Veterinary Parasitology 189 (2012) 387–389

Contents lists available at SciVerse ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Efficacy of albendazole and moxidectin and resistance to ivermectin against *Libyostrongylus douglassii* and *Libyostrongylus dentatus* in ostriches

Lara Pereira de Souza[a], Rosane Teixeira Lelis[a], Igor Rio Apa Granja[b], Renato Augusto DaMatta[a], Clóvis de Paula Santos[a,*]

[a] *Laboratório de Biologia Celular e Tecidual, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, 28013-602 Campos dos Goytacazes, RJ, Brazil*
[b] *Rioapavestruzes, 28013-600 Guarani, MG, Brazil*

ARTICLE INFO

Article history:
Received 17 February 2012
Received in revised form 18 April 2012
Accepted 20 April 2012

Keywords:
Libyostrongylus douglassii
Libyostrongylus dentatus
Anthelmintic resistance
Ostrich
Albendazole
Moxidectin
Ivermectin

ABSTRACT

Anthelmintic resistance has emerged globally as a problem amongst nematode of livestock and has been particularly well documented in equine and small ruminants. There are no studies regarding the efficacy of anthelmintics against the hematophagous nematodes in ostriches, *Libyostrongylus dentatus*; and just a few on *L. douglassii*. Here the efficacy of albendazole, ivermectin and moxidectin were evaluated against these two species in an ostrich farm in Minas Gerais state, Brazil. The feces were collected on the day of treatment and after 13 days of an oral dose of albendazole (6 mg/kg), or an injected dose (0.2 mg/kg) of ivermectin or moxidectin. The fecal egg count reduction test and coprocultures were performed to determine possible resistance against the drugs used. An efficacy of 60% was found for ivermectin, while albendazole and moxidectin were 100% effective. Both worm species appeared to have reduced sensitivity to ivermectin.

© 2012 Elsevier B.V. Open access under the Elsevier OA license.

1. Introduction

Libyostrongylus dougassii and *L. dentatus* are haematophagous parasites located in the proventriculus and ventriculus of ostriches (Ederli et al., 2008a, 2008b). These helminths are widely distributed in Brazil (Andrade et al., 2011a). Parasitism by *L. douglassii* may cause anemia, weight loss, anorexia, proventriculitis, killing young ostriches (Reinecke, 1983) and occasionally adults (Reinecke, 1983; Bastianello et al., 2005; Santos et al., 2010). Heterophilic inflammatory infiltration near nematodes has also been associated with mixed infections of *L. douglassii* and *L. dentatus* (Andrade et al., 2011b).

Libyostrongylus control is curative or preventive similar to that of other nematodes of production animals (Santos et al., 2010). Anthelmintics have been widely used in livestock for the control of parasites. Because of heavy reliance on these drugs and their widespread use anthelmintic resistant parasites emerged as a major problem (Coles, 2005). This phenomenon was first reported by Malan et al. (1988) in South African ostriches that received levamisole at 30 mg/kg to control *Libyostrongylus* infection; since then, no other report was published on this subject. However, anthelmintic resistance may be common among *Libyostrongylus* but has not been well documented.

Management practices in Brazil, from 17 farms located in 9 states, revealed that the majority of anthelmintic management schemes are based on the annual treatment with ivermectin (Andrade et al., 2011a). Fenbendazole and albendazole use was also reported but anthelmintics were not rotated on any of the examined farms (Andrade et al., 2011a). The objective of the present study was to evaluate the efficacy of ivermectin, albendazole and moxidectin against *Libyostrongylus* in ostriches raised on a farm in the

* Corresponding author. Tel.: +55 22 2739 7180; fax: +55 22 2739 7178.
E-mail address: cps@uenf.br (C.d.P. Santos).

0304-4017/© 2012 Elsevier B.V. Open access under the Elsevier OA license.
http://dx.doi.org/10.1016/j.vetpar.2012.04.030

state of Minas Gerais, Brazil with a history of ivermectin use.

