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PII: S0304-4017(13)00362-2
DOI: http://dx.doi.org/doi:10.1016/j.vetpar.2013.06.011
Reference: VETPAR 6873

To appear in: *Veterinary Parasitology*

Received date: 8-3-2013
Revised date: 6-6-2013
Accepted date: 7-6-2013

Please cite this article as: Arias, M.S., Sanchís, J., Francisco, I., Francisco, R., Piñeiro, P., Cazapal-Monteiro, C., Cortiñas, F.J., Suárez, J.L., Sánchez-Andrade, R., Paz-Silva, A., THE EFFICACY OF FOUR ANTHELMINTICS AGAINST *Calicophoron daubneyi* IN NATURALLY INFECTED DAIRY CATTLE, *Veterinary Parasitology* (2013), http://dx.doi.org/10.1016/j.vetpar.2013.06.011

THE EFFICACY OF FOUR ANTHELMINTICS AGAINST *Calicophoron daubneyi* IN NATURALLY INFECTED DAIRY CATTLE

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Abstract

The paramphistomicidal activity of four anthelmintics in dairy cattle naturally infected by *Calicophoron* (*Paramphistomum*) *daubneyi* was evaluated. Seventy Friesian adult cows were treated at drying-off (19 albendazole; 23 netobimin; 13 closantel and 15 oxyclozanide), and 21 remained untreated as controls. The anthelmintic efficacy was determined by estimating the faecal egg count reduction (FECR) values for each of the anthelmintics. The reduction in the number of cows shedding eggs in the faeces was also estimated.

The *Calicophoron daubneyi* egg-output was not fully suppressed following the administration of any of the parasiticides. The FECR values ranged from 0 to 26% in the cows receiving albendazole or netobimin, with 11-39% of cattle becoming negative after therapy. Better results were achieved with closantel and oxyclozanide, with FECR values of 97-99% and CPCR (cattle positive by coprology reduction) percentages of 85-93%.

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The observation of a similar efficacy with closantel and oxyclozanide against *C. daubneyi* led us to recommend the administration of closantel in those countries where oxyclozanide is not available.

Key words: rumen fluke, dairy cattle, deworming, closantel

Introduction

Infection of ruminants by Paramphistomidae flukes occurs by feeding forage contaminated with metacercariae, which excyst in the intestine (Sanchís et al., 2013). During migration of immature flukes to their final location (rumen, reticulum), severe pathological disorders including acute catarrhal and haemorrhagic inflammation of the jejunum occur. The adult stages are associated with inflammation of the mucosa and mucoid diarrhoea (Rolfe & Boray, 1993). Consequently, clinical signs of anaemia, hypoproteinemia and oedema, together with a reduction in feed conversion, weight gain, and milk production are observed (Spence et al., 1996; Kamaraj et al., 2010).

Although this has been a neglected infectious trematode disease in ruminants, it has recently emerged in many countries (Mage et al., 2002; Šoštaric et al., 2011; Arias et al., 2011; Taylor, 2012; Gordon et al., 2013). Control of rumen fluke infection is mainly achieved through the application of trematocidal drugs, although two areas should be considered: (1) fasciolicidal drugs are frequently administered to control liver fluke (*Fasciola*), and (2) due to milk withdrawal requirements, these drugs are used mainly during the dry period only. Only certain salicylanilides seem effective against both liver and rumen flukes (Murphy et al., 2008), and among these, oxyclozanide is the reported drug of choice against paramphistomes (Rojo-Vázquez et al., 2012). A 56.1-98.1% reduction of adult flukes after a single administration of oxyclozanide and 100% after 2

doses administered 3 days apart have been observed (Rolfe & Boray, 1987). Nevertheless, oxyclozanide is not available or licensed in some countries (Rolfe and Boray, 1988).

In the current investigation, the efficacy of four anthelmintics (albendazole, netobimin, closantel and oxyclozanide) against the rumen fluke *Calicophoron daubneyi* in naturally infected dairy cows has been analysed.

