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Challenges of nematode control in ruminants: Focus on Latin America

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ABSTRACT

Gastrointestinal nematodes (GIN) of ruminants (cattle, sheep and goats) are ubiquitous and can cause severe injuries to infected animals and significant losses in farming revenues. GIN are able to survive severe environmental and host conditions, but mankind has developed a number of ingenious methods for parasite control. The commerce and use of modern anthelmintic drugs with a broad spectrum of activity has been a solid tool for nearly 40 years, however the continuous use of these drugs, has led to the selection of populations of drug-resistant worms worldwide. At present, the ever-growing agricultural systems in Latin America are facing many challenges and cannot rely on the far-reaching objective of parasitic elimination from the host or the environment. The lack of extensive programs for monitoring drug resistance exacerbates the negative consequences of reduced efficacy, which is evident in some areas with the increase in mortality rate even after treatment. Experts agree that new schemes of parasitic control are needed and should be based on the strategy of targeted selective treatment where affected hosts are identified and treated accordingly. In this article, we will focus our discussion on the challenges for the control of GIN in Latin America by 2020 imposed by reduced drug efficacy. We will evaluate phenotypic and molecular markers, methods for single-animal evaluation, and the implementation of schemes for anthelmintic treatment that address parasites in refugia.

1. Introduction

Latin America has one of the world largest meat and milk production herds, occupying 25% of the world's livestock productive land area of 80 millions km^2 (Thornton, 2002). Farming systems range from highly technical to rural and tribal herds providing a significant source of income and wealth. The same amplitude occurs in sanitary management practices with an undesirably high death rate due to parasitic infections and poor nutritional status. Farm animals (cattle, sheep and goats) suffer considerably from heavy infections of gastrointestinal nematodes (GIN), which are more evident in young animals prior to the development of some level of immunity. The abomasal nematode *Haemonchus* sp. is pathogenic during the migration of the L_4 larvae to the pits on the walls of the gastric glands and during feeding by the adult worms. Due to its extensive pathogenicity, *Haemonchus* is one of the most important parasites of ruminants (Kassai, 1999). Similar importance may be attributed to *Cooperia* sp., *Ostertagia* sp., *Trichostrongylus* sp., *Teladorsagia* and *Oesophagostomum*, but these GINs

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cause lesions in other sections of the gastrointestinal tract.

Affected animals are often first noticed when they lag behind other animals, have a staggering gait, breathe faster, and appear weak, symptoms that may ultimately end in death. Chronic infections lead to extreme weight loss, lethargy, pallid mucosae/anemia (in the case of *Haemonchus* infection), and the presence of submandibular and ventral abdominal edema. Infection is seldom caused by a single species of parasite; so clinical parasitism is often the result of infection with multiple species of GIN.

Clinical signs and history of infection are often insufficient for a specific diagnosis of GIN infections and field Veterinarians are reluctant on use laboratory exams. Patent infections can be determined by simple flotation techniques, which may even provide a quantitative estimate of worm burden (e.g. the faecal egg count - FEC, using a McMaster technique). Although FEC assays are popular, a low FEC may be encountered in heavy pre-patent parasitic infections when the bulk of the pathogenic worms are in the larval stage (Eysker and Ploeger, 2000). An important disadvantage of the FEC is the limited correlation between values of eggs per gram (EPG) and the impact on production from GIN infections. Levels of parasite-specific antibodies and serum pepsinogen may also be used to diagnose specific infection in cattle (Charlier et al., 2010).

Parasite-related problems are recurrent in ruminants, and density-dependent phenomena play a major role in how the host–parasite relationship must be seen and managed. Therefore, most of the topics that we include in this review consider these phenomena. Our objective is to discuss the scientific literature, recognizing that articles with similar content are available (Sutherland and Leathwick, 2010). This review is also a complement to Torres-Acosta et al. (in press) summarizing the advances and opinions in Latin America of current efforts (decisions of production/clinical threshold treatment, diagnostic methods, molecular markers, and herbal medicine) and the challenges that face us for the next decade.