2. Materials and methods

The study was performed in an ostrich farm located in the municipal district of Guarani in the state of Minas Gerais. The production of ostriches on the farm began in 2004 and since then, ivermectin has been used twice a year for the control of parasites. The anthelmintic test used 16 adult ostriches for each drug evaluated. The birds were treated with an oral dose of albendazole (6 mg/kg) and an injectable dose (0.2 mg/kg) of ivermectin or moxidectin. The dosages for ivermectin and moxidectin were based on the literature that reports the use of these compounds to ostriches (Pennycott and Patterson, 2001; Bastianello et al., 2005). These doses were the same as recommended by the manufacturer to other livestock animals. Although albendazole has not been used in ostriches, the rationale of the authors of the articles cited above was followed, and the recommended manufacturer dosage for the same types of animals was adopted. The brand names of these drugs and the company that manufactures them were as follow: albendazole, "*Ricobendazole oral*", manufactured by "Ouro Fino"; ivermectin, "*Ivomec injetavel 50 ML – Ivermectina Merial 1%*", manufactured by "Merial Brasil"; moxidectin, "*Cydectin NF 500 ML – Fort Dodge – Moxidectina 1%*", manufactured by "Fort Dodge". All birds used were infected with both *Libyostrongylus* species.

The feces were collected from each ostrich, on the day of treatment and after 13 days, with the aid of a disposable plastic bag immediately after defecation, avoiding the part that contacted the soil or the vegetation (Andrade et al., 2011a). Two grams of feces were used for quantifying the number of eggs per gram (EPG), according to the modified technique of Gordon and Whitlock (1939). This technique uses the Mac Master chamber that detects above 50 EPG. The efficacy of the drugs was calculated as $E = 100 [1 - (Xt/Xc)]$; Xt and Xc are the arithmetic mean of EPG before (c) and after (t) 13 days of anthelmintic treatment for each group (Coles et al., 1992). The anthelmintic resistance was confirmed if the % of the fecal egg count reduction was <95% (Coles et al., 1992). Fecal cultures were performed in samples positive for eggs after treatment, the infective larvae were identified as before (Ederli et al., 2008b) and a mean of all the animals calculated.

3. Results and discussion

The efficacy of the anthelmintics varied. Ivermectin had an efficacy of 60%, while albendazole and moxidectin of 100% (Table 1). The farm studied here used ivermectin twice a year for 7 years without rotation of the drug, clearly indicating that this period was sufficient to select resistant individuals in the helminth population. Ivermectin is one of the most widely drugs used in ostrich breeding in Brazil (Andrade et al., 2011a). This is probably due to the low cost and lack of guidance in anthelmintic management.

Although, ivermectin and moxidectin belong to the same class and share the same mode of action, the pharmacokinetic profiles of these drugs are significantly distinct

Table 1
Anthelmintic effect of albendaloze, ivermectin and moxidectin against *Libyostrongylus douglassii* and *L. dentatus* in ostriches in the state of Minas Gerais, Brazil.

Anthelmintic	EPG												Variance of reduction	% Efficacy (95% CL)
	Pre-treatment						Post-treatment							
	Mean[c]	Standard deviation	Variance	Variations in counts	95% confidence limits (CL)		Mean	Variance	Standard deviation	Variations in counts	95% confidence limits			
					Minimum	Maximum					Minimum	Maximum		
Albendazole[a]	1631	1275	1,626,292	200–4000	952	2311	0	0	0	0–0	0	0	0	100 (100)
Ivermectin[b]	1113	1056	1,115,833	100–3300	550	1675	450	1,186,667	1089	0–4400	−130	1030	0.42	60 (−58/90)
Moxidectin[b]	2000	3817	1,457,200	200–14,900	−34	4034	0	0	0	0–0	0	0	0	100 (100)

[a] Oral dose of 6 mg/kg.
[b] Injectable dose of 0.2 mg/kg.
[c] Mean values of 16 animals per group.

and these differences may have important implications for the development of resistance (Sangster, 1999). This probably explains the differences in efficacy observed here.

Infective larvae of *L. dentatus* (56.7%) and *L. douglassii* (43.3%) were recovered on the coprocultures from ostriches treated with ivermectin. This result indicates that both *Libyostrongylus* species have acquired resistance to ivermectin showing that the prolonged use of the same drug has selected resistant individuals. This also suggests that both species behave very similarly.

Despite the results found here, ivermectin was effective against *L. douglassii* in ostriches in Scotland (Pennycott and Patterson, 2001). Moreover, fenbendazole alone or combined with resorantel (Fockema et al., 1985; Malan et al., 1988) and moxidectin has also been effective against *Libyostrongylus* (Bastianello et al., 2005). The efficacy of these drugs indicates that they are adequate to control *Libyostrongylus*. However, further work needs to be done to better understand the efficacy of the available drugs against nematodes of ostriches. Furthermore, there are no data from official bodies such as the FDA (USA) or ANVISA (Brazil) approving the use of any anthelmintic compound for ostriches although the subject has been discussed in the USA (Bren, 2002).

