The study was conducted in the "Benito Juarez" Zoological Park of Morelia, located in Juarez Roadway, Felix Ireta, P.Z. 58070 Morelia, Michoacán, México, from January to February, 2019. The study protocol was approved by the Ethics Committee of the Mexican Association of Zoo, Wildlife and Exotic Pet Veterinarians (AMMVEZOO, A.C.).

2.2. Animals and sample analysis

Ectoparasites from forty-seven birds were included in this study: 3 Ghigi golden pheasants (*Chrysolophus pictus luteus*),14 golden pheasants (*Chrysolophus pictus*), 3 Swinhoe's pheasants (*Lophura swinhoii*), 3 common pheasants (*Phasianus colchicus*), 3 silver pheasants (*Lophura nycthemera*), 1 Reeve's pheasant (*Syrmaticus reevesii*), 2 Lady Amherst's pheasants (*Chrysolophus amherstiae*), and 18 West Mexican chachalacas (*Ortalis poliocephala*) with natural infestations of lice, on all parts of the bird's body were collected using the transparent adhesive tape technique whilst wearing gloves and the samples were placed individually in 70 % alcohol. Due to the abundant burden of parasites in all regions of the body, no count was made, they were only considered positive with the presence of at least one parasitic form in some part of the body. All animals were identified, and the following epidemiological data were collected: age, genus, species, and body weight. The samples obtained were placed on a slide and drops of a solution of lactophenol cotton blue (LCB) were added (Tantalean, 2010). The samples were later observed under a microscope (Leica DFC300 FX®, Leica microsystems), using the objectives 10× and 40 × . Ectoparasite identification was carried out using the morphological characteristics described by Taylor et al. (2016) and Hendrix and Robinson (2017), which all corresponded to *Goniodes pavonis* lice species. The samples were collected on day 1 of treatment with post-treatment assessment carried out on day 28, on the 1 st and 28th the same evaluations were made. The diet and the environment was not modified and no other pharmacological treatment was applied during the study period.

2.3. Treatment

The animals (29 pheasants and 18 chachalacas) were randomly divided in two groups: the treatment group (G1), which included 24 birds (15 pheasants and 9 chachalacas), that were treated with 2.50 mg/kg of afoxolaner (NexGard®, Boehringer Ingelheim, Lyon, France), the dose was similar to that reported by Yarto et al. (2019); and the control group (G2), with the other 23 remaining birds (14 pheasants and 9 chachalacas), which did not receive any treatment.

2.4. Statistical analysis

Data collected from the sample of birds included in this study were analyzed using the Tukey's student range test since the data did not show a normal distribution (Data were subjected to a normality test Shapiro–Wilk). Since it is a categorical variable (the number of pre-treatment positive and negative animals, and the number of post-treatment positive and negative animals) a value of 2 was given to each positive animal and a value of 1 to each negative animal in order to convert the categorical variable into a numeric variable for analysis. For the comparison of body weights, a matched pairs test was applied, initial weight and final weight were compared in each group and for each species.

Table 1
Comparison of the mean number of ectoparasite-positive birds pre- and post-treatment with afoxolaner with the untreated control group.

	Day 1 positive (%)	Day 28 positive (%)
Treatment n = 24	2.00^a (100)	1.00^b (0)
Control n = 23	2.00^a (100)	2.00^a (100)
CV	0	34.39
SEM	0	0.07

Ectoparasite positive birds were designated with the number 2 and negative birds were designated with the number 1, number 2 in the table means that 100% of the birds were positive and the mean was "2" and number 1 in the table means that 100% of the birds were negative and the mean was 1, ^{ab}Values with different superscript letters within a column are significantly different, alpha < 0.05. CV = coefficient of variation; SEM = standard error of the mean.

3. Results

At the beginning of this study, both of the groups were positive for ectoparasite infection (100 % of the birds were parasitised). A group comparison after treatment showed a significant difference; the treated group (group 1) had a lower number of positive animals than the untreated group (Table 1). The body weight of the chachalacas treated with afoxolaner remained the same at the end of the study, the weight of the chachalacas of the control group decreased significantly (Table 2); pheasants treated with afoxolaner increased their weight significantly, untreated pheasants showed no difference in this study, as seen in Table 2.

