Risk factors associated with occurrence of anthelmintic resistance in sheep of resource poor farmers in Limpopo province, South Africa

Morutse Mphahlele[1], Ana M. Tsotetsi-Khambule[2], Rebone Moerane[3] and Majela L. Mashiloane[4], Oriel M.M. Thekisoe[1],

[1]Unit for Environmental Sciences and Management, North-West University, Potchefstroom, 2531, South Africa

[2]Epidemmiology, Parasites and Vectors Programme, ARC-Onderstepoort Veterinary Research, Onderstepoort, 0110, South Africa

[3]Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110, South Africa

[4]Livestock Research Unit, Mara Research station, Louis Trichardt, 0920, South Africa

Corresponding author: Morutse Mphahlele

Unit for Environmental Sciences and Management, North-West University, South Africa

Email: morutse.broilerchicks@gmail.com

Abstract

Anthelmintic treatment is the most common way of controlling nematode infections in ruminants even though several countries have reported anthelmintic resistance (AR), resulting in limitation for sustainable small ruminant production. The aim of the present study was to evaluate the knowledge of resource poor sheep farmers in Limpopo province on the use of anthelmintics. A questionnaire survey regarding helminthosis control practices was administered to farmers of small ruminant flocks in five districts of Limpopo province, South Africa, namely Capricorn, Sekhukhune, Waterberg, Vhembe and Mopani. A total of 77 resource poor farmers who owned 40 - 100 sheep were interviewed between June and August of 2017 using a structured questionnaire with a combination of qualitative and quantitative, open-ended questions. None of the farmers weighed their sheep before dosing them instead visual appraisal of individual weight was the most common means of estimating the anthelmintic dose. Farmers that owned sheep for more than 10 years were better informed than their inexperienced counterparts in terms of identifying symptoms of gastrointestinal parasites infection and knowledge of infection mode ($p<0.05$) although no significant difference was found to exist between the three groups of farmers with regard to the usage and correct application of anthelmintics ($p>0.05$). Majority of the respondents (57%) were aware of gastrointestinal nematodes (GI) that infect sheep although only 34% knew how animals become infected. The results from the present study also revealed that 57% of the respondents can identify the clinical symptoms of gastrointestinal nematodes infection. Although 67.5% of farmers mentioned that they never dose their sheep, 32.5% use anthelmintics at varying times in a year. The above information is an indication of risks associated with occurrence of anthelmintic resistance in the study areas. *In vivo* and *in vitro* AR studies should be conducted in flocks with high treatment frequencies to establish the degree of resistance if there's any using faecal egg count reduction tests. Furthermore, there is a need to give training to resource-poor farmers of small stock on proper application of anthelmintic treatment and to educate them on how to prevent development of AR.

Key words: Gastrointestinal nematodes; anthelmintic resistance; under dosing; treatment frequency, resource poor farmers, Limpopo province.

Introduction

Gastrointestinal nematodes of small ruminants are distributed worldwide and are particularly important parasites of ruminants in all regions across the tropical and sub-tropical countries (Martinez-Valladares 2013). These infections cause low productivity due to stunted growth, poor weight gain, feed utilization, feeding and water intake, lower meat, wool and milk production, cost of treatment and mortality in young animals (Paddock 2010). Anthelmintic treatment is the most common way of controlling nematode infections in ruminants even though several countries have reported anthelmintic resistance (AR), representing a limitation for sustainable small ruminant production (Domke et al. 2011).

Anthelmintic resistance occurs when parasites, usually eliminated by a given dose, suddenly survive the treatment and since resistance is inherited, the surviving worms will pass their resistance alleles to their progeny (Sangster 1999). Resistance against drugs belonging to the same anthelmintic drug class is called side-resistance, whereas cross- and multidrug-resistance refers to resistance against two or multiple drugs belonging to different anthelmintic drug classes (Torres-Acosta et al. 2012). AR development can be limited by ensuring that the nematode parasites are exposed to an effective anthelmintic drug concentration and to consider the timing and frequency of anthelmintic drug treatments so that only a small proportion of the population is exposed to the anthelmintic (Sargison 2016).

The AR is a problem in countries such as Australia, New Zealand, South Africa, and many Latin American countries (Dolinská et al. 2012; Torres-Acosta et al. 2012). In Europe, anthelmintic resistance has been reported in the Slovak Republic (Čerñanská et al. 2006), Spain (Alvarez-Sanchez et al. 2001), Italy (Traversa et al. 2007), Greece (Papadopoulos et al. 2001), the United Kingdom (Bartley et al. 2006; Taylor et al. 2009), and the Netherlands (Borgsteede et al. 2007).

