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Short communication

Frequency of cattle farms with ivermectin resistant gastrointestinal nematodes in Veracruz, Mexico

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A B S T R A C T

This study reports the percentage of cattle farms with ivermectin (IVM) resistant gastrointestinal nematodes (GINs) in Veracruz, Mexico, and identifies the GIN genera involved in the resistances. It also describes management practices of anthelmintic (AH) use on the surveyed farms. Twenty-one farms were assessed by means of the faecal egg count reduction test using the McMaster technique. Only two farms had GIN populations susceptible to IVM (9.5%). The proportion of farms with IVM resistant GIN was 71.4% (15/21). Seven of these farms had less than 80% egg count reductions. *Haemonchus* and *Cooperia* were the genera most commonly found in the resistant populations, followed by *Oesophagostomum*. Inappropriate AH treatment practices were identified from the completed questionnaires. Further management practices such as selective treatment and quarantine treatments are proposed to further reduce the spread of IVM resistance between farms.

1. Introduction

Ivermectin (IVM) and other macrocyclic lactones (ML) are the most common anthelmintic (AH) products used to control gastrointestinal nematodes (GIN) in cattle worldwide (Wolstenholme et al., 2004). However, the intensive and frequent use of this anthelmintic group has resulted in the development of IVM resistance in several hosts (McKellar and Jackson, 2004). Reports of ML resistance in GIN populations of cattle have emerged in several parts of the world. It is well known that the rapid development of GIN populations resistant to broad-spectrum AH drugs is an additional threat affecting the efficacy and sustainability of these important medications as parasite control tools (Leathwick et al., 2012). Thus, it is important for all countries to monitor the development and spread of resistant worm populations on different farms within a region or country.

In Mexico, the situation of ML resistance in GIN populations on cattle farms has been scarcely studied. The first report of resistance on cattle farms was generated recently for IVM in the hot humid tropics of Mexico (Encalada-Mena et al., 2008). Although the latter described the situation at only five farms where 100% frequency of IVM resistance was found, that survey stimulated the interest of other groups in Mexico to study the incidence of IVM resistance in other regions. A second report by Canul-Ku et al. (2012) in a sub humid hot tropical region in Mexico (Yucatán) also found a high frequency of IVM resistant GIN populations on cattle farms (11/14; 78.6%). These studies suggested the need to further investigate the situation of IVM resistance in other more humid tropical zones of Mexico such as Veracruz, the state of Mexico with the highest number of cattle (2,454,171 representing 10.5% of the national inventory) (INEGI, 2010). Cattle farmers in this area depend on grazing (Castillo-Gallegos et al., 2005) and, consequently, GINs represent an important threat to animal health in the region.

The objective of the present study was to estimate the percentage of cattle farms with GIN resistant to IVM in Veracruz, Mexico, including the identification of the genera involved and, to identify anthelmintic practices used on the surveyed farms.

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2. Materials and methods

2.1. Study area and cattle farms selected

This study was carried out from January 2012 to April 2013 in five municipalities (Martinez de la Torre, Misantla, Nautla, Tlapacoyan and Vega de Alatorre) in the state of Veracruz, Mexico (24°4′N, 97°03′W). The climate is humid tropical with an average annual temperature of 23.4 ± 0.5 °C, annual rainfall of 1991 ± 392 mm and relative humidity of 85 ± 6%. Thirty-two cattle farms, where grazing is practiced, were included in the survey. However, only 21 farms were completely evaluated for AH resistance. Selection and sampling were made by convenience, i.e., not randomly selected.

At each farm, grazing calves 3–8 months old and 50–150 kg live weight were used. The inclusion criteria for farm selection were as follows: (i) herds with a population of calves (3–8 months of age), (ii) calves not dewormed during the last 60 days prior to the study, and (iii) herds with calves excreting greater or equal to 150 eggs per gram (EPG) of faeces (individual calf values not herd mean value) of the strongylida order. From a total of 1283 calves sampled from 32 herds, only 503 calves from 21 herds complied with the selection criteria. The major factor that limited the number of herds evaluated was the lack of animals with a faecal egg count (FEC) greater or equal to 150 EPG (even after visiting each farm on three or four occasions).