4. Discussion

The number of ectoparasites in the birds treated with afoxolaner in this study decreased to zero over a 28-day period. These results are similar to those reported by Yarto et al. (2019), who administered 2.5 mg kg of afoxolaner to peacocks infested with *Goniodes pavonis* lice, reporting that the treatment with afoxolaner significantly decreased the prevalence of positive infections of peacocks positive for lice (p = 0.02) compared with the control group, the haematocrit improved in the afoxolaner-treated group from a baseline of 46.4% –54.7% at 35 days post-treatment. Unlike other acaricides, isoxazolines have been shown to be safe and effective in in lice removal (Kohler-Aanesen et al., 2017) and mites in other species. Brauneis et al. (2017) administered fluralaner at a dose of 0.5 mg/kg of live body weight at 7-day intervals to healthy hens which were later experimentally infested with 200 adults

Table 2
Comparison of the mean initial weight and final weight in kilograms of birds treated with afoxolaner and untreated birds

	Initial weight	Final weight	P-Value
Chachalacas			
Treatment	0.912	0.90	0.27
Control	1.058	1.030	0.04
Pheasants			
Treatment	0.850	0.870	0.004
Control	1.176	1.175	0.48

Matched pairs test, alpha < 0.05.

of *Dermanyssus gallinae*. This isoxazoline resulted in a rapid rate of death in mites within 4 h after the infestation and for 12 days after the start of the treatment. A 98.7–100% efficacy was achieved within the first 24 h and maintained over 15 days, showing an efficacy and sustained effect against bird ectoparasites similar to that obtained in our study. In a study by Hinkle et al. (2018), the efficacy and safety of using fluralaner in a water-soluble formulation for infestations with *Ornithonyssus sylviarum* in laying hens was assessed and they showed a reduction of 90 % in mite number from day 6 to days 19 and 22 in groups treated with 0.25, 0.5, and 1.0 mg/kg fluralaner. On day 19, the average mite count was lower in the 0.5 and 1.0 mg/kg groups than the group treated with 0.25 mg/kg, and in the group treated with 1.0 mg/kg than the group treated with 0.5 mg/kg, with no adverse effects exhibited in the treated birds. The results of this study, showed that oral administration of 2.5 mg/kg afoxolaner in pheasants and chachalacas resulted in an effective treatment for lice infestations. No adverse effects attributed to afoxolaner treatment were observed. Treated birds showed an increase in body weight compared with untreated birds, although *Goniodes pavonis* is not a bloodsucking lice, it causes discomfort that is related to the amount of parasites in each animal, these results agree with Millán et al. (2004) who reported a poor body condition in infested partridges (*Alectoris rufa*) that had a large number of *Goniodes dispar* lice.

5. Conclusion

This study demonstrated that the oral administration of afoxolaner is an effective option for the treatment of *Goniodes pavonis* infestations in other zoo birds (pheasants and chachalacas) belonging to the same subfamily (Phasianinae) of the one already reported (Peacocks) since no adverse effects attributed to the treatment were seen, however, experimental toxicological tests with different dose levels are needed to verify drug safety concerns.

Declaration of Competing Interest

None.

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CRediT authorship contribution statement

Enrique Yarto Jaramillo: Conceptualization, Resources, Methodology, Writing - review & editing, Project administration. **Camilo Romero Núñez:** Conceptualization, Methodology, Software, Validation, Formal analysis, Data curation, Writing - original draft, Supervision, Project administration. **María de los Á. Álvarez Zavala:** Resources, Methodology, Writing - review & editing, Visualization. **Eduardo Cruz López:** Resources, Methodology, Writing - review & editing, Visualization. **Josué Rangel Díaz:** Resources, Methodology, Writing - review & editing, Visualization. **Laura Miranda Contreras:** Validation, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Evelyn Galicia Franco:** Investigation, Data curation, Writing - review & editing, Visualization. **Rafael Heredia Cárdenas:** Software, Validation, Formal analysis, Data curation, Writing - review & editing.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2020.109065.

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