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Risk factors for anthelmintic resistance development in cattle gastrointestinal nematodes in Argentina

Fatores de risco para o desenvolvimento de resistência anti-helmíntica em nematódeos gastrintestinais de bovinos na Argentina

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Abstract

Risk factors for anthelmintic resistance (AR) on bovine ranches were studied. Data were derived from a survey made to 50 ranch owners, who had conducted a faecal egg-count-reduction test. The questionnaire contained descriptors of bovine ranch management and nematode control. A case-control design study was undertaken and AR cases were present in 26 herds. Associations between the binary outcome variable (AR versus not AR) and risk factors recorded in the questionnaire were evaluated. Variables associated with the presence of AR at P< 0.15 and/or odds ratio (OR) > 2 were subjected to a multivariable logistic regression model. The main effects contributing to general AR (avermectin AVM and/or benzimidazole) in the final model were total number of annual treatments (OR 7.68; 95% CI 2.4 to 28.3) and use of more than 75% of AVM in the past (OR= 18.6; 95% CI 1.3 to 97.3), whereas for AVM resistance alone were total number of AVM annual treatments (OR= 11.5; 95% CI 2.9 to 45.5) and number of AVM Nov-Jan treatments (OR= 5.8; 95% CI 1.71 to 47.9). The results showed that treatment frequency, date of treatment and frequency of treatment in the past with a single drug were the main risk factors involved in AR development.

Keywords: Nematode, cattle, anthelmintic resistance, risk factor.

Resumo

Fatores de risco para resistência anti-helmíntica (AR) em fazendas de criação de bovinos foram estudados de dados obtidos de um levantamento em 50 propriedades. Em todas foram conduzidos testes de redução de contagem de ovos (opg) e um questionário preenchido pelos proprietários sobre o manejo e o controle de verminose nessas fazendas. Um estudo com desenho de caso controlado foi realizado e casos de AR estavam presentes em 26 rebanhos. Associações foram avaliadas entre a variável binária produzida (AR versus sem AR) e fatores de risco registrados nos questionários. Variáveis associadas com o resultado de interesse a P<0.15 e/ou razão de prevalência (OR) > 2 foram usados num modelo de regressão logística multivariável. Os principais efeitos contribuintes para AR geral (avermectina AVM e/ou benzimidazole), no modelo final, foram número total de tratamentos anuais (OR 7,68; 95% IC 2,4 a 28,3) e uso no passado mais que 75% de AVM (OR= 18,6; 95% IC 1,3 a 97,3), e para resistência à AVM foram número total de tratamentos anuais (OR=11,5; 95% IC 2,9 a 45,5), número de tratamentos com AVM de novembro a janeiro (OR= 5,8; 95% IC 1,71 a 47,9). Estes resultados mostraram que a frequência dos tratamentos, a época do ano em que foram feitos os tratamentos e a frequência dos tratamentos no passado com uma única droga foram os principais fatores de risco implicado no desenvolvimento de AR.

Palavras-chave: Nematóide, gado, resistência anti-helmíntica, fator de risco.

Introduction

Anthelmintic resistance (AR) in gastro-intestinal nematodes of cattle has become a problem in many countries, where several survey reports indicate widespread AR (KAPLAN, 2004). Moreover, the number of reports in the literature published over the past five years suggests that the problem is rapidly increasing (SUTHERLAND; LEATHWICK; 2011). Numerous studies about bovine nematode AR have been reported worldwide, mostly in regions where production systems are based on grazing management, such as New Zealand, Brazil, Colombia, UK and the United States. AR has been reported for all the broad-spectrum anthelmintics

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(levamisole, benzimidazoles and macrocyclic lactones) used for cattle (PAIVA et al., 2001; FAMILTON et al., 2001; COLES, 2002; WAGHORN et al., 2006; MÁRQUEZ et al., 2008; GASBARRE et al., 2009). In Argentina, the first cases of macrocyclic lactone and benzimidazoles resistance in cattle were reported by Anziani et al. (2001) and Fiel et al. (2001). Cases of multiple anthelmintic and nematode species resistance were detected later (MEJÍA et al., 2003; ANZIANI et al., 2004). Some recent surveys made in Argentina have attempted to quantify regional AR occurrence in cattle ranches (CARACOSTANTOGOLO et al., 2005; SUAREZ; CRISTEL, 2007).