2.2. Experimental design to evaluate resistance

To diagnose the presence of GIN populations resistant to IVM, the Faecal Egg Count Reduction Test (FECRT), recommended by the World Association for the Advancement of Veterinary Parasitology was used (Coles et al., 1992). On day 0, within each farm, a sample of faeces was collected directly from the rectum of each calf (using a labeled plastic bag) to calculate the FEC. Faeces were transported in a plastic cooler (4–5 °C) to the Animal Health Laboratory for analysis. Faecal egg counts were determined using the modified McMaster technique with a sensitivity of 50 EPG (Rodríguez-Vivas and Cob-Galera, 2005). Coprocultures were made to identify the genus of GIN infective (L_3) larvae present in faeces. Then, on day 1, calves were distributed according to their parasite loads (balanced) into two experimental groups: a control group having 10–15 calves maintained without treatment and a treated group with 10 to 15 calves treated with IVM (Endectin®, Novartis, México; Reg. Sagarpa No. Q-0715-038) (intramuscular injection using a dose of 0.2 mg kg^{-1} live weight). Prior to treatment, animals were weighed individually using the same mobile weighing scale (TRU-TEST MP600®) so as to administer the correct dose of IVM, thereby avoiding variability among doses used for treatments. Fourteen days after treatment, another faecal sample was obtained from each calf to calculate the FEC as mentioned previously. A new coproculture per group was performed to identify the genus of GINs (L_3 stage) involved.

2.3. Questionnaire

A questionnaire was applied to every farm owner/manager to obtain information related to herd size, cattle breeds, production objective (dual purpose, milk or breeding stock), the family of AH currently used (ML, benzimidazole or imidazothiazole), frequency of AH treatments per year, correct dosage or not (e.g., animal weight measured or not), deworming new animals on arrival at farm and whether or not rotations of AH were used.

2.4. Statistical analysis

For each cattle farm, AH resistance was calculated following Coles et al. (1992) and RESO© software (CSIRO, 1990, Animal Health Division) by means of the formula:

$$\text{Percentage reduction} = \left(\frac{1-T}{C}\right) \times 100\%$$

where T is the FEC arithmetic mean of the treated group and C is the FEC arithmetic mean of the control group after treatment (at day 14).

The confidence interval (95%) was calculated by means of the RESO software. A cattle farm was considered to have resistant parasites when the FEC reduction percentage was <95% and the lower limit of the confidence interval was <90%. A population was considered suspect when the cattle farm complied with one of the two mentioned criteria and it was considered susceptible when none of the criteria was fulfilled.

A univariate analysis was performed using 2 × 2 contingency tables of exposure variables in Epi Info 3.5.3 (Center for Disease Control and Prevention, 2008) to estimate the association level with the response variables (susceptibility, low resistance or resistance to AHs). Exposure variables with P-values ≤0.20 were included in a logistic-binomial regression model (multivariate analysis) using Statgraphics 15.2.06 (StatPoint Inc., 2007). A P-value ≤0.05 was considered significant.

3. Results

3.1. Cattle farms with gastrointestinal nematodes resistant to ivermectin

Only two cattle farms had GIN populations susceptible to IVM (Table 1). The percentage of cattle farms with IVM resistant GIN was 71.4% (15/21). The percentage of FEC reduction on resistant farms varied from 0% to 93% (Table 1). Seven resistant farms had less than 80% of FEC reduction. The remaining 19% of cattle farms (4/11) were identified as suspect of IVM resistance.

3.2. Identified genera

On day 0, GIN infections on the cattle farms were evaluated and involved the following genera: *Haemonchus, Cooperia, Ostertagia* and *Oesophagostomum*, with the order of resistance to IVM being *Haemonchus*, *Cooperia* and *Oesophagostomum* (Table 2).

3.3. Questionnaire survey

The information related with anthelmintic practices used on the farms is shown in Table 3. In this study, 71.42% (15/21) of the cattle farms corresponded to dual-purpose system (milk and meat production). The most widely used family of AH was ML (85.71%; 18/21), followed by benzimidazole (47.61%; 10/21) and imidazothiazole, (28.57%; 6/21). All studied farms have used IVM for the control of GIN. Most producers do not weigh the animals before applying AH treatments (85.71%; 18/21). Also, most farmers mentioned they rotate between families of AH (90.5%; 19/21) and dewormed new animals on arrival (85.71%, 16/21). In this study, no factors were found to be associated with the presence of GIN populations resistant to IVM (P > 0.05).