The most commonly used anthelmintic classes are: macrocyclic lactones, benzimidazoles and imidazothiazoles (Cezar et al. 2010). Many reports of AR are cases of benzimidazoles or levamisole, but the number of cases of resistance to the macrocyclic lactone, namely, ivermectin is increasing (Papadopoulos 2008). Reports of resistance to doramectin and moxidectin are less common (Papadopoulos et al. 2012).

In South Africa, farmers control nematode infections in their livestock primarily by means of anthelmintic drugs, namely ivermectin, albendazole and levamisole (Tsotetsi et al. 2013). Ninety percent of South African sheep farms harbour nematode strains resistant to at least one class of anthelmintics and 40% of South African farms have been found to house strains of nematodes resistant to three or more classes of anthelmintics (Van Wyk et al. 1997).

Van Wyk (1999) reported that the AR of *H. contortus* in South Africa is possibly the highest that has so far been recorded in the world with emerging strains that may soon be impossible to treat with any of the existing anthelmintics.

Although farmers are doing their best to combat nematode infections, the severity of anthelmintic resistance has led to a decrease in the efficacy of anthelmintics (Tsotetsi et al. 2013). In South Africa AR have been reported in both commercial and resource-poor farming sectors, with the commercial sector being described as being the worst in the world (Vatta & Lindberg 2006).

Table 1: Anthelmintic Resistance evaluated by the Fecal Egg Count Reduction Test (FECRT) in small ruminants in South Africa

Area and period resistance reported	Drug	Host animal	Source
Limpopo province, Tompi Seleka	Benzimidazole and Rafoxanide	Sheep	Van Wyk et al. 1999
Northwest Province, Mafikeng	Rafoxanide, Levamisole and Fenbendazole	Goats	Bakunzi 2003
Mpumalanga, Emerlo	Benzimidazole, Levamisole, Ivermectin and Rafoxanide	Sheep	Van Wyk et al. 1999
Kwazulu Natal	Benzimidazole, Levamisole, Ivermectin and Rafoxanide	Sheep	Van Wyk et al. 1999
Gauteng Province,	Albendazole, Levamisole and Ivermectin	Goats	Tsotetsi et al. 2013
Hammanskraal	Levamisole and Ivermectin	Sheep	Tsotetsi et al. 2013
Gauteng province, Nigel	Albendazole, Levamisole and Ivermectin	Goats	Tsotetsi et al. 2013
	Albendazole and Ivermectin	Sheep	Tsotetsi et al. 2013

The risk of under dosing and a continued use of one class of anthelmintics, irrespective of efficacy status are frequently encountered factors enhancing development of anthelmintic resistances (Čerñanská et al. 2008; Aga et al. 2013). In an Ethiopian study, a questionnaire survey to assess community's current knowledge revealed that majority of the respondents had poor or no information on economic importance of gastrointestinal nematode and anthelmintic drugs utilization practices (Belina et al. 2017). Furthermore, two studies in Norway and Slovakia revealed that in the majority of the small holder sheep flocks, determining the anthelmintic dose by visual appraisal of the weight of the heaviest lamb or ewe before the start of the drenching operation was a common practice and insufficient weight estimations for calculating the correct anthelmintic dose may lead to development of AR (Domke et al. 2011; Kupčinskas et al. 2016).

The aim of the present study was to evaluate the resource poor farmer's knowledge on anthelmintic use in Limpopo province, South Africa.

Materials and Methods

Study area description

This study focused on Limpopo Province located in the north of South Africa (Figure 1A & B). It is one of the developing provinces in South Africa and is particularly vulnerable to climate change impacts, due to its exposure to extreme weather events (Tennant & Hewitson 2002; Cook et al. 2004). The province has three distinct climatic regions: the Lowveld region which is characterized by a semi-arid climate(s), the Middle- and Highveld that is considered semi-arid, and Escarpment that experiences sub-humid climate (Limpopo Department of Agriculture 2008).

The province experiences long sunny days and dry weather conditions on most days. During the summer months, warm days are often interrupted by a short-lived thunderstorm (Limpopo Department of Agriculture 2008). It can get very hot in October to March, with average temperatures rising to 27ºC in summer and 20ºC in winter. The bulk of the precipitation occurs in summer, and annual rainfall totals range from about 400 - 600 mm over most of the province (Anon 2007). Limpopo is a province having a high number of rural dwellers dependent on natural resources and farming is of considerable importance (Reason et al. 2005; Thomas et al 2005).