Material and Methods

Experimental design

The present research was conducted in NW Spain (Galicia), where raising bovine livestock is the main agricultural activity. Galicia has a maritime climate with high rainfall providing conditions that favour the free-living stages of *Calicophoron daubneyi* (Sanchís et al., 2013). During a period of 14 weeks, faecal samples were individually taken from 91 Holstein cows with natural paramphistomosis. These ruminants are kept in 3 stables, and fresh forage is provided when climatic conditions favour its growth (all year, excluding January-February) (Arias et al., 2010). In the dry period, cows are maintained on pastures with heifers. Deworming occurred during the dry season, before the cows re-entered the stable.

Seventy cattle were orally treated with dewormer during the dry period and 21 remained untreated as controls. A total of 19 cows received a single dose of albendazole (10 mg / Kg b.w., Albendex, SP Veterinaria SA, Tarragona, Spain), 23 were given netobimin (20 mg / Kg b.w., Hapasil, Intervet-Schering Plough, Spain), 13 received closantel (10 mg / Kg b.w., Endoex, SP Veterinaria, SA, Tarragona, Spain) and 15 were given oxyclozanide (15 mg / Kg b.w., Oxyzan, LABA, Perú).

Copromicroscopic analysis

C. daubneyi eggs were observed through the copromicroscopic sedimentation technique using individual samples of five grams of faeces (Díaz et al., 2007). The egg-counts were presented as the number of eggs per gram of faeces (EPG).

The *C. daubneyi* eggs and *Fasciola* eggs were differentiated based on their morphological characteristics (Arias et al., 2011; Gordon et al., 2013).

Indexes of Efficacy

The efficacy of the anthelmintics was determined by calculating the reduction in the faecal egg counts (FECR) and in the numbers of cattle positive by coprology (CPCR), using the following formulae:

% FECR: $(1 - (\text{Average egg-output}_{\text{post-treatment}} / \text{Average egg-output}_{\text{pre-treatment}})) \times 100$

% CPCR: $(1 - (\text{Positive Cows}_{\text{post-treatment}} / \text{Positive Cows}_{\text{pre-treatment}})) \times 100$

Statistical analysis

At each farm, the egg-output values were analysed using the non-parametric Kruskal-Wallis test. The indexes of efficacy were analysed by means of the χ^2 test.

The non-parametric Spearman test was employed to study the existence of a correlation between the results from the diverse groups. All tests were performed using SPSS for Windows (20.1). The results were considered significant when $P< 0.05$.

Results

Faecal examination

The dynamics of *C. daubneyi* eggs in the cattle is drawn in Figure 1. At the beginning of the study, EPG counts ranging from 270 to 353 were observed. The controls exhibited the highest values of egg-output throughout the study. The administration of albendazole and netobimin did not suppress the presence of eggs in the faeces (Fig. 1), and values near 200 EPG at the 5th wpt (week post-treatment) were counted. The egg-output counts increased between the 5th wpt and the end of the study, especially in those cattle receiving netobimin.

As reflected in Figure 1, the egg-output decreased at 1 wpt in the cattle treated with oxyclozanide and at 2 wpt when administering closantel. Then, values close to 0 were recorded until the end of the study.

There were statistically significant differences between the dewormed cows and the controls (χ^2= 138.281, *P*= 0.001) as well as among the treated cattle (χ^2= 185.371, *P*= 0.001). Significant differences were established between the cattle receiving albendazole and closantel (Z= -8.810, *P*= 0.001), albendazole and oxyclozanide (Z= -10.000, *P*= 0.001), netobimin and closantel (Z= -9.125, *P*= 0.001) and netobimin and oxyclozanide (Z= -10.282, *P*= 0.001).

Significant, positive correlations were recorded between the egg counts in the cows treated with: albendazole and netobimin (Correlation Coefficient, CC= 0.749, *P*= 0.001), albendazole and controls (CC= 0.585, *P*= 0.001), netobimin and controls (CC= 0.612, *P*= 0.001) and closantel and oxyclozanide (CC= 0.819, *P*= 0.001). On the contrary, the EPG values in the oxyclozanide-treated cattle and in the controls were negatively correlated (CC= -0.199, *P*= 0.015).