2. The rise and fall of modern anthelmintics (AH)

Efficient GIN control was only possible after the introduction of the broad-spectrum compounds, and well-organized livestock producers clearly received enormous advantages from their regular use once these compounds were introduced in Latin America. Although AH provided cattle farmers with the means of controlling worm infestations, the problem of anthelmintic resistance (AR) was recognized as a serious obstacle, threatening the sustainability of ruminant production (Nari and Hansen, 1999; Anziani et al., 2004).

The development of AR was a clear consequence of imposing a strong selection pressure on the parasitic population. Coles (2002) suggested that the apparent lack of AR in cattle, due to large parasitic populations in refugia and less-frequent AH treatments, was only temporary. The situation today is very complex, where intensive ruminant production is unsustainable in many beef/milk-producing countries due to the loss of efficacy of nearly all the AH drugs (Mello et al., 2006; Suarez and Cristel, 2007; Demeler et al., 2009; Gasbarre et al., 2009; Almeida et al., 2010a; Perri et al., in press).

3. Detection of AR in GIN of ruminants

The general consensus is that early detection of AR is an important factor for sustainable chemical control and although AR is widespread in most Latin American countries, insufficient preventive programs were set in place to prevent the expansion of AR. The challenges though are to detect subtle changes in drug efficacies to prevent AR, to determine which AH class should be used in quarantine animals and programs of drug rotation, and in extreme cases, the removal of the compound from the farm's management program. One clear example is ivermectin, which can be found in more than 65 different brands, ranging greatly in quality and price in Brazil. In most Latin American countries there are few restrictions on the purchase of medicines, and farmers are able to buy them with no veterinary input (Molento, 2007). Therefore ivermectin has often disappointing treatment results due to the easy access of large quantities of antiparasiticides by resource-poor farmers. It has also been shown that once a farmer chooses an anthelmintic, perhaps based on price, this can reduce the efficacy of all other medicines from the same family, accelerating side-resistance (Molento et al., 2004a).

Although popular and inexpensive, the faecal egg count reduction test (FECRT) has low sensitivity and repeatability for the detection of AR due to its high variation between hosts (Miller et al., 2006). Even though the density-dependent effect may be suppressed after treatment, low faecal egg output may complicate interpretations when comparing post-treatment egg counts, to pre-treatment EPG. These basic technical features, plus the very low number of faecal exams that are routinely performed in Latin American laboratories, makes FECRT mostly applied only for research purposes.

3.1. In vitro tests: a promising AR diagnostic tool?

The phenotypic characterization of AR can be performed using *in vitro* tests, which in some cases may be more rapid, economical, and practical than FECRT. *In vitro* tests are widely used for basic research in Latin America (Borges et al., 2007; Macedo et al., 2009; Mencho et al., 2009; Almeida et al., 2010b; Hernández-Villegas et al., 2011). However, and as pointed out by von Samson-Himmelstjerna et al. (2009a), considerable effort must still be devoted to standardizing the techniques with different isolates from laboratories in different countries to achieve similar results and to make the tests suitable for routine use.

The egg hatch test (EHT) is recommended to detect benzimidazole resistance due to the benzimidazole action on eggs, and the good repeatability and reproducibility of the test (von Samson-Himmelstjerna et al., 2009a). Meanwhile, the larval development test (LDT) and the larval feeding inhibition test (LFIT) tests were developed to detect resistance to ivermectin and levamisole (Hubert and Kerboeuf, 1992). The necessity to have reliable *in vitro* tests for the macrocyclic lactones (ML) has stimulated the development of assays based on parasitic motility (D'Assonville et al., 1996; Molento and Prichard, 2001; Kotze et al., 2006). Demeler et al. (2010) performed a ring test to evaluate a LMIT for detection of ivermectin resistance and observed high reproducibility among different laboratories in Europe. The encouraging results may suggest that LMIT could be considered as a diagnostic test for ivermectin resistance in the near future.