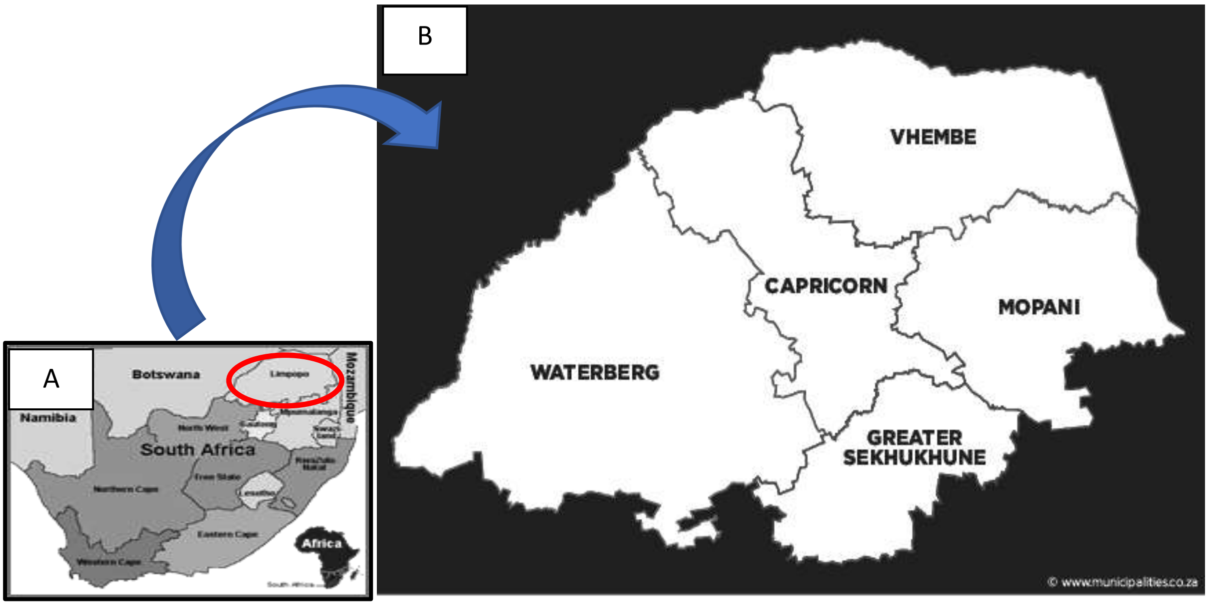

Figure 1: Map showing study areas. (A) Map of South Africa showing Limpopo province circled in red, (B) Limpopo province showing five districts of Limpopo (Limpopo local government 2012).

Questionnaire survey

A total of 77 resource poor farmers derived from the extension officers and animal health technician's data base in Limpopo province, who owned 40 - 100 sheep were interviewed between June and August of 2017 using a structured questionnaire with a combination of qualitative and quantitative, open-ended questions. The farmers in Capricorn, Sekhukhune and Vhembe districts converged at the dipping tanks with the exception of Waterberg and Mopani districts where farmers were visited in their respective homes. All the farmers were interviewed individually. The questionnaire was composed of two sections, whereby the first section was related to main characteristics of the farm management such as flock size, breed, grazing conditions and housing time. The second section was dedicated to helminth parasite control practices. Questions regarding among other things mode of infection, clinical signs linked to parasitism, time and reason for anthelmintic treatment, anthelmintic products used, dose determination and mode of application were included.

Statistical analysis

Data collected was manually coded and analyzed using descriptive statistics and frequencies. Microsoft® Excel 2016 and SAS Statistics (Version 9.4) statistical package were also used to analyze the data. Chi-square analysis was used to compare the proportions of yes to no in response to different variables.

Results

A total of 77 questionnaires (100%) were returned. Demographics revealed that 88% of the respondents were older people aged over 40 years, of which 50 (73.5%) were males and 18 (26.4%) were females. The results presented in Table 2 show that there was a numerical difference between the male and the female farmers in terms of knowledge on the risk factors that are associated with the development of anthelmintic resistance but no significant difference ($p > 0.05$) between the two genders was observed.