The fecal egg count reduction test is one of the most important methods to detect anthelmintic resistance because it can be used for all drug groups and is inexpensive as compared to other *in vivo* test such as the "controlled test". Although we have performed this classic test for detection of anthelmintic resistance and it clear indicates resistance in the case of ivermectin, care must be taken in the interpretation of data generated by this test because it has not been completely validated for ostriches.

Since the production of ostrich has spread in several countries the use of anthelmintic for control of nematode parasites is a fact. However, it is necessary to better understand the metabolism and pharmacokinetics of anthelmintics in ostriches. Further studies in other properties should be performed to better understand the sensitivity of *Libyostrongylus* to anthelmintics. Moreover, producers need to be aware of the correct anthelmintic management and the consequences of not following it properly.

Acknowledgments

The authors would like to thank Andrèa Carvalho César for proof reading the manuscript, referees for their helpful suggestions and the fostering agencies Fundação Carlos Chagas Filho do Rio de Janeiro (FAPERJ), Coordenação de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

References

Andrade, J.G., Lelis, R.T., DaMatta, R.A., Santos, C.P., 2011a. Occurrence of nematodes and anthelmintic management of ostrich farms from different Brazilian states: *Libyostrongylus douglassii* dominates mixed infections. Vet. Parasitol. 178, 29–133.

Andrade, J.G., Carvalho, E.C.Q., Santos, C.P., DaMatta, R.A., 2011b. Mixed infection with *Libyostrongylus dentatus* and *Libyostrongylus douglassii* induces a heterophilic inflammatory infiltrate in the proventriculus of ostriches. Avian Pathol. 40, 367–370.

Bastianello, S., McKenna, P.B., Hunter, J., Julian, A.F., 2005. Clinical and pathological aspects of *Libyostrongylus* infection in ostriches. Surveillance 32, 3–6.

Bren, L., 2002. Treating minor species: a major animal health concern. FDA veterinarian, v. XVII, n° 5 set/oct, 7–12.

Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35–44.

Coles, G.C., 2005. Anthelmintic resistance – looking to the future: a UK perspective. Res. Vet. Sci. 78, 99–108.

Ederli, N.B., Bonadiman, S.F., Moraes Neto, A.H.A., DaMatta, R.A., Santos, C.P., 2008a. Mixed infection by *Libyostrongylus douglassii* and *L. dentatus* (Nematoda: Trichostrongylidae) in *Struthio camelus* (Ratites: Struthioniformes) from Brazil with further morphological characterization of adults. Vet. Parasitol. 151, 227–232.

Ederli, N.B., de Oliveira, F.C.R., Lopes, C.W.G., DaMatta, R.A., Santos, C.P., Rodrigues, M.L.A., 2008b. Morphological diagnosis of infective larvae of Libyostrongylus douglassii (Cobbold, 1882) Lane, 1923 and *L. dentatus* Hoberg, Lloyd and Omar, 1995 (Nematoda: Trichostrongylidae) of ostriches. Vet. Parasitol. 155, 323–327.

Fockema, A., Malan, F.S., Cooper, G.G., Visser, R.E., 1985. Anthelmintic efficacy of fenbendazole against *Libyostrongylus douglassii* and *Houttuynia struthionis* in ostriches. J. S. Afr. Vet. Assoc. 56, 47–48.

Gordon, N.M., Whitlock, H.V., 1939. A new technique for counting nematode eggs in sheep faeces. J. Comm. Cient. Indust. Res. Org. 12, 50–52.

Malan, F.S., Gruss, N.A., Roper, N.A., Ashburner, A.J., Du Plessis, C.A., 1988. Resistance of *Libyostrongylus douglassii* in ostriches to levamisole. J. S. Afr. Vet. Assoc. 59, 202–203.

Pennycott, T., Patterson, T., 2001. Gastrointestinal parasites in ostriches (*Struthio camelus*). Vet. Rec. 148, 155–156.

Reinecke, R.K., 1983. Veterinary helminthology. Butterworth, Durban, p. 295.

Sangster, N.C., 1999. Pharmacology of anthelmintic resistance in cyathostomes: will it occur with the avermectin/milbemycins? Vet. Parasitol. 85, 189–204.

Santos, C.P., Andrade, J.G., DaMatta, R.A., 2010. An update on *Libyostrongylus*, a gastro-intestinal nematode of ostriches. In: Lamann, G.V. (Ed.), Veterinary Parasitology. Nova Science Publishers, pp. 179–192 (Chapter 7).