During the last 20 years, in central Argentina technologies for nematode infection control in bovines have been based on the over-use of broad anti-parasitic drugs. Endectocides and benzimidazoles were the most frequently used anthelmintics (SUAREZ, 2002). In this region, anthelmintic treatments were frequently applied from autumn (weaning) to mid winter, with an additional drench in late winter or late spring during the fattening period of cattle. This practice is supported by the hypothesis that AR was caused by frequent anthelmintic treatments (PRICHARD et al., 1980). Many ranch advisors on nematode control recommended adopting suppressive drenches, such as monthly treatments. In other cases, they recommended strategic management practices, such as treatment and move to a clean annual crop (oat or rye) when the number of larvae in pastures is low (SUAREZ; LORENZO, 2000) or treatment during late spring or summer when weather conditions are deleterious to larval survival. Veterinarians also frequently proposed rotating between anthelmintic drugs to avoid AR.

In the last years, information has been generated about which practices are highly selective for AR in sheep nematodes (LEATHWICK et al., 2009). It has been postulated that in many situations all these recommendations, such as suppressive or strategic treatments, have accelerated the selection for AR by permitting only the survival of resistant larvae at the moment when there are no nonresistant larvae in refugia (VAN WYK, 2001; VAN WYK et al., 2006). However, very little is known about selection for AR in cattle nematodes (STAFFORD; COLES, 1999) despite the urgent need for studies exploring factors associated with AR development.

The objective of this study was to examine the association between the development of AR and farm practices for the control of cattle gastrointestinal nematodes and other farm management activities.

Materials and Methods

Farm selection

A case-control study was undertaken with data obtained from interview surveys (either by telephone or face to face) made to 50 cattle ranch owners who had conducted a faecal egg-count-reduction test (FECRT) on their herds no more than three years before. The sampled farms were selected based on the AR status (half of them had AR and the other half did not). The cattle ranches were located mostly in central Argentina, in the following regions: Humid Pampas (n=15), Subhumid Pampas (n=24) and Semiarid Pampas and North Patagonian region (n=11). AR was present in 26 of the ranches. Avermectin (AVM) resistance was present in 25 herds, benzimidazole (BZD) resistance in 11 herds and AR to both drugs in 10 herds. The surveyed farms were mainly mixed cow-calf and finishing or only finishing operations.

Data of 23 FECRTs were obtained from Suarez and Cristel (2007) and data of 27 FECRTs were kindly provided by the following colleague parasitologists: O. Anziani, C. Descarga, C. Rossanigo, M. Buffarini, R. Sanabria, J. Romero, F. Olaechea, C. Entrocasso, C. Fiel. In all cases, FECRTs and resistance were confirmed by a mean egg count reduction percentage of less than 95% (100(1-[T2/C2])) and the 95% confidence level of less than 90%, according to the protocol of the WAAVP (COLES et al., 1992). Ivermectin (IVM), fenbendazole or albendazole (BZD) and levamisole (LVM) were tested. Ranches were defined as either AR (IVM, BZD or IVM and BZD resistant) or not AR for the case-control analysis.

Questionnaire

The survey questionnaire comprised 29 questions, including descriptors of the ranch management and gastrointestinal nematode control and other questions addressing the principal AR development hypotheses. The questionnaire was pilot-tested by 10 owners and the final questionnaire took approximately 20 minutes for completion. Some variables, such as AVM use as external parasiticide, drench gun calibration or cropping practices in the ranch, were not incorporated to the analysis because most participants had responded similarly.

Several hypotheses, such as herd annual treatment frequency, under-dosing, treatment recommended by veterinarians by monitoring diagnoses from egg counts or live weight gain were tested in the questionnaire. The refugia hypothesis (VAN WYK, 2001) was analysed through questions on the percentage of annual or safe pastures used, treatment and movement to safe pastures or enclosure feed system for weaning calves during a short period (less than 3 months), treatment of bovines older than two years, and drenching during November-January. Likewise, a refugia index (0 to 1) was developed using the following variables: more than 25% of annual crops (0.25), treatment of two-year-old or adult bovines (0.25), treatment and move to clean pastures (0.25), treatment before move to temporary enclosure feedlot system (0.25); and the indicative level was more than the average farm level (≥0.36).

Statistical methods

Associations between the binary outcome variable (AR versus not AR) and ranch-level risk factors were analyzed. A screening test was carried out using either the Chi-square test for independence for categorical variables or univariable logistic-regression analysis for continuous independent variables. Likewise, a simple linear regression was used to test dependent variables as percentage of anthelmintic drug efficacy and continuous independent variables.

Variables associated with the outcome at P<0.15 or odds ratio (OR) > 2 were evaluated using multivariable logistic-regression model. The ORs calculated from the estimated coefficients in the final models were used to measure the strength of the association.

The relative contribution of each factor to the probability of AR presence or absence was determined by applying the multivariable logistic regression model (DI RIENZO et al., 2008). Backward elimination was used to determine which factors could be excluded from the model based on a likelihood ratio Chi-square statistic corresponding to *P* = 10% at each step.

Results

Mean FECRT efficacies for IVM in ranches with (n= 25) and without (n= 25) resistance were 69.66 ± 23.3 % and 97.57 ± 2.3 %, respectively, and for BZD with (n= 11) and without (n= 39) resistance were 78.91 ± 22.9 % and 98.58 ± 1.7 %, respectively. Figure 1 shows the level of FECRT in each ranch with and without AR. LVM resistance was not present. The responses of the surveyed owners about the categorical or continuous variables evaluated that were not associated with ranch AR status by univariable analysis are the following: type of cattle system, cattle breed, region, partial feedlot practices, treatment of calves before move to feedlot, treatment according to the live weight gain of the growing lot, anthelmintic dose estimation, stocking rate (heads per ha), proportion of ranch annual crops, proportion of ranch perennial and natural pastures, and number of annual BZD treatments in the past.

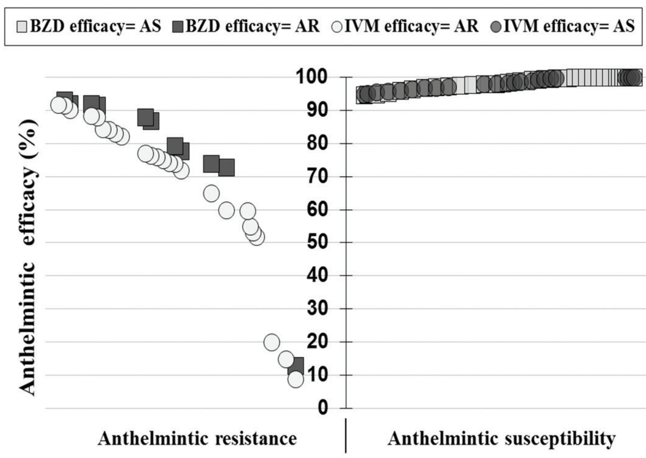

Figure 1. Level of FECRT in the farms with and without AR.

Regarding the owners' responses about ranch herd ivermectin (IVM) or benzimidazole (BZD) resistant status, the variables more than 75% of IVM was used in the past (OR= 3.2; P< 0.05) or more than 75% of BZD was used in the past (OR= 0.8; P< 0.30) were tested.

Finally, 13 variables were included in the multivariable model (Tables 1 and 2); these variables reflected the frequency of treatments hypothesis (n= 3), the annual period of the treatments (n= 4), the type of control plan or methods included (n= 2), refugia hypothesis (this aspect is partially included in the other variables, n= 2), ranch practices (n=2) and use of egg counts and veterinarian advice (n= 1).

The results of linear regression between continuous variables and the percentage of efficacies obtained from ivermectin and benzimidazole FECRTs are presented in Table 3. These results show that there is a significant correlation between the number of annual total anthelmintic drenches and the average IVM and BZD efficacy of the ranch and between the number of annual avermectin drenches and IVM efficacy. These results are presented in Figure 2, which shows a significant decrease in drug efficacy with increasing annual herd anthelmintic drenches.

The final logistic regression model of risk factors for general AR is presented in Table 4. This model explains that the variables such as frequency of annual treatments (OR 7.68; *p*<0.002) and recurrent use of practically one anthelmintic drug in the past (OR 18.62; *p*<0-028) were significantly associated with risk of AR development. Likewise, the main effects contributing to IVM resistance (Table 5) were total number of AVM annual treatments (OR=11.50; *p*<0.0005), number of AVM Nov.-Jan. treatments

Table 1. Responses of cattle owners according to ranch herd anthelmintic resistance (AR) status and results of categorical variables univariable analysis that were included in multivariable model. AR: anthelmintic resistance, OR: odds ratio.

Variable	Level of variable	AR Yes	AR no	X^2, (*P*)	OR
Type of anthelmintic control plan	Strategic programmed	21	9	9.74	7.0
	Programmed and after tactic drenches (in view of symptoms, BC, epg or LWG)	5	15	(0.002)	
Treat in November –January period	Yes	17	8	5.13	3.78
	No	9	16	(0.023)	
Treat and move to safe pasture	Yes	14	9	2.17	2.42
	No	9	14	(0.14)	
Complement drugs with other control methods	Yes	6	10	1.98	0.45
	No	20	14	(0.15)	
Use diagnostic pursuit with egg counts and veterinarian advice to worm control	No or sporadically	21	14	2.99	3.0
	Yes, periodic epg of the growing lot and veterinarian advice	5	10	(0.084)	

BC: body condition; epg: eggs per gram; LWG live weight gain.

Table 2. Answers of cattle owners according to ranch herd anthelmintic resistance (AR) status and results of continuous variables univariable analysis that were presented to multivariable model.

Variable	Level of variable	AR yes	AR no	*P*	OR/AF*
Young cattle (<18 month of age)/total stock relation	≤ 0.89	11	15	2.03	2.27
	≥ 0.90	15	9	0.15	
Number of annual anthelmintic treatments	≤ 3 treatments	25	9	19.7	41.6
	≥ 3.1 treatments	1	15	0.0000	
Number of annual avermectin treatments in the past	≤ 2.5 treatments	6	19	15.7	12.6
	≥ 2.5 treatments	20	5	0.000	
Number of anthelmintic treatments during Nov-Jan.	0 treatments	9	15	3.89	3.78
	≥ 0.1 treatments	17	9	0.05	
Number of avermectin treatments during Nov-Jan.	0 treatments	13	20	6.18	5
	≥ 0.1 treatments	13	4	0.01	
Refugia index (0-1)	≤ 0.35	12	16	2.13	2.3
	≥0.36	14	8	0,14	
Number of autumn-winter drenches	≤ 3 treatments	1	12	7.83	24
	≥ 3.1 treatments	10	5	0.006	

*OR: Odds Ratio/ AF: attributable fraction.

Table 3. Correlation (c. cor.) and lineal regression between continuous variables and the percentage of anthelmintic efficacies. The ranch herd efficacy of avermectin (AVM) and benzimidazole (BZD) was calculated by means of the average of the result of FECRT with both drugs.

Explanatory variable	Dependent variable	c. cor.	r2	b	const	*P*
Number of annual anthelmintic treatments	Average of ranch herd AVM and BZD efficacies	–0.31	0.09	–3.51	100.7	0.031
Number of annual avermectin treatments	Percentage of AVM efficacy	–0.48	0.229	–7.59	100.05	0.0004
Number of annual benzimidazole treatments	Percentage of BZD efficacy	–0.14	0.02	–1.52	96.2	0.32
Refugia index	Average of ranch herd AVM and BZD efficacies	–0.16	0.03	–11.0	92.8	0.25
Ranch herd stocking rate (head per ha)	Average of ranch herd AVM and BZD efficacies	–0.14	0.02	–2.16	93.2	0.34
Young cattle (<18 month of age)/total stock relation	Average of ranch herd AVM and BZD efficacies	–0.07	0.01	–4.7	92.1	0.62

(OR= 5.83; p<0.10) and the use of AVM in the past as minor effect (OR= 4.45; p<0.19).

Discussion

This study has the advantage that all the herds have been tested and their AR status obtained in parasitological laboratories. However, using this methodology it is difficult to ensure the representativeness of the process of ranch herd sample selection. The selection of the owners of cattle ranches that had made a FECRT during the last 3 years is quite biased, because in Argentina it is not easy to locate those farmers or their advisors. Adoption of the FECRT by cattle owners was very low and we used FECRT information from the literature and from research trials kindly provided by several colleagues. Therefore, our effort was focused on the quality and confidence of AR diagnosis to have a reliable farm sample; thus, we obtained a reliable but limited small sample size. An additional limitation is the field method used for AR diagnosis. FECRT is widely adopted because all family of drugs can be evaluated, no sophisticated laboratory equipment is required and its cost is relatively low. However, it is well known that this test has low sensitivity for incipient AR in a nematode population; FECRT is only capable of detecting AR at an advanced stage, when at least 25% of the nematode population has the resistant phenotype (MARTIN et al., 1989), but in practical terms this is the test that we can use under field conditions. Currently, there are more sensitive genetic tests, but only to evaluate BZD resistance and it is too expensive to be used in routine field diagnosis (HUMBERT et al., 2001).

Even though the logistic regression method overestimates odds ratios in studies with small to moderate sample size (NEMES et al., 2009), these present results support the frequency of treatment hypothesis and the frequency of use the same drug in the past on the development of AR (PRICHARD et al., 1980). The use of more than 75% of AVM in the previous survey years mostly implies the use of the same type of drug and is directly correlated with AVM resistance. Likewise, the use of only AVM during the last years refers also to the lack of annual rotation of drug classes.

Previous studies in the Pampas region demonstrate that late spring and summer conditions were associated with rapid development and migration of third-stage larvae (L_3) to herbage, but also with high mortality rate and low L_3 detection after initial recoveries (SUAREZ; LORENZO, 2000). In addition, larva availability for tracer calves on naturally infected pastures during this period was generally very small (SUAREZ, 1990; SUAREZ; LORENZO, 2000). Then, under the refugia hypotheses Nov-Jan period can be considered the time when more resistant selection pressure is exerted on the nematode populations in the Pampas region.

In New Zealand, an analysis of sheep AR through a field survey indicated refugia as an important risk factor (HUGHES et al. 2007). Their results showed that the prevalence of AR was higher on farms on which more than 50% of lambs were weaned onto paddocks not grazed for a long period by lambing ewes and where lambs were not always returned to the same paddock after drenching. Other surveys conducted with small ruminants showed that underdosing, repeated use of one class of drug and refugia size at treatment time can be associated with AR (SILVESTRE et al., 2002).

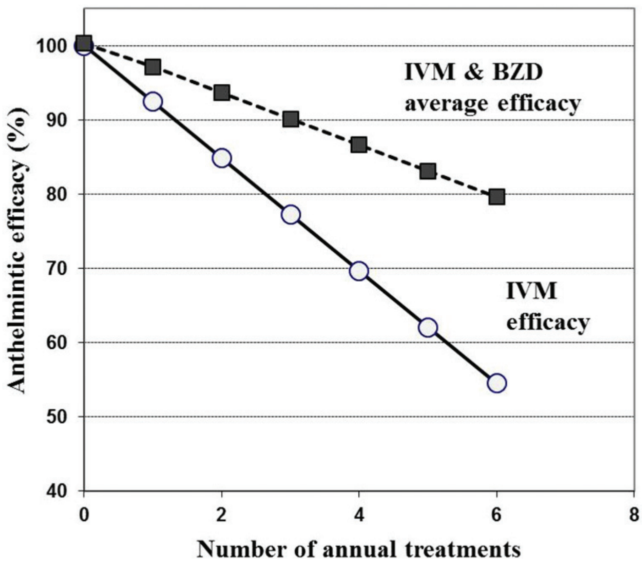

Figure 2. Correlation between number of drug annual treatments and the percentage of ivermectin (IVM) efficacy (y= 100.5+-7.59x; p<0,031) and the average of ivermectin and benzimidazole (BZD) efficacy (y= 101.7+-3.51x ; p<0,0004).

Our results also indicate that the number of autumn-winter drenches alone contributed to the development of AR (X^2 7.83; p<0006). The average number of autumn-winter herd drenches given in the last 3-5 years was 2.91 and the odds ratio of AR of herds exposed to more than three treatments was 24 times higher than not exposed herds (Table 2). This contribution of autumn-winter treatments to the development of AR is not so clear, because this period is the most favourable for the survival of infective larvae in pastures (SUAREZ; LORENZO, 2000; SUAREZ, 2001) and minimum resistant selection could be exerted. However, most of these grazing systems use annual crop pastures (oats and ryegrass) during the winter and when animals are first introduced to these pastures, larva availability for grazing animals is negligible (SUAREZ, 1990). A strategy involving treatment and then moving stock to clean crops without refugia may be a suitable method for AR selection. Similarly, selection can be produced with another herd management strategy: owners during the fattening period standardized the live weight of acquired or weaning calves by drenching and move to feedlots; then after a short period, the animals are moved again to pastures. Treatment and move to feedlot yards with negligible nematode presence should also select for AR.

These results reinforce the hypothesis of increased frequency of anthelmintic application as the principal risk factor; however, it is difficult to separate this hypothesis from that of refugia, because it is associated with the factor that autumn-winter drenching is frequently used along with Nov-Jan drenching and the approach involving treatment before move to annual crops. The latter two factors reduce the number of larvae in refugia.

In Argentina, as in other countries (STAFFORD; COLES, 1999; SOUTELLO et al., 2007; SUAREZ et al., 2011), there is evidence that cattle owners might be using more anthelmintics than required to prevent bovine meat and milk losses. This overuse of anthelmintics, especially injectable and persistent drugs (mainly avermectins) seems to decrease larvae in refugia, because when drug profiles decline over time there will be a period when resistant

Table 4. Multivariable logistic-regression model of all drug anthelmintic resistance status.

Variable	Values of variable	*b*	S.E. (*b*)	Odds Ratio	Wald 95% UL	Wald 95% LL	Chi square	*P*
Number of annual treatments		2.04	0.66	7.68	2.12	27.7	9.67	0.002
Use more than 75% of avermectin in the past	- Yes - No	2.92	1.34	18.6	1.35	256.3	4.78	0.028
Number of treatments during Nov-Jan.		0.93	1.17	2.53	0.26	8.79	0.63	0.42
Refugia Index		–0.77	1.94	2.15	0.05	97.1	0.15	0.69
Type of anthelmintic control plan	- Strategically programmed - Programmed followed by tactic* drenches	0.27	0.97	1.32	0.20	8.79	0.08	0.77
Constant		–9.17	2.80	0.0001	0.0000	0.03	10.7	0.001

*Tactic drenches according to symptoms, body condition, epg or LWG. BC: body condition; epg: eggs per gram; LWG live weight gain.

Table 5. Multivariable logistic-regression model of avermectin resistance status.

Variable	Values of variable	*b*	S.E. (*b*)	Odds Ratio	Wald 95%		Chi square	*P*
					LL	UL		
Number of annual treatments with avermectins		2.44	0.70	11.5	2.9	45.5	12.08	0.0005
Number of annual treatments with avermectins in Nov-Jan		1.76	1.07	5.83	1.71	47.8	2.70	0.10
Use more than 75% of avermectins in the past	-Yes -No	–1.49	1.15	4.45	0.46	1.67	1.67	0.19
Constant		–5.47	1.61	0.004	0.000	0.09	11.55	0.0007

larvae are able to establish but susceptible genotypes are not. As resistance builds up in a population, this mechanism will become evident as a reduction in the period of protection against ingested larvae, as previously observed in sheep (SUTHERLAND et al., 1997) and cattle (V.H. Suarez, unpublished data). Coles et al. (2006) proposed that an AVM reduced period of protection of 50% could be considered a possible case of AR.

Another outcome factor that was associated with AR, but also strongly related to the number of annual drenches, was the type of anthelmintic control plan. One type was the strategic programmed nematode control, whereby owners programmed drug application at a fixed date regardless of the epidemiological knowledge that focused on the probable larval challenge or worm burden size. The other type was a control plan that generally involves a programmed treatment (at weaning and/or then a short period later) followed by tactic drenches based on different herd criteria (symptoms, body condition score, live weight gain or egg counts) and sometimes based on the epidemiological studies in our region (FIEL et al., 1994; SUAREZ et al., 1999). This latter type of control reduced the number of drenches and was generally associated with the veterinarian advice, which used the egg counts and live weight gains of the herd to make drench decisions. The latter factor shows that veterinary advice could be profitable in reducing the number of treatments to those that the herd truly needs, therefore reducing the risk of AR development.

Likewise, some management strategies that employ refugia-based methods to prolong the efficacy of current cattle anthelmintics should be suggested to veterinarians and cattle owners. Nematode control methods, such as targeted timed whole herd drenches or targeted selective treatments (KENYON et al., 2009) based, for example, on production data (herd live weight gain or body condition score) serve to reduce the number of drenches, minimising pasture contamination with resistant parasites and favouring dilution with susceptible genotypes.

In conclusion, these results show that treatment frequency, the period of the year for treatment and the past treatment frequency with a single drug were the main risk factors involved in AR development and mostly support the frequency of treatments hypothesis. These results also show a need for reducing the number of treatments by applying regional epidemiological knowledge, refugia criteria and control advice and herd monitoring by professionals.

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