The Brazil-Argentina Council of Biotechnology (CBAB) has been financing a hands-on two-week course for the last two years in Curitiba, Brazil, where researchers from Brazil, Argentina, Uruguay, Paraguay, and Colombia were trained in basic *in vitro* and molecular techniques for the diagnosis of drug resistance (M. Molento, personal communication). Efforts such as this are improving regional technical expertise and accelerating the spread of *in vitro* tests as a routine practice as well as the use of PCR-based protocols and bioinformatics.

3.2. Molecular targets for resistance: will they be available by 2020?

Molecular analysis of GIN, such as PCR-based diagnostics, sequence comparisons, and pyrosequencing, are expected to provide precise quantification of alleles associated with AR (Beech et al., 2010; Cudeková et al., 2010). Although until recently, three codons containing single nucleotide polymorphisms (SNPs) were assigned and could be used to detect resistance to benzimidazoles, von Samson-Himmelstjerna et al. (2009b) evaluated 11 laboratory isolates of H. contortus and reported five new allelic variations possibly associated with resistance to the same product. These authors evaluated 11 laboratory isolates of *H. contortus* and reported five new allelic variations possibly associated with resistance to the same product. Resistance genotype diagnosis is progressively being implemented in Latin America and Gromboni et al. (2009) reported polymorphic differences from single larvae of *H. contortus* determining alleles containing A, T and AT using ARMS-PCR from sheep in Brazil. The Leu256Phe mutation in the GluClα3 gene of *C. oncophora* (Njue et al., 2004) is the only SNP considered to be a marker for ivermectin resistance and it is also the focus of a new initiative that is on going to determine the frequency of GluClα3 SNPs in cattle, sheep and goat parasites in Brazil (S.M. Niciura, personal communication).

As we are seeing in most of the research groups worldwide, including in Argentina, Brazil and Mexico, the arrival of genome sequencing techniques will quickly improve our knowledge on parasite biology and physiology. This could also have a significant impact on drug resistance diagnosis leading to promptly changes in parasite control strategies.

3.3. Can we rely on targeted selective treatment (TST)?

"To deworm or not deworm"? Corwin and Stromberg (1995) asked that question almost two decades ago, and although the technology of animal production has improved, the answer is still unknown for most farmers. From our experience, TST is not adequately practiced in the sheep and goat industry in Latin America (Torres-Acosta et al., in press), perhaps because the concept of TST is very challenging for the traditional farmer and to some of the struggling local animal industries. The amount of information behind the concept and overcoming the old and fixed drug-base recommendations contribute to the lack of acceptance of TST. Cabaret et al. (2009) reported that the concept of treating animals using TST was not seen as feasible by conventional farmers, but organic farmers were more in favor of using phenotypic markers for the control of GIN in France. The issue of implementing TST involves knowing how to apply it, the extent of its application, and the degree of its flexibility. Therefore, efforts must be made to educate producers through technical publications and practical training courses.

Selecting individual animals for treatment and not treating a large part of the flock is the basis for the concept of refugia. Mathematical models and field trials have shown that TST can slow the development of resistance (Leathwick et al., 2008; Gaba et al., 2010). Individual treatment of cattle has been considered from reports of resistance in *Cooperia* sp., *Haemonchus* sp., *Ostertagia* sp., *Trichostrongylus* sp., and *Dictyocaulus* sp. in many regions, including Brazil and Argentina. Below we will discuss some TST techniques that are being applied when evaluating single-animal rather than changing farm management.

3.4. Drenching animals based on faecal egg counts

Animals with higher faecal nematode egg excretion normally harbor the largest worm burdens. However, the FEC as a marker for TST has not received sufficient attention. A study testing the utility of FEC in New Zealand (Leathwick et al., 2006) treated animals with EPG counts of 500 or higher and compared the results with those from conventional treatment. The strategy achieved a marginal reduction in frequency of AH use that had no effect on productivity. Kenyon et al. (2009) suggested that the main drawback of the use of FEC is that farmers cannot determine FECs without the aid of a laboratory, which is quite costly if one must regularly sample a fraction of the flock. This observation is in agreement with field observations in Latin America even though a small number of farmers have a modest laboratory on their property to perform FECs.

3.5. The FAMACHA© guide

The FAMACHA© method was developed in South Africa as an aid in identifying anemic animals that may harbor severe infections of *H. contortus*. The sensitivity and specificity of identifying anemia with this tool has been explored in sheep and goats (Van Wyk and Bath, 2002; Moors and Gauly, 2009; Reynecke et al., 2011a). The use of FAMACHA© can significantly reduce the frequency of AH treatment and can have a positive impact on production revenues (Mahieu et al., 2007; Molento et al., 2004b, 2009). The use of FAMACHA© and FEC for parasitic control was surveyed by an online interview (www.farmpoint.com.br) with farmers from 13 states in Brazil; where 55% reported using FAMACHA©, and 26% used FEC. These figures may represent approximately 20% of the Brazilian herd (35 million animals) clearly indicating that 10 years after its validation the application of FAMACHA© in Brazil is influencing sanitary management, reducing drug use, improving farm revenue, and aiding the selection of animals for resilience (M. Molento, personal communication). The disadvantage of this technique is the possible presence of other parasites causing anemia or of nonparasitic causes of anemia. These difficulties have been studied with the aim of improving the system focusing on training and intervals for examinations (Reynecke et al., 2011b).

We consider the FAMACHA© system a very powerful technique when applying TST to reduce parasite chemical exposure. A number of universities and research units offer practical courses covering high-tech farms to rural and tribal herds in Latin America and this will only be strengthen in the near future.

3.6. Body condition score (BCS)

This tool is suggested as a marker for helminth infections of non-hematophagous worms (i.e. *Trichostrongylus colubriformis* or *Teladorsagia circumcincta*). A monthly record of BCS can identify animals with mixed parasitic infections that cause signs of decreased feed conversion, dehydration, and/or anorexia. Honhold et al. (1993) found a low correlation between BCS and FEC when used to determine the need for treatment. Van Wyk et al. (2006) reported encouraging data regarding the phenotypic correlation between BCS, hematocrit values, and FEC from a farm infested with *T. colubriformis*. The main advantage of BCS is its practicality and low cost. The main disadvantage is again a lack of specificity for parasitic infections.

3.7. Live weight change (LWC)

Although not used in practice in Latin America, live weight is a good phenotypic marker of resilience in young ruminants. Animals should be weighed on a regularly basis, and an AH treatment is only applied to those lambs that fail to reach a certain threshold of LWC (Kenyon et al., 2009). The main advantage of this evaluation is its practicality, which can be enhanced if farmers use automated systems (Stafford et al., 2009). This marker has been suggested for those geographical regions where *H. contortus* and other hematophagus worms are not abundant. The disadvantage of this TST protocol is its lack of specificity, because a poor LWC in a growing lamb may not represent the effects of its worm burden.

4. The arrival of new technologies

4.1. Improving nutrition to reduce dependence on AH drugs

In 1945, Fraser suggested that sheep could take care of their own parasites if the farmers took care of their sheep. Today, a solid body of scientific evidence supports this argument, especially from the field of nutrition–parasite interactions and their effects on GIN infections (Coop and Kyriazakis, 2001; Athanasiadou et al., 2008; Hoste et al., 2008a). Farmers in Latin American countries experience good pasture availability during spring, summer, and most of autumn but usually do not provide animals with the appropriate amount of supplements during the dry winter months (June, July, and August) when significant losses in bodyweight can occur. Farmers also do not see immediate advantages of providing supplemental feed and prefer to drench their animals even when parasitic challenge is negligible.

Establishing the economic viability of any nutritional manipulation for the control of GIN and reduction of dependence on AH drugs is therefore important. Torres-Acosta et al. (2004) and Martínez-Ortíz-de-Montellano et al. (2007) have demonstrated the economic feasibility of supplementary feeding for enhancing resilience to GIN in goats in Mexico. With the participation of agronomists and nutritionists, practitioners must ultimately find the quantity of supplement required to achieve optimal balance between productivity and sanitation in a given farming system.

4.2. Screening for plant-extracts

Due to the enormous variety of plants and to the interest in obtaining new active compounds, research in phytotherapy for the control of GIN of sheep and goat has been extensive in the last decade, and many potential crude extracts or solutions have been developed in Latin America. Recent reviews have also reported a boost in the AH efficacy of tannin-rich bioactive materials under *in vitro* and *in vivo* conditions (Hoste et al., 2006, 2008b; Alonso-Diaz et al., 2010).

In Brazil, the extracts of *Eucalyptus citriodora*, *E. globulus*, and *E. staigeriana* at 5% (v/v) caused 100% inhibition in the EHT in gastrointestinal nematodes of goats (Chagas, 2004). An ethanol extract from the seeds of *Melia azedarach* was most active on eggs of *H. contortus*, with 100% reduction, and the ethanol extract from leaves showed the best inhibition in the LDT, with 91.6% reduction in larval development (Maciel et al., 2006). Oliveira et al. (2009) determined the efficacy of an extract of *Cocos nucifera* against GIN of sheep. The efficacies in the EHT and LDT at 5 mg/mL and 80 mg/mL were 100% and 99.8%, respectively. Hernández-Villegas et al. (2011) evaluated the efficacies of leaf extracts from *Phytolacca icosandra* against infective L_3 larvae and eggs from *H. contortus* collected from sheep in Mexico. The ethanol extract from *P. icosandra* showed 55.4% inhibition of migration at 2 mg/mL in LMIT assays and 72.6% inhibition of hatching at 0.15 mg/mL in EHA.

4.3. Challenges for the use of active plant extracts

Once the *in vitro* and *in vivo* results are robust and can be replicated, the next steps would be the development of new throughput methodologies for analysis of the many potential chemicals in the extracts, the purification of fractions, and the isolation of candidate compounds, even using the *Caenorhabditis elegans* model (L. Katiki, personal communication), for plant extract screening.

We would then need to determine the direct or indirect mechanisms of action of the compounds on the parasites and the possible effects on the hosts. Problems with solubility and absorption by the gastrointestinal tract are the main obstacles to developing herbal formulations with good bioavailability and AH efficacy. Many multidisciplinary teams are being formed in Latin America joining universities, research institutes, and drug companies from around the world to develop new antiparasitic drugs (Chagas, 2008; Woods and Knauer, 2010). Herbal medicine is in great demand as a promising alternative to synthetics for parasitic control (Burke et al., 2009; Molento, 2009), and as Athanasiadou et al. (2007) reported, products derived from plant extracts are a mixture of bioactive substances and are therefore expected to provide a weaker selection pressure on the parasitic population compared to commercial AHs.

4.4. *Hopes and dreams behind vaccine technology*

Vaccination would prevent rather than cure infections and represents a more sustainable and environmentally friendly form of parasitic control compared to drugs. The Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Organization for Economic Co-Operation and Development (OECD) regard vaccines among the most cost-effective methods for promoting both human and animal health. With the exception of the vaccine against *Dictyocaulus viviparus*, however, no vaccines are currently available commercially for the control of helminth infections in ruminants. More than 40 years of research have resulted in the identification of several antigens or mixtures of antigens that confer high levels of protection against many species of helminths (Knox et al., 2001), but few of these experimental vaccines have proceeded to or successfully passed the next phase of development, which is the large-scale, cost-effective production of the vaccine (Geldhof et al., 2007; Redmond and Knox, 2006).

A group from Brazil and Argentina are investigating the molecular variability of two regions of the H11 gene from *H. contortus* to verify whether the presence of nucleotide polymorphisms in the complete gene could result in post-translational modifications. Thirty-three nucleotide substitutions were found on one fragment (29 transitions and four transversions), and 17 substitutions were found on another (16 transitions and one transversion). Inside the exonic regions, the SNPs identified resulted in 10 amino acid substitutions (two synonymous, seven non-synonymous, one stop codon). This is the first analysis of the molecular variability of the H11 gene and the results provide a measure of the minimum nucleotide mutational rate in Brazilian isolates (Pondelek et al., 2011). Vaccines against parasites are unlikely to become available for the control of worms on farms in the next decade, but a vaccine for the control of *Haemonchus* spp. in sheep and cattle may be an exception as demonstrated recently (Bassetto et al., 2010). The authors' vaccinated dairy calves three times with a gut membrane from *H. contortus* immunogen and then challenged the animals with *H. contortus* and *H. placei*. The vaccinated animals showed a significant 100% reduction of egg counts for *H. contortus* and 68.4% reduction in worm count for *H. placei*.

4.5. *Would online advice do the job?*

Farmers and practitioners welcome new information, and the internet is reasonably available on farms in most developed countries. This is definitely not the case for most developing countries. Changes in management have not been evident until recently, and although the industry has become aware of the losses incurred from inappropriate dosing times, implementing even a simple new strategy is still very difficult (Torres-Acosta et al., in press).

A few software-based initiatives (WORMBOSS, Decision Tree, Receiver Operating Characteristic – ROC, ParaCalc) offer creative and flexible models that incorporate large amounts of information on host–parasite relationships, climatic conditions, and management practices (Ploeger et al., 2008; Reynecke et al., 2011c, J. Charlier, personal communication). A major concern, however, is that these tools may be too complex for the average practitioner to use. Farmers and veterinary practitioners thus need an easy-to-use system that can integrate parasitic control into the daily management routine. An important feature of the Decision Tree system is that inputs of a farm's past, present, and future grazing management can predict future parasitic control (AWI/CRC, 2009; Ploeger et al., 2008). Descriptions of WORMBOSS and the Decision Tree can be found at http://wormboss.com.au and http://www.parasietenwizer.nl (click on the English icon for translation).

5. Concluding remarks

Vercruysse and Claerebout (2001) defined a number of threshold conditions for choosing to treat cattle with an AH drug (i.e. therapeutic, production-based, and preventive thresholds) for a wide variety of situations, accounting for host category, farm system, parasitic fauna, and environment. Their objective was to define concepts having a wide range of technical alternatives for deciding whether to treat an animal. Although these indications were appropriate and were based on scientific literature, we are still trying to adopt them, considering the difficulties that are listed above. We think that once the deficiencies of chemical control are sufficiently recognized the efforts in the application of improved TST strategies will get adequate attention. The interests of both the pharmaceutical and the fast-growing livestock industries would be best served by addressing these issues in Latin America.

EPG exams and FECRT will still make a strong impact worldwide where *in vitro* and molecular tests are expected to be important tools for AR surveys before 2020. Further studies are necessary to make them more practicable for routine diagnostics. New validated tests and surveys for resistance are required, and the extent of AR in bovine nematodes must be better understood. We must face the difficulties of parasitic control from now until 2020 and embrace several internet-based tools of control of GIN that can integrate local climate, host response, farm management, labor costs, and the impact on the environment. The aims of these efforts are to promote a sustainable livestock production for a growing human population and to increase the welfare of our animals.

Conflict of interest statement

The authors have no financial or personal relationship with individuals or organizations that could inappropriately influence or bias the paper entitled "Challenges of nematode control in ruminants: Focus on Latin America".

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