4. Discussion

This study determined the percentage of cattle farms with GINs resistant to IVM. It showed a high prevalence of cattle farms with IVM resistant GIN. This is consistent with previous reports of ML

Table 1
Percentage of egg reduction and status of resistance to ivermectin of gastrointestinal nematodes in cattle farms from Veracruz, Mexico.

Herds	Pre-treatment				Post-treatment				% Red. EPG	C.I. 95%	Status
	Control		Treated		Control		Treated				
	N	FEC/mean	N	FEC/mean	N	FEC/mean	N	FEC/mean			
1	10	695	10	667	10	325	10	317	3	0–65	*R*
2	13	1100	15	979	10	683	14	496	27	0–80	*R*
3	12	317	13	327	12	258	13	27	90	45–98	*R*
4	11	364	11	409	11	232	11	41	82	0–98	*R*
5	14	696	15	657	14	671	14	221	67	30–84	*R*
6	13	953	15	853	13	454	15	420	75	40–89	*R*
7	15	400	13	331	15	200	13	46	89	63–97	*R*
8	10	520	14	750	10	150	14	86	95	83–99	LR
9	10	320	10	315	10	205	10	20	46	0–87	*R*
10	11	314	11	405	11	164	11	0	100	–	*S*
11	12	908	12	979	12	17	12	38	93	59–99	*R*
12	12	338	12	367	12	12	10	17	85	54–95	*R*
13	10	240	10	315	10	645	10	890	89	22–98	*R*
14	11	164	12	200	11	205	12	42	96	84–99	LR
15	13	412	13	588	13	275	13	51	0	0–88	*R*
16	12	321	11	300	12	146	11	25	90	58–98	*R*
17	12	238	12	267	12	217	12	0	100	–	*S*
18	13	412	13	588	13	265	13	31	95	87–98	LR
19	11	264	12	271	11	273	12	142	96	83–99	LR
20	10	335	10	335	10	625	10	50	77	0–96	*R*
21	12	367	12	517	12	304	12	17	85	39–96	*R*

N – number of animals in experimental groups; FEC – faecal egg count (mean); % Red. EPG – percentage reduction in eggs per gram; C.I – confidence interval; R – resistant; LR – low resistance or suspect; S – susceptible.

Table 2
Genera of gastrointestinal nematodes of cattle recovered post-treatment from coprocultures of treated groups with ivermectin and control groups.

Farm	Percentage of GIN genera in control groups					Percentage of GIN genera in the treated groups					Status
	Haem	Oster	Coop	Stron	Oeso	Haem	Oster	Coop	Stron	Oeso	
1	0	0	64	0	36	5	0	95	0	0	R
2	54	0	10	0	36	0	0	84	0	16	R
3	88	0	12	0	0	34	0	66	0	0	R
4	16	0	70	14	0	0	0	89	5	6	R
5	24	0	34	40	2	50	0	40	4	6	R
6	60	0	34	3	3	45	0	39	3	0	R
7	73	0	10	2	15	60	0	23	2	9	R
8	86	0	7	1	6	46	0	32	1	12	LR
9	76	0	5	2	8	65	0	2	8	7	R
10	67	0	20	12	1	38	0	0	6	0	S
11	91	0	0	1	8	74	0	2	9	15	R
12	72	0	21	1	6	84	0	10	3	3	R
13	61	0	32	0	7	68	0	0	13	20	R
14	79	0	12	0	6	23	0	0	0	4	LR
15	58	0	29	0	13	65	0	25	2	5	R
16	77	0	18	2	3	56	0	18	0	4	R
17	67	0	31	0	2	57	0	1	1	0	S
18	72	0	25	1	2	68	0	15	4	0	LR
19	77	0	15	1	6	78	0	3	5	13	LR
20	67	0	26	4	3	85	0	7	3	3	R
21	75	0	16	7	2	93	0	0	5	2	R

Haem – *Haemonchus* spp.; Oeso – *Oesophagustomum* spp.; Coop – *Cooperia* spp.; Oster – *Ostertagia* spp.; Stron – *Strongyloides* spp. R – resistant; LR – low resistance or suspect of IVM resistance; S – Susceptible.

resistance in cattle nematodes from several parts of the world. The findings are also consistent with previous studies in Mexico where frequencies of IVM resistance on cattle farms vary from 78.9% (Canul-Ku et al., 2012) to 100% (Encalada-Mena et al., 2008; Alegría-López et al., 2015). The high prevalence reported in different zones of Mexico could be due to the common use of ivermectin, which is the preferred anthelmintic drug of cattle farmers in Mexico (Pérez-Cogollo et al., 2010). In the present study, most farmers used IVM to control nematodes two or three times per year. However, they eventually used the same AH to control other parasites such as ticks and/or flies. Thus, the frequent use of IVM seemed to increase the possibility of generating IVM resistant parasite populations. Additional information of potential contributing factors to resistance development such as selection pressure from AHs, sources of refugia from unselected GIN on the farm, environmental factors affecting the hosts or parasites, as well as differences in management practices, might explain the differences of resistance between studies in Mexico. However, it is possible that the frequency of IVM resistant GIN populations could be close to 80% of farms in hot tropical zones of Mexico. This information should be disseminated to farmers in Mexico as part of a campaign to reduce the speed of development of IVM resistance in the country.

In this study, the genus most frequently identified as IVM resistant or suspect of IVM resistance was *Haemonchus* followed by *Cooperia*. The high biotic potential of *Haemonchus* strains (Coyne et al., 1991) compared to other GIN genera could be related to

Table 3
Farm **c**haracteristics, status of resistance and management of anthelmintics on cattle farms in Veracruz, Mexico.

	History and management of anthelmintics									Status
Cattle farm	Zootechnical purpose	Production system	Herd Size	Correct dose (0.2 mg kg^{-1})	Frequency of treatments per year	Weight measured for correct dose?	Rotation of anthelmintics	Dewormed new animals?	Cattle have contact with other cattle farms?	
1	Dual purpose	Extensive	≥100	No	3	No	Yes	No	Yes	R
2	Dual purpose	Extensive	≥100	No	3	No	No	No	Yes	R
3	Dual purpose	Extensive	≥100	No	1	No	Yes	No	Yes	R
4	Dual purpose	Extensive	≥100	Yes	4	No	Yes	No	No	R
5	Milk	Extensive	<99	No	2	No	No	No	No	R
6	Breeding stock	Extensive	≥100	No	6	No	Yes	Yes	Yes	R
7	Breeding stock	Extensive	≥100	Yes	2	No	Yes	Yes	Yes	R
8	Breeding stock	Extensive	≥100	No	4	No	Yes	Yes	No	LR
9	Dual purpose	Extensive	≥100	No	4	No	Yes	Yes	Yes	R
10	Dual purpose	Extensive	≥100	No	2	No	Yes	Yes	Yes	S
11	Dual purpose	Extensive	<99	No	4	No	Yes	Yes	Yes	R
12	Dual purpose	Extensive	≥100	No	3	No	Yes	Yes	Yes	R
13	Dual purpose	Extensive	≥100	No	2	No	Yes	Yes	No	R
14	Dual purpose	Extensive	≥100	No	4	No	Yes	Yes	Yes	LR
15	Breeding stock	Extensive		Yes	1	No	Yes	Yes	Yes	R
16	Dual purpose	Extensive	≥100	No	2	No	Yes	Yes	Yes	R
17	Dual purpose	Extensive	≥100	No	2	No	Yes	Yes	Yes	S
18	Dual purpose	Extensive	≥100	No	3	No	Yes	Yes	Yes	LR
19	Breeding stock	Extensive	≥100	No	2	No	Yes	Yes	Yes	LR
20	Dual purpose	Extensive	≥100	No	3	No	Yes	Yes	No	R
21	Dual purpose	Extensive	≥100	No	2	No	Yes	Yes	Yes	R

R – Resistant; LR – Low Resistance or suspect of IVM resistance; S – susceptible.

the higher frequency of AH resistant isolates. The second genus found resistant to IVM was *Cooperia* in more than 80% of farms with GIN resistance. Our results were similar to those obtained by Cotter et al. (2015), Demeler et al. (2009) and Suárez and Cristel (2007), who reported tests with *Cooperia oncophora* resistant to IVM. It is known that *Cooperia* is the dose-limiting nematode genus for MLs in cattle (Sutherland and Leathwick, 2011). *Cooperia* has been reported as one of the most prevalent parasites on tropical cattle farms in Mexico (von Son de Fernex et al., unpublished data). Finally, in this study *Oesophagostomum* IVM resistant populations were also found.

The findings on the IVM use, as well as the general AH drug use on Veracruz cattle farms, need to be discussed in the light of the implications towards the correct form of use for the MLs in tropical regions of Mexico, i.e., Veracruz. It is evident that the current management practices are leading inexorably towards a more serious IVM resistance problem on cattle farms in Veracruz. With the present study there can be at least four practical AH management suggestions:

(a) Selective treatment of young animals. Previous AH resistance surveys performed with cattle in Mexico showed the great difficulty that exists when trying to find cattle with more than 150 EPG year round (Canul-Ku et al., 2012). In the present study, such difficulty was present even in very young grazing calves. Those animals with low FEC do not need AH treatment. Thus, farmers might be able to keep considerable refugia of susceptible parasites by treating only the few animals that have poor growth. Alternatively, farmers could learn how to obtain a faecal sample and use a simple flotation technique to identify only those animals that have a substantial FEC. The research groups in Mexico, and other tropical areas, still have to answer an important question: What is the FEC that should trigger treatment to reduce the negative impact of GIN in cattle, either young or old.

(b) Treating animals with the right dose. This survey showed that farmers are not weighing animals prior to AH use. For most farmers working with cattle in Mexico, the main problem to comply with weighing is the lack of a weighing scale and/or a chute to contain animals that will be treated. Thus, farmers rely a lot on the visual assessment of weight. The latter is true also for small ruminant farmers in Mexico (Torres-Acosta et al., 2012). This problem can be solved in two ways: Either the farmers invest in a scale and then keep using it, or farmers could use girth measuring tape to determine an estimated weight.

(c) Using quarantine treatments. This methodology could be very useful on farms, such as those included in the present survey, that exchange animals frequently. When farmers exchange animals from one farm to another, they also transfer their parasites, both internal and external. The latter means that farmers need to limit the introduction of parasites from any exchange. One alternative is to give a quarantine treatment. This means treating the incoming animals with a combination of AH drugs of all different families (MLs, benzimidazoles, imidazothiazole). Each animal must be treated with the correct dose and route of administration. After treatment, animals are kept inside a quarantine pen, preferably with concrete floor, for at least 3 days. If the animals are negative to GIN eggs after the three-day quarantine, they can enter the ranch premises. If animals are still positive, veterinarians and farmers ought to decide if the number of incoming animals represents a danger for the existing GIN population on the ranch. In this study, 85.7% of producers dewormed new animals with broad spectrum AH. However, animals were immediately introduced to pastures. Thus part of the quarantine treatment management is not performed correctly at present. This practice should be accompanied with more information such AH resistance diagnosis in the farm of origin and the level of GIN infection of incoming animals.

(d) Using a combination of anthelmintics. This strategy is based on the combined use of different AH classes with similar spectrum of activity and different mechanism of action against resistant GIN (Leathwick, 2013; Bartram et al., 2012; Leathwick et al., 2012). Modeling studies demonstrated that administration of multiple AH actives can have benefits in delaying the development of AH resistance (Leathwick et al., 2015). A field study using a combination AHs increased "refugia" of unselected genotypes slowing the development of AH resistance (Leathwick et al., 2012). The latter might occur by improving drug efficacy against nematode resistant, even if resistance to one o more products of the constituent actives is present (Sutherland, 2015; Bartram et al., 2012). Even more, a recent study performed in sheep farms showed evidence of reversion towards AH susceptibility in GIN in response to "resistance management program" where a combination of AH was included (Leathwick, 2013).

The findings on the percentage of cattle farms with GINs resistant to IVM, and the AH drug use on Veracruz cattle farms, helped to identify different scenarios (depending of the characteristic of each farm) where there is the possibility of implementing "resistance management programmes" where a combination of AH products might be included. However, additional work needs to identify strategies to minimize the identified AH resistance risk practices leading to maintain a refuge of unselected parasites. As stated by Sutherland (2015) "the challenge for researches and advisors is now to improve adoption of properly designed and implemented resistance management programmes". Further studies should be designed to evaluate strategies aiming to minimize the problem of AH resistance in tropical cattle farms.

5. Conclusions

A high proportion of cattle farms surveyed in Veracruz, Mexico, were classified as having IVM resistant worm populations (71.4%), while only two farms had GIN populations susceptible to IVM (9.5%). The worm genera involved in the IVM resistance in Veracruz were *Haemonchus* and *Cooperia*, followed by *Oesophagostomum*. Inappropriate AH treatment practices were recorded on cattle farms.

Conflict of interest

The authors of this manuscript have no financial or personal relationships with other people or organizations that could inappropriately influence or bias the content of the paper.

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