Deworming efficacy

123 The percentages of the FECR are summarised in Table 1. The cows receiving
124 albendazole reached FECR values between 0 and 27% and those given netobimin
125 reached 0-26%. Significant differences between these two treatments were not observed
126 (χ^2= 1.929, *P*= 0.165).
127 In the cattle treated with closantel, the values of FECR were ≥92% at the 2nd wpt and
128 approximately 98-99% in those receiving oxyclozanide by 1 wpt (χ^2= 5.848, *P*= 0.016).
129 As Table 1 shows, administration of albendazole or netobimin provided a less than 40%
130 reduction of cows positive by coprology (CPCR), with the highest CPCR values in the
131 2-3 wpt (χ^2= 0.259, *P*= 0.611). The CPCR values were ≥85% by the 2nd wpt in the
132 ruminants that received closantel and 93% in the oxyclozanide-treated cows (Table 1)
133 (χ^2= 13.690, *P*= 0.001).
134

135 **Discussion**

136 Infection of cows by gastrointestinal nematodes and paramphistomes can reduce milk
137 production by approximately 0.4 – 3 L per day (Spence et al., 1996; Mohan, 2011).
138 Oxyclozanide has been reported to be the best anthelmintic against paramphistomosis in
139 cattle (Díaz et al., 2006; Murphy et al., 2008). In the current investigation, the
140 administration of oxyclozanide to Holstein cows that were naturally infected by the
141 rumen fluke *Calicophoron daubneyi* reduced the egg-output in 98-99% from the first
142 week after treatment, in agreement with the results of Rolfe & Boray (1987).
143 Because oxyclozanide is not available in Spain, and in some other European and
144 American countries, albendazole, netobimin and closantel were also assayed against
145 paramphistomosis in dairy cattle. The results of deworming with albendazole or
146 netobimin were non-satisfactory because of the faecal egg counts were only slightly
147 reduced, in agreement with the findings of Mage & Reynal (1990), which found a 9%

148 FECR after the administration of a dosage of 10 mg albendazole / Kg b.w. Rolfe &
149 Boray (1987) reported a 49% reduction of adult paramphistomes using netobimin, while
150 Mage & Reynal (1990) did not observe diminution in the faecal egg counts.
151 None of the cows given closantel, except two, passed *C. daubneyi* eggs by the 2nd week
152 after treatment. These results differ from previous data, indicating the ineffectiveness of
153 a 7.5 mg / Kg b.w. dosage against adult rumen flukes (Rolfe & Boray, 1987). Several
154 features could help to clarify this apparent discrepancy. First, in the current
155 investigation, the efficacy of closantel against mature flukes was assessed based on
156 reduction of faecal egg counts. Second, a higher dosage than before (10 mg / Kg b.w.
157 versus 7.5 mg / Kg b.w., respectively) was assayed. Third, different routes for
158 administrating the closantel were chosen: oral (present work) versus intraruminal
159 injection (Rolfe & Boray, 1993).
160 There are no accepted criteria for establishing a successful flukicide treatment; so, as
161 indicated for nematodes, reduction on faecal egg counts (FECR) is applied (Gordon et
162 al., 2013). Several points could influence the FECR values, such as the initial (pre-
163 treatment) EPG counts or the number of animals remaining positive after being
164 dewormed. Thus, high FECR percentages could reflect a limited number of individuals
165 passing moderate EPG counts or an elevated number of treated animals with low egg-
166 output. Other disadvantages of estimating the FECR percentages from this parameter
167 are associated with the presence of adult flukes in the definite host. As a consequence,
168 the effect on immature stages could not be measured. In the current research, the finding
169 that 7% and 15% of cattle passed *C. daubneyi* eggs after receiving oxyclozanide and
170 closantel, respectively, corroborates these anthelmintics are active against adult rumen
171 flukes. Because variations were not observed between the egg-output and the cattle
172 positive to coprology values throughout 14 weeks post-treatment, efficacy against

immature paramphistomes is also concluded. On the contrary, more than 60% of cows remained positive after treatment with albendazole or netobimin, suggesting that these anthelmintics have a reduced effect on juvenile and/or adult rumen flukes.