Table 2: The percentages of yes between male and female on questions relating to risk factors associated with development of anthelmintic resistance

Risk factors	Female	Male	Chi square value	Pr > Chi square
Knowledge of symptoms of infection	15.91	84.09	2.27	*0.13
Knowledge of mode of infection	15.38	84.62	1.02	*0.31
Usage of anthelmintics	15.63	84.37	1.32	*0.24
Knowledge of dosage calculation	17.39	82.61	0.41	*0.51

*Pr > Chi square = Probability at which the proportions of yes are different

Of all the respondents, only 1 farmer (1.7%) was aged between 21 – 30 and 8 (13.5%) were aged between 31 – 40 years and there was no significant difference between the farmers falling in different age group categories in respect of their knowledge of symptoms of infection, knowledge of mode of infection, usage of anthelmintics and knowledge of dosage calculation ($p > 0.05$). All in all, the highest number of female farmers was found in Sekhukhune district (38.8%) and the lowest was in Waterberg and Capricorn districts with 2 (11.1%) female farmers each.

The results indicated that visual appraisal of individual weight was the most common means (100%) of estimating the anthelmintic dose used in sheep. Table 3 shows that only a small percentage (4.35%) of farmers from Vhembe district stated that they know how to calculate anthelmintic dose as compared to the farmers in Capricorn, Mopani, Sekhukhune and Waterberg districts at 30.43, 34.78, 13.04 and 17.39 respectively ($p < 0.05$) and these correspond with a similarly small percentage (4.55) of farmers with some knowledge of symptoms of internal parasites infection and the probability at which the proportions of yes are different was highly significant ($p < .0001$).

Table 3: The percentages of yes from farmers in the five districts of Limpopo province on questions relating to risk factors associated with development of anthelmintic resistance

Risk factors	**Capr**	**Mop**	**Sekh**	**Vhembe**	**Waterberg**	**Chi square value**	**Pr > Chi square**
Knowledge of symptoms of infection	27.27	25.00	29.55	4.55	13.64	24.83	*<.0001
Knowledge of mode of infection	43.31	19.23	19.23	7.69	11.54	16.10	*0.0029
Usage of anthelmintics	25.00	31.25	18.75	12.50	12.50	13.83	*0.0079
Knowledge of dosage calculation	30.43	34.78	13.04	4.35	17.39	17.51	*0.0015

Capr = Capricorn; Mop = Mopani; Sekhu = Sekhukhune. *Pr > Chi square = Probability at which the proportions of yes are different

Majority of the respondents (57%) were aware of gastrointestinal nematode (GI) that infect sheep although only 34% know how animals become infected. The results in Table 4 reveals that the farmers that owned sheep for more than 10 years were better informed than their inexperienced counterparts in terms of identifying symptoms of internal parasites infection and knowledge of infection mode ($p<0.05$) although no significant difference was found to exist between the three groups of farmers with regard to the usage and correct application of anthelmintics ($p>0.05$).

Table 4: The percentages of yes between experienced and inexperienced farmers in Limpopo province on questions relating to risk factors associated with development of anthelmintic resistance

Risk factors	**Less than 5 yrs.**	**6 - 10 yrs.**	**More than 10 yrs.**	**Chi square value**	**Pr > Chi square**
Knowledge of symptoms of infection	15.91	20.45	63.64	5.21	*0.07
Knowledge of mode of infection	7.69	23.08	69.23	6.55	*0.03
Usage of anthelmintics	18.75	18.75	62.50	1.26	*0.53
Knowledge of dosage calculation	21.74	17.39	60.87	0.27	*0.87

*Pr > Chi square = Probability at which the proportions of yes are different

The farmers with a lower level of education had a better awareness of three out of the four risk factors that are associated with the development of anthelmintic resistance than those with matric qualification ($p<0.05$) (Table 5).

Table 5: The percentages of yes between farmers of different education levels in Limpopo province on questions relating to risk factors associated with development of anthelmintic resistance

Risk factors	Below matric	Post matric	Chi-square value	Pr > chi square
Knowledge of symptoms of infection	63.64	36.36	3.05	*0.08
Knowledge of mode of infection	57.69	42.31	3.62	*0.05
Usage of anthelmintics	53.13	46.87	8.98	*0.002
Knowledge of dosage calculation	52.17	47.83	5.95	*0.01

*Pr > chi-square = probability at which the proportions of yes are different

About 67.5% of resource poor sheep farmers in Limpopo province never use anthelmintics although 32.4% of them had deworming schedule for their sheep. However, the Chi square analysis of the five districts of Limpopo province yielded a statistical difference of $p=0.0079$ and $p=0.0015$ for farmers' usage of anthelmintics and knowledge of dosage calculation respectively, which was highly significant (Table 3).

Table 6: Knowledge on clinical manifestation/signs of gastrointestinal infection

Clinical symptoms	Frequency	Frequency %
Nasal discharge	6	17.1
Rough coat	10	28.5
Loss of body condition	11	31.4
Worms of in the faeces	3	8.5
Diarrhea	3	8.5
Bottle jaw	2	5.7

Results of the present study also revealed that 57% of the respondents can identify the clinical symptoms of gastrointestinal nematodes infection with loss of body condition and rough coat mentioned 11 times (31.4% frequency) and 10 times (28.5% frequency) respectively (Table 7).

Table 7: Helminths control practices of resource poor farmers in Limpopo province

Helminth control factor	Frequency	Frequency %
Anthelmintic class		
Benzimidiazole	26	70.3
Macrolytic lactones	9	24.3
Levamisole	1	2.7
Praziquantel + levamisole	1	2.7
Treatment frequency		
Once per year	3	3.9
Twice per year	9	11.7
Three times in a year	13	16.8
Never	52	67.5
Frequency of active AI change (Months)		
3 months	10	66.67
4 months	2	13.33
6 months	3	20.00

Where AI = Active ingredient

Forty-eight percent of the respondents know the infection occurrence months, with 65% of them mentioning summer months when it is hot and wet, 19% citing winter and small percentages (8%, 5% and 3%) saying infections occur during spring, all year round and at the beginning of both winter and summer respectively (Figure 2).

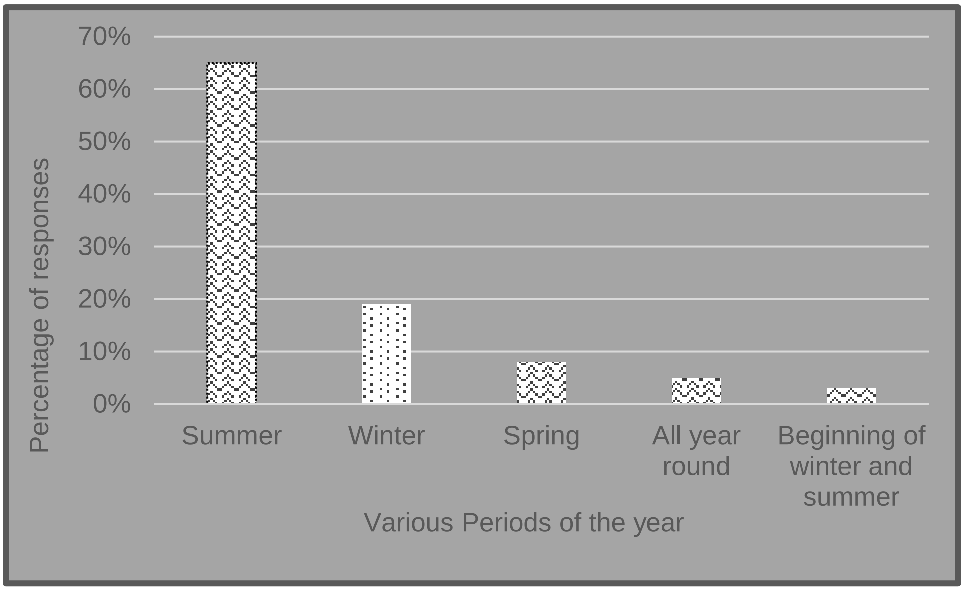

Figure 2: Periods of helminth infections according to resource poor farmers' knowledge in Limpopo province

Table 7 shows that 67.5% of the farmers never use commercial anthelmintics whilst 32.5% apply anthelmintics at varying intervals during the course of the year. Benzimidazoles were the most common anthelmintic class used in sheep. Macrocyclic lactones were mentioned 9 times (24.3% frequency) as the preferred drug for the control of gastrointestinal nematodes in sheep with levamisole and praziquantel levamisole combination mentioned once for each (Table 7).

Discussion

The main factors for selection for anthelmintic resistance are, a high treatment frequency (Chartlier et al. 1998), under-dosing and the use of the same anthelmintic class over several years (Sargison et al. 2007). These factors, alone or in combination, together with certain types of farm management can accelerate the development of AR (Jackson 2009; Jabbar et al. 2006).

None of the resource poor farmers in the present study weighed their sheep before dosing but relied solely on the visual appraisal of an animal to determine its weight and wrong weight estimation may be a cause of under dosing. Bakunzi (2003) attributed anthelmintic resistance observed in goats in a study conducted in Mafikeng to under dosing as a result of limited financial resources. Kumsa et al (2010) also reported that animal owners in Ethiopia do not have information about anthelmintic rotation and similar to the findings of the two South African studies, the farmers treat their animals only by visual estimation of animals' body weight to determine the required doses.

Underestimation of real weight has potential to lead to under-dosing, which can contribute to the development of AR (Chartier et al. 2001). A poor drenching practice can result in under-dosing of a drug and select for AR (Smith et al. 1999); Wolstenholme et al. 2004). It is not only the incorrect estimation of animal live-weight that causes under-dosing but also incorrect calibration of drench guns and in consideration of ensuring a correct dose, one has to estimate the weight as accurately as possible, preferably by an individual weighing of each animal (Waller 1997). Weighing the heaviest animal before drenching all animals with a slightly over-calculated dose can also be considered as an appropriate way to ensure correct anthelmintic dose (Waller 1994).

Results of the current study indicated that only 32.4% of resource poor farmers had deworming schedule for their animals and although frequency of treatments with anthelmintics varied among farmers, 16.8% of them treated their animals three times a year and these results are in agreement with the result in an Ethiopian survey that showed only 17.78% of the farmers had deworming schedule and 68.33% of the respondents did not deworm their animals (Belina et al. 2017). Although most of the farmers (67.5%) never treat their animals with anthelmintics, there is still a percentage of farmers (32.4%) made up of those farmers who treat their animals once a year (3.9%), twice a year (11.7%) and three times a year (16.8%) with anthelmintics. Those farmers who treat their flocks two or more times per year, do so without coprological checking for the necessity of treatment or its efficacy afterwards and that puts the flocks at risk of anthelmintc resistance.

The results of the present study also revealed that 57% of the farmers in Limpopo province are aware of gastrointestinal nematodes and these results are following a trend of the findings of a study conducted in Gauteng Province, South Africa that revealed that about 88% of the farmers are aware of veterinary helminthosis (Tsotetsi et al. 2013). On the contrary, a study conducted by Belina et al (2017) in Ethiopia revealed that 83.61% of the respondents had poor to no information on anthelmintic drugs utilization practices and 78.89% of interviewed respondents did not know the issue of anthelmintics resistance and its future impact.

The majority of resource poor sheep farmers in Limpopo province still had poor understanding on GI nematode infection which is in line with the results of parasite control practices on gastrointestinal nematode in two separate Ethiopian studies (Aga et al. 2013; Melaku et al. 2013). Contrary to their lack of understanding on gastrointestinal nematode infection, the farmers had knowledge of clinical symptoms and loss of body condition was cited more than any other symptom which was consistent with the results of an earlier study that reported that GI nematode infections was higher in animals with poor body condition (Mohammed et al. 2015). This could be explained by the fact that loss of body condition could be due to factors such as parasitic infections which lead to lower immunological response against infective stage of the parasites (Mohammed et al. 2015; Muktar et al. 2015).

The results of the present study also showed that a 67.5% of sheep farmers in Limpopo province never use anthelmintics which in itself pre-determines lower risk of AR in Limpopo Province South Africa. That is a notion supported by earlier workers that higher treatment frequencies increase the selective advantage for resistant parasites, allowing for an increase in the proportion of resistant parasites over time (Sargison 2011) but contrary to the results of the study by Van Wyk et al. (1999) in Limpopo province that revealed AR was still a problem even in sheep flocks where anthelmintic treatment was less intensive. Although a high risk of resistance is eliminated by the large number of farmers that never use anthelmintics in their flocks, some degree of risk may still exist on some farms that use commercial anthelmintics because the survey also revealed that knowledge of AR, the prevalence of gastrointestinal nematodes and proper usage of anthelmintics is lacking. Similar to the findings of studies conducted in the southern part of Ethiopia and Cuba, the present study established that benzimidazoles group of anthelmintics especially albendazole was the most commonly used by resource poor farmers in Limpopo province to deworm their sheep (Kumsa et al. 2010; Arece et al. 2004). On the other hand, levamisole was found to be used very rarely in South Africa, as in many European countries (Čerňanská et al. 2008).

Conclusion

In the present study, important observations and opinions emerged that reflect serious constraints for resource poor farmers in Limpopo province of South Africa. Among other things, present study has shown that the anthelmintic drenching practices such as incorrect dosages used in sheep flocks of resource poor farmers in Limpopo province, South Africa may contribute to the development of AR. None of the sheep flocks had sufficient weight estimation for calculating correct anthelmintic dose and that represents a high risk for under-dosing anthelmintics in these flocks. In order to avoid or slow down the emergence of AR, correct use of anthelmintics and on-farm training about gastrointestinal nematodes infecting small stock must be provided. Such training initiatives should focus on aspects such the importance of correct dosage, when to alternate anthelmintic classes, treatment frequency and new treatment strategies, such as targeted drenching in combination with faecal egg counts. We also suggest that government veterinary services at district and municipality levels purchase the weighing scales which animal health technicians can always carry along during branding, vaccination and dipping campaigns so that they can also assist farmers with weighing in order to ensure they can administer accurate doses of anthelmintic.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

MM, AMT-K, RM and OMMT planned the study. MM conducted the experiment and drafted the manuscript. AMT-K, RM and OMMT reviewed the manuscript. MLM conducted statistical analysis.

References

Aga, T.S., Tolossa, Y.H. and Terefe, G., 2013. Parasite control practices and anthelmintic efficacy field study on gastrointestinal nematode infections of Horro sheep in Western Oromiya, Ethiopia. African Journal of Pharmacy and Pharmacology, 7(47):2972-2980.

Álvarez-Sánchez, M.A., Mainar-Jaime, R.C., Pérez-García, J., Monteagudo-Rodríguez, M. and Martín-Gómez, S., 2001. Anthelmintic resistance in small-ruminant flocks in Spain: extension in the Leon province (NW). In

Abstracts to the 18th International Conference of the World Association for the Advancement of Veterinary Parasitology August 26 – 30, 2001, Stresa, Italy, 2001, pp.155.

Anon, 2007. Pietersburg: The Columbia Encyclopaedia, (6th edition) Columbia University Press. [Online] Available: www.encyclopedia.com (April 4, 2008).

Arece, J., Mahieu, M., Archimède, H., Aumont, G. and Fernández, M., 2004. Comparative efficacy of six anthelmintics for the control of gastrointestinal nematodes in sheep in Matanzas, Cuba. Small Ruminant Research, 54: 61-67.

Bartley, D.J., Nonnan, A.A., Jackson, E., Sargison, N., Mitchell, G.B.B. and Jackson, F., 2006. A small scale survey of ivermectin resistance in sheep nematodes using the faecal egg count reduction test on samples collected from Scottish sheep. Veterinary Parasitology, 137: 112 – 118. DOI: 10.1016/j.vetpar.2005.12.014

Belina, D., Giri, A., Mengistu, S. and Eshetu, A., 2017. Gastrointestinal Nematodes in Ruminants: The Parasite Burden, Associated Risk Factors and Anthelmintic Utilization Practices in Selected Districts of East and Western Hararghe, Ethiopia. Journal of Veterinary Science and Technology, 8:2.

Borgsteede, F.H.M., Dercksen, D.D. and Huijbers, R., 2007. Doramectin and albendazole resistance in sheep in the Netherlands. Veterinary Parasitology, 144: 180 – 183. DOI: 10.1016/j.vetpar.2006.09.031.

Čerñanská, D., Várady, M. and Čorba, J., 2006. A survey on anthelmintic resistance in nematode parasites of sheep in the Slovak Republic. Veterinary Parasitology, 135: 39 – 45. DOI: 10.1016/j.vetpar.2005.09.001.

Čerñanská, D., Várady, M., Čudeková, P. and Čorba, J., 2008. Worm control practices on sheep farms in the Slovak Republic. Veterinary Parasitology, 154: 270 – 276. DOI: 10.1016/j.vetpar.2008.03.026.

Cezar, A.S., Toscan, G., Camillo, G., Sangioni, L.A., Ribas, H.O. and Vogel, F.S.F., 2010. Multiple resistance of gastrointestinal nematodes to nine different drugs in a sheep flock in southern Brazil. Veterinary Parasitology, 173: 157 – 160. DOI: 10.1016/j.vetpar.2010.06.013.

Chartier, C., Pors, I., Hubert, J., Rocheteau, D., Benoit ,C. and Bernard, N.,1998. Prevalence of anthelmintic resistant nematodes in sheep and goats in Western France. Small Ruminant Research, 29: 33-41.

Chartier, C., Soubirac, F., Pors, I., Silvestre, A. and Hubert, J., 2001. Prevalence of anthelmintic resistance in gastrointestinal nematodes of dairy goats under extensive management conditions in southwestern France. Journal of Helminthology, 75: 325-330.

Cook, C., Reason, C.J.C. and Hewitson, B.C., 2004. Wet and dry spells within particularly wet and dry summers in the South African summer rainfall region. Climate Research, 26: 17-31. doi:10.3354/cr026017, http://dx.doi.org/10.3354/cr026017.

Domke, A.V.M., Chartier, C., Gjerde, B., Leine, N. and Vatn, S., 2011. Worm control practice against gastro-intestinal parasites in Norwegian sheep and goat flock. Acta Veterinaria Scandinavica, 53: 29.

Dolinská, M., Königová, A. and Várady, M., 2012. Is the micro-agar larval development test reliable enough to detect ivermectin resistance? Parasitology Research, 111: 2201 – 2204. DOI: 10.1007/s00436012-2944-4.

Jabbar, A., Iqbal, Z., Kerboeuf, D., Muhammad, G., Kha,n M.N. and Afaq, M., 2006. Anthelmintic resistance: The state of play revisited. Life Sciences, 79: 2413-2431.

Jackson, F., 2009. Worm control in sheep in the future. Small Ruminant Research, 86: 40-45.

Kumsa, B., Debela, E. and Megesa., 2010. Comperative efficacy of Albendazole, Tetramisole and Ivermectin against gastrointestinal nematodes in naturally infected goats in Ziway Oronia Reginal State (South Ethiopia). Journal of Animal and Veterinary Advances, 9: 2905 - 2911.

Kumsa, B., Tolera, A. and Nurfeta, A., 2010. Comparative efficacy of seven brands of Albendazole against naturally acquired gastrointestinal nematodes in sheep in Hawassa, southern Ethiopia. Turkish Journal of Veterinary & Animal Sciences, 34(5):417-425 doi: 10.3906/vet-0712-28.

Kupčinskas, T., Stadalienė, I., Šalomskas, A., Trusevičius, P. and Varady, M., 2016. Worm-control practices and prevalence of anthelmintic resistance using in vivo FECRTs on smallholder sheep farms in Lithuania. Helminthologia, 53(1): 24 – 30.

Limpopo Department of Agriculture, 2008. Background. [Online] Available: http://www.lda.gov.za/index.php?option=com_content&view=article&id=60&Itemid=55 (April 4, 2008).

Limpopo Local Government, 2012. The Mapping of Agricultural Commodity Production in the Limpopo province, South Africa.

Martínez-Valladares, M., Martínez-Pérez, J.M., Robles-Pérez, D., Cordero-Pérez, C. and Famularo, M.R., 2013. The present status of anthelmintic resistance in gastrointestinal nematode infections of sheep in the northwest of Spain by in vivo and in vitro techniques. Veterinary Parasitology, 191: 177 – 181. DOI: 10.1016/j. vetpar.2012.08.009.

Melaku, A., Bogale, B., Chanie, M., Fentahun, T. and Berhanu, A., 2013. Study on utilization and efficacy of commonly used anthelmintics against gastrointestinal nematodes in naturally infected sheep in North Gondar, North-Western Ethiopia. African Journal of Pharmacy and Pharmacology, 7: 679-684.

Mohammed, A., Disassa, H., Kabeta, T., Zenebe, T. and Kebede, G., 2015. Prevalence of Gastrointestinal Nematodes of Sheep in Gursum Woreda of Eastern Hararghe Zone, Oromia Regional State, Ethiopia. Researcher, 7: 45-54.

Muhammad, N.K., Mohammad, S.S., Mohammad, K.K. and Zafar, A.H., 2010. Gastrointestinal heliminthiasis: Prevalence and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan. Parasitology Research.

Muktar, Y., Belina, D., Alemu, M., Shiferaw, S. and Belay, H., 2015. Prevalence of Gastrointestinal Nematode of Cattle in Selected Kebeles of Dire Dawa Districts Eastern Ethiopia. Advanced Biology Research 9: 418-423.

Odoi, A., Gathuma, M.J., Gachuiri, KC. and Omore, A., 2007. Risk factors of gastrointestinal nematode parasite infection in small ruminants kept in smallholder mixed farmers in Kenya. BMC Veterinary Research, 3: 1-11.

Paddock, R., 2010. Breed, Age and Sex Wise Distribution of *Haemonchus contortus* in Sheep and Goats in and around Rawalpindi Region, Pakistan. Medical Veterinary Journals, 12: 60-63.

Papadopoulos, E., 2008. Anthelmintic resistance in sheep nematodes. Small Ruminants Research, 76: 99 – 103. DOI: 10.1016/j.smallrumres.2007.12.012.

Papadopoulos, E., Gallidis, E. and Ptochos, S., 2012. Anthelmintic resistance in sheep in Europe: A selected review. Veterinary Parasitology, 189(1):85 – 88. DOI: 10.1016/j.vetpar.2012.03.036.

Papadopoulos, