Journal of Agricultural Research, Development, Extension, and Technology, 3(1), 55-59 (2021)
Full Paper

Anthelmintic resistance of gastro-intestinal nematodes to albendazole, levamisole and ivermectin in Murrah buffaloes

Khan L. Junatas and Elizabeth C. Molina

College of Veterinary Medicine, University of Southern Mindanao, Kabacan, Cotabato

Received: March 6, 2021
Revised: July 5, 2021
Accepted: August 8, 2021

Abstract

Resistance in buffaloes to anthelmintic treatment has already been reported. However, there is paucity of information about anthelmintic resistance in Murrah buffaloes in the Philippines. In the present study, 36 Murrah buffaloes (24 aged >1 to 2 years and 12 aged >2 to 3 years) naturally-infected with gastrointestinal nematodes were observed to determine the efficacy of albendazole (ABZ), levamisole (LEV) and ivermectin (IVM). The efficacy of the drugs was determined through the fecal egg count reduction test (FECRT). The presence of anthelmintic resistance was confirmed if the fecal egg count reduction (FECR) is lower than 95% and if the lower limit of the 95% confidence interval is lower than 90%. Resistance to the anthelmintics albendazole and levamisole was exhibited by gastro-intestinal nematodes infecting Murrah buffaloes in this study. On the other hand, Ivermectin still showed efficacy against gastro-intestinal nematodes but only in buffaloes less than two years old. Resistance to ivermectin was already demonstrated in buffaloes >2 to 3 years of age. The observed resistance in albendazole and levamisole may be explained by the regular use of these two drugs in the farm's parasite control program. The study suggests that better strategies may be considered by the farm for a more sustainable control against helminthiasis.

Keywords - albendazole, anthelmintic resistance, buffaloes, ivermectin, levamisole

Introduction

Gastro-intestinal parasites affect livestock including buffaloes, and infection could result in significant losses to production. Losses may be in the form of reduced growth, poor reproductive performance, reduced milk production, and overall lower production which may cause significant economic losses to the producer. Anthelmintic treatment is one of the major strategies in the control of these parasites. However, over the years control has become increasingly difficult because of the development of resistance to anthelmintic drugs. Presently, there are no reports on anthelmintic resistance of gastro-intestinal nematodes infecting buffaloes in the Philippines. Further, there is a lack of information on the types of anthelmintics that are still effective against these parasites in buffaloes.

Anthelmintic resistance is the inherited reduction in the susceptibility of the nematode population toward the drug action (Geerts & Gryseels, 2000). This means that there is a genetically transmitted loss of sensitivity (Köhler, 2001) thereby decreasing the number of parasites being affected which were previously sensitive to the same drug. This phenomenon is well-documented in cattle, goats, sheep, pig, and horses in a number of countries (Coles et al., 2006; Gerwert et al., 2002; Love & Coles, 2002; Van Zeveren, 2007). However, limited information on anthelmintic resistance in buffaloes is available. In the Philippines, only few studies give emphasis to the potential significance of anthelmintic resistance. The only reported observations in the country were benzimidazole resistance in goats and sheep (Ancheta et al., 2004) and resistance to albendazole (ABZ), triclabendazole and bromofenofos of *Fasciola* sp. in water buffaloes (Venturina et al., 2015). Therefore, there is a need to obtain more data to establish whether this phenomenon exists in nematodes affecting buffaloes as this will provide a basis for planning a sustainable program for control against helminthiasis in these animals which are important commodities in the country's economy. Judicious use of drugs that are still effective against

Corresponding author email: Elizabeth C. Molina, emolina@usm.edu.ph

gastro-intestinal nematodes in these animals would preserve their efficacy. These could then be used in combination with other strategies for a more effective and sustainable control of gastro-intestinal nematodes.

Methodology

Ninety-four naturally-infected Murrah buffaloes, regardless of sex, at the Philippine Carabao Center at the University of Southern Mindanao, Kabacan, North Cotabato, Philippines were preselected and screened for eggs of gastro-intestinal nematodes, which are morphologically distinct from other gastro-intestinal parasites. The pre-set criterion for inclusion in this study was a fecal egg count of 100 (per gram of feces) or more. From the 94 animals, 36 were selected based on this criterion. These 36 animals were apparently healthy, aged >1 to 3 years old, and had mild infection (fecal egg counts ranging from 120-690) based on the categories provided by Hansen and Perry (1994). Female buffaloes that were included in this study were not pregnant. The animals were divided into two groups based on age (24 buffaloes aged >1 to 2 years and 12 buffaloes aged >2 to 3 years). Each group was subdivided into four sub-groups, with nine buffaloes per drug treatment (6 aged >1 to 2 years, and 3 aged >2 to 3 years). Sub-groups 1, 2 and 3 were treated with albendazole (ABZ) (10 $mg{\cdot}kg^{1}$ PO; Valbazen®, Pfizer), levamisole (LEV) (8 $mg{\cdot}kg^{-1}$ PO;Latigo 500®, Univet), and ivermectin (IVM), (0.2 $mg{\cdot}kg^{-1}$ SC; Ivomec®, Merial), respectively. Sub-group 4 remained untreated. Approximately five grams of fecal samples were collected from each buffalo intra-rectal by insertion of the hand with gloves moistened with water on days 7, 14, and 21 post-treatment. The fecal samples were placed in covered plastic cups that were appropriately labeled. They were then transported to the laboratory and promptly examined. Egg counts in individual samples were obtained using the Modified McMaster method (detailed by Coles et al., 1992) with a sensitivity of 30 eggs per gram (Roberts & Sullivan, 1950) to obtain data on egg counts. The Meander system was observed in counting the eggs under low power magnification (100X) of a microscope. The data reported in this paper are based on the average egg counts within each sub-group.

The efficacy of the anthelmintics was based on the post-treatment percentage fecal egg count reduction (% FECR) determined as the reduction of egg excretion at 21 days using the following formula adopted from Besvir et al. (1986) where the fecal egg count of each treatment group was compared to that of the control group:

$$\%\ FECR = \frac{\text{Average no. of eggs in control group} - \text{Average no. of eggs in treated group}}{\text{Average no. of eggs in control group}} \times 100\%$$

To confirm the presence of anthelmintic resistance, the data were analyzed using the RESO FECRTv4 software program (AusVet Animal Health) to determine the upper and lower confidence interval limits (95%) as recommended by Coles et al. (1992). Presence of anthelmintic resistance was confirmed if the fecal egg count reduction (FECR) is lower than 95% and if the lower limit of the 95% confidence interval is lower than 90%. If only one of these criteria is met, there is only suspect resistance (Coles et al., 1992).

All procedures performed in the study were in accordance with the ethical standards of the Animal Care and Use Committee (IACUC) of the University of Southern Mindanao.

Results and Discussion

The efficacy of ABZ, LEV, and IVM against common gastro-intestinal nematode infection after one-dose treatment is shown in Table 1. In both age groups, the post-treatment egg counts were lower than the pre-treatment egg counts. Although the three drugs exhibited quite high efficacy in buffaloes aged >1 year to 2 years, there was an indication of resistance of the parasites to both albendazole and levamisole in this age group, while there was an indication of low resistance was noted for ivermectin. A lower efficacy was observed for the three drugs in buffaloes aged >2 years to 3 years suggesting resistance of the parasites to the anthelmintic compounds in this age group of animals. These results indicate that gastro-intestinal nematodes infecting Murrah buffaloes in the Philippines are already resistant to commonly used anthelmintics. A similar scenario had been widely reported with different species of nematodes that infect cattle, sheep, and goat (Anziani et al., 2004; Borgsteede et al., 2007; Soutello et al., 2007). The regular use of these drugs as part of the control measures against helminth parasitism in the farm may have contributed to the observed resistance

Table 1. Efficacy of different anthelmintics (at Day 21) post-treatment against gastro-intestinal nematode infection in Murrah buffaloes with one-dose treatment.

Age	Drug	Dose ($mg \cdot kg^{-1}$)	n	Fecal egg count (eggs per gram)		% Fecal egg count reduction	Status**
				Pre-treatment	Post-treatment		
>1 year to 2 years	ABZ	10	6	175	20	90 (97; 70)	Resistant
	LEV	8	6	220	20	90 (97; 70)	Resistant
	IVM	0.2	6	250	10	95 (99; 59)	Low
	Control		6	170	205	0	
>2 years to 3 years	ABZ	10	3	270	180	–29 (70; 0)	Resistant
	LEV	8	3	200	75	46 (94; 0)	Resistant
	IVM	0.2	3	180	20	86 (95; 57)	Resistant
	Control		3	300	140	0	

* Upper confidence interval limit (95%) and lower confidence interval limit (95%) are in parentheses.
** Status of helminths (whether resistant or susceptible to the drug)
ABZ = albendazole; LEV = levamisole; IVM = ivermectin

to albendazole and levamisole. Farm records revealed that since its establishment in 1995, the regimen against helminthiasis has employed these two drugs. Each year, buffaloes in the farm are treated every six months when they are 6 months or older while calves are drenched once a month until they reach 3 months of age. On the other hand, ivermectin still shows efficacy in buffaloes >1 to 2 years of age in this study as only low resistance was noted in this sub-group. This drug is occasionally used considering its cost and availability.

Drugs are powerful selectors for resistant alleles; frequent treatments with the same drugs allow for continued selection of these resistant worms and increase their frequency thereby causing the rapid appearance of resistant nematodes (Soutello et al., 2007; Wolstenholme et al., 2004). In controlled trials, Barton (1983) and Martin et al. (1989) have proven that high treatment frequency induced resistance more strongly than less frequent dosing regimens. Considering the short generation time and high fecundity of the common helminths infecting livestock, it is possible for these parasites to develop resistance to anthelmintics as a result of frequent exposure to the same drugs or to the same class of anthelmintics. Over time, these parasites could achieve a significant level of evolution producing generations of worms that exhibit a certain degree of resistance to anthelmintics. This decrease in susceptibility to anthelmintics is caused by a change in the gene frequency of susceptible worm population resulting in drug selection (Sangster, 1999). Hence, worms will remain alive after tolerating drug medication. This capability is expressed in the increase of resistant alleles (Fleming et al., 2006), passage of increased number of parasite eggs, higher survival rate of adult worms in hosts, and high number of larval load in the pasture after treatment (Vercruysee, 2005).

In the present study, the regular use of albendazole and levamisole as part of the control measures against parasites could have contributed to the observed anthelmintic resistance to these two drugs, while macrocyclic lactone ivermectin, which was occasionally used, was still active against nematodes in buffaloes >1 to 2 years old. Prior studies have also shown the relationship of drug frequency to anthelmintic resistance. For example, Ancheta et al. (2004) reported that anthelmintic resistance in goats in the Philippines was associated with the frequency and duration that benzimidazole drenches have been used. In Pakistan, Jabbar et al. (2008) showed that the frequent use of oxfendazole (which, together with albendazole, belong to the same class of anthelmintic—benzimidazole) resulted in low efficacy and the development of anthelmintic resistance. Soutello et al. (2007) also reported resistance to macrocyclic lactone moxidectin due to the high frequency of usage. Similarly, resistance to moxidectin was reported by Condi et al. (2009) in Brazil. Interestingly, it was also reported by Geerts et al. (1987) that resistance in cattle still developed with long-term use of the drug, even if annual treatment frequency with levamisole was low.

The result of the present study provided basis for the farm to consider better strategies that would result in a more sustainable control against helminthiasis. Since resistance to albendazole and levamisole is already present, the farm may consider ceasing their use as part of their parasite control program. Ivermectin could still be used especially for animals under 2 years of age.

Other strategies in parasite control may also be considered. For example, Shalaby (2013) identified two strategies to overcome anthelmintic resistance: the selective treatment approach and the combination drug approach. The selective treatment approach is done by giving medication only to animals that require treatment. Shalaby argued that this approach is the key point in controlling and delaying the development of resistance. By contrast, the combination drug strategy is done by using two anthelmintics from different chemical classes. This approach can also delay anthelmintic resistance due to the synergistic effect of these two drugs. Hence, for the farm to maximize the benefits of combination drug strategy, it should determine which drugs of different classes cause no or low resistance and use these drugs in combination in their control program. Shalaby also mentioned alternate methods of parasite control such as pasture management and improved nutrition. These are other strategies that the farm can employ, in combination with the measures stated above, to have a more effective and sustainable parasite control program.

Conclusion

Resistance to the anthelmintics albendazole and levamisole is already exhibited by gastro-intestinal nematodes infecting Murrah buffaloes in this study. Ivermectin is still shows efficacy, but only in buffaloes less than 2 years old; resistance to the drug was already demonstrated in buffaloes >2 to 3 years of age.

Disclosure Statement

No potential conflict of interest was declared by the authors.

References

Ancheta, P.B., Dumilon, R.A., Venturina, V.M., Cerbito, W.A., Dobson, R.J., LeJambre, F.L., Villare, E.C. & Gray, G.D. (2004). Efficacy of benzimidazole anthelmintics in goats and sheep in the Philippines using larval development assay. *Veterinary Parasitology*, *120*, 107-121.

Anziani, O.S., Suarez, V., Guglielmo, A.A., Warnke, O., Grande, H. & Coles, G.C. (2004). Resistance to benzimidazole and macrocyclic lactone anthelmintics in cattle nematodes in Argentina. *Veterinary Parasitology*, *122*, 303-306.

Barton, N.J. (1983). Development of anthelmintic resistance in nematodes from sheep in Australia subjected to different treatment frequencies. *International Journal for Parasitology*, *13*, 125-132.

Besvir, J., Rapic, D., Dzakula, N., Blagovic, S. & Pompe-Gotal, J. (1986). Fasinex (triclabendazole)-new fasciolicide. *Praxis Vet*, *34*, 239-242.

Borgsteede, F.H.M., Dercksen, D.D. & Huijbers, R. (2007). Doramectin and albendazole resistance in sheep in The Netherlands. *Veterinary Parasitology*, *144*, 180-183.

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

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., Von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A. & Vercruysse, J. (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, *136*, 167-185.

Condi, G.K., Soutello, R.G.V. & Amarante, A.F.T. (2009). Moxidectin-resistant nematodes in cattle in Brazil. *Veterinary Parasitology*, *161*, 183-287.

Fleming, S.A., Craig, T., Kaplan, R.M., Miller, J.E., Navarre, C. & Rings, M. (2006). Anthelmintic resistance of gastro-intestinal parasites in small ruminants. *Journal of Veterinary Internal Medicine*, *20*, 435-444.

Geerts, S., Brande, J. Kumar, V. & Biesermans, L. (1987). Suspected resistance of Ostertagia ostertagi in cattle to levamisole. *Veterinary Parasitology*, *23*, 77-82.

Geerts, S. & Gryseels, B. (2000). Drug resistance in human helminths: current situation and lessons from livestock. *Clinical Microbiology Reviews*, *13*, 207-222.

Gerwert, S., Failing, K. & Bauer, C. (2002). Prevalence of levamisole and benzimidazole resistance in *Oesophagostomum* populations of pig-breeding farms in North Rhine-Westphalia, Germany. *Parasitology Research*, *88*, 63-68.

Hansen, J. & Perry, B. (1994). *The epidemiology, diagnosis and control of helminth parasites of ruminants*. Nairobi, Kenya: International Laboratory for Research on Animal Diseases.

Jabbar, A., Iqbal, Z., Saddiqi, A.H., Babar, W. & Saeed, M. (2008). Prevalence of multiple anthelmintic resistant gastro-intestinal nematodes in dairy goats in a desolated tract (Pakistan). *Parasitology Research*, *103*, 29-35.

Köhler, P. (2001). The biochemical basis of anthelmintic action and resistance. *International Journal of Parasitology*, *31*, 336-345.

Love, S.J.C. & Coles, G.C. (2002). Anthelmintic resistance in sheep worms in New South Wales, Australia. *Veterinary Record*, *150*, 87.

Martin, P.J., Anderson, N. & Jarret, R.G. (1989). Detecting benzimidazole resistance with faecal egg count reduction tests and in vitro assays. *Australia Veterinary Journal*, *66*, 236-240.

Roberts, F.H.S. & O'Sullivan, P.J. (1950). Methods for egg counts and larval cultures for strongyles infesting the gastro-intestinal tract of cattle. *Australian Journal of Agricultural Research*, *1*(1), 99-102.

Shalaby, H.A. (2013). Anthelmintics resistance; how to overcome it? *Iranian Journal of Parasitology*, *8*, 18-32.

Soutello, R.G.V., Seno, M.C.Z. & Amarante, A.F.T. (2007). Anthelmintic resistance in cattle nematodes in northwestern São Paulo State, Brazil. *Veterinary Parasitology*, *148*, 360-364.

Van Zeveren, A.M., Casaert, S., Alvinerie, M., Geldhof, P., Claerebout, E. & Vercruysee, M. (2005). Anthelmintics. In C.M. Kahn (Ed.). *The Merck veterinary manual* (pp. 2117-2122). Whitehouse Station, NJ: Merck and Co.

Venturina, V.M., Alejandro, M.A., Baltazar, C.P., Abes N.S. & Mingala, C.N. (2015). Evidence of *Fasciola* spp. resistance to albendazole, triclabendazole and bromofenofos in water buffaloes (*Bubalus bubalis*). *Annals of Parasitology*, *61*(4), 283-9.

Vercruysee, J. (2007). Experimental selection for ivermectin resistance in *Ostertagia ostertagi* in cattle. *Veterinary Parasitology*, *150*, 104-110.

Wolstenholme, A.J., Fairweather, I., Prichard, R., Von Samson-Himmelstjerna, G. & Sangster, N.G. (2004). Drug resistance in veterinary helminths. *Trends in Parasitology*, *20*, 469-476.