In addition to the efficacy, another critical point regarding control of parasites in dairy cattle is the withdrawal time. While a period >30 days is suggested for the closantel, only 3 days are needed for the oxyclozanide (Chartier et al., 2012). The observation of a similar efficacy of closantel and oxyclozanide against *C. daubneyi* led us to recommend the administration of closantel in those countries where oxyclozanide is not available.

Conflict of interest

All authors declare the absence of any financial or personal interests that could inappropriately influence the current work.

The final article has been approved by all authors.

Acknowledgements

This work was partly supported by the Research Projects 10MDS261023PR (Xunta de Galicia, Spain) and AGL2012-34355 (Ministerio de Economía y Competitividad, Spain; FEDER). Dr. MS Arias is recipient of a "Parga Pondal" postdoctoral research fellowship (Xunta de Galicia, Spain). Jaime Sanchís was given a research grant (Edmundus 17; Banco de Santander – USC, Spain) for developing his PhD Thesis at the University of Santiago de Compostela (Spain).

References

Arias, M., Lomba, C., Dacal, V., Vázquez, L., Pedreira, J., Francisco, I., Piñeiro, P., Cazapal-Monteiro, C., Suárez, J.L., Díez-Baños, P., Morrondo, P., Sánchez-

198 Andrade, R., Paz-Silva, A., 2011. Prevalence of mixed trematode infections in
199 an abattoir receiving cattle from northern Portugal and north-west Spain. Vet
200 Rec. 168, 408.
201 Arias, M., Piñeiro, P., Hillyer, G.V., Suárez, J.L., Francisco, I., Cortiñas, F.J., Díez-
202 Baños, P., Morrondo, P., Sánchez-Andrade, R., Paz-Silva, A., 2010. An
203 approach of the laboratory to the field: assessment of the influence of cattle
204 management on the seroprevalence of fascioliasis by using polyclonal- and
205 recombinant-based ELISAs. J Parasitol. 96, 626-631.
206 Charlier J, Hostens M, Jacobs J, Van Ranst B, Duchateau L, Vercruysse J. (2012).
207 Integrating fasciolosis control in the dry cow management: the effect of closantel
208 treatment on milk production. PLoS One. 7: e43216.
209 Díaz, P., Lomba, C., Pedreira, J., Arias, M., Sánchez-Andrade, R., Suárez, J.L., Díez-
210 Baños, P., Morrondo, P., Paz-Silva, A., 2006. Analysis of the IgG antibody
211 response against Paramphistomidae trematoda in naturally infected cattle.
212 Application to serological surveys. Vet Parasitol. 140, 281-288.
213 Díaz, P., Pedreira, J., Sánchez-Andrade, R., Suárez, J.L., Arias, M.S., Francisco, I.,
214 Fernández, G., Díez-Baños, P., Morrondo, P., Paz-Silva, A., 2007. Risk periods
215 of infection by *Calicophoron daubneyi* (Digenea:Paramphistomidae) in cattle
216 from oceanic climate areas. Parasitol Res. 101, 339-342.
217 Gordon, D.K., Roberts, L.C., Lean, N., Zadoks, R.N., Sargison, N.D., Skuce, P.J., 2013.
218 Identification of the rumen fluke, *Calicophoron daubneyi*, in GB livestock:
219 possible implications for liver fluke diagnosis. Vet Parasitol. doi:
220 10.1016/j.vetpar.2013.01.014.
221 Mage, C., Bourgne, H., Toullieu, J.M., Rondelaud, D., Dreyfuss, G., 2002. *Fasciola*
222 *hepatica* and *Paramphistomum daubneyi*: changes in prevalences of natural

infections in cattle and in *Lymnaea truncatula* from central France over the past 12 years. Vet Res. 33, 439-47.

Mohan, SK., 2011. Amphistomosis- an unnoticeable threat. JIVA, 9, 60-61.

Murphy, T.M., Power, E.P., Sánchez-Miguel, C., Casey, M.J., Toolan, D.P., Fagan, J.G., 2008. Paramphistomosis in Irish cattle. Vet Rec. 162, 831.

Paraud, C., Gaudin, C., Pors, I., Chartier, C., 2009. Efficacy of oxyclozanide against the rumen fluke *Calicophoron daubneyi* in experimentally infected goats. Vet J. 180, 265-267.

Rojo-Vázquez, F.A., Meana, A., Valcárcel, F., Martínez-Valladares, M., 2012. Update on trematode infections in sheep. Vet Parasitol. 189, 15-38.

Rolfe, P.F., Boray, J.C., 1987. Chemotherapy of paramphistomosis in cattle. Aust Vet J. 64, 328-332.

Rolfe, P.F., Boray, J.C., 1988. Chemotherapy of paramphistomosis in sheep. Aust Vet J. 65, 148-150.

Rolfe, P.F., Boray, J.C., 1993. Comparative efficacy of moxidectin, an ivermectin/clorsulon combination and closantel against immature paramphistomes in cattle. Aust Vet J. 70, 265-267.

Sanchís, J., Sánchez-Andrade, R., Macchi, M.I., Piñeiro, P., Suárez, J.L., Cazapal-Monteiro, C., Maldini, G., Venzal, J.M., Paz-Silva, A., Arias, M.S., 2013. Infection by Paramphistomidae trematodes in cattle from two agricultural regions in NW Uruguay and NW Spain. Vet Parasitol. 191, 165-171.

Šoštaric, B., Beck, R., Mihaljević, Ž., Vicković, I., 2011. Paramphistomosis - emerging or reemerging disease of ruminants in Croatia. Vet Stanica 42, 296-297

246 Spence, S.A., Fraser, G.C., Chang, S., 1996. Responses in milk production to control of
247 gastrointestinal nematode and paramphistome parasites in dairy cattle. Aust Vet
248 J. 74, 456-459.
249 Taylor, M.A., 2012. Emerging parasitic diseases of sheep. Vet Parasitol. 189, 2-7.
250

Figure captions

Figure 1.- Dynamics of *C. daubneyi* eggs in cattle naturally infected and treated with anthelmintics. The results are the means ± 2 SD. (–): controls; (☼): albendazole (10 mg / Kg b.w.); (●): netobimin (20 mg / Kg b.w.); (✎): closantel (10 mg / Kg b.w.); (□): oxyclozanide (15 mg / Kg b.w.). WPT: weeks post-treatment.

Table 1.- Values of FECR (faecal egg count reduction) and CPCR (coprology positive cattle reduction) in cattle naturally infected by *Calicophoron daubneyi* and treated with albendazole (ABZ) (10 mg / Kg b.w), netobimin (NB) (20 mg / Kg b.w), closantel (CLO) (10 mg / Kg b.w) and oxyclozanide (OXY) (15 mg / Kg b.w). Results are expressed as percentages and 95% Confidence Interval (CI).

	ABZ		NB		CLO		OXY	
WPT	FECR	CPCR	FECR	CPCR	FECR	CPCR	FECR	CPCR
1	7 (4-10)	16 (0-32)	-	30 (12-49)	18 (13-23)	23 (0-46)	99 (97-100)	93 (81-100)
2	1 (0-3)	37 (15-59)	21 (16-25)	39 (19-59)	92 (88-95)	85 (65-100)	98 (96-99)	93 (81-100)
3	0 (0-1)	37 (15-59)	17 (13-22)	39 (1-59)	98 (97-100)	92 (78-100)	98 (96-99)	93 (81-100)
5	27 (22-32)	21 (3-39)	26 (21-31)	30 (12-49)	98 (97-100)	92 (78-100)	98 (96-99)	93 (81-100)
7	9 (6-13)	16 (0-32)	-	22 (5-39)	98 (97-100)	92 (78-100)	99 (97-100)	93 (81-100)
9	3 (1-5)	11 (0-24)	-	22 (5-39)	98 (97-100)	92 (78-100)	98 (96-99)	93 (81-100)
11	20 (16-25)	11 (0-24)	-	22 (5-39)	97 (95-99)	92 (78-100)	98 (96-99)	93 (81-100)
13	16 (12-20)	11 (0-24)	-	22 (5-39)	97 (95-99)	92 (78-100)	98 (96-99)	93 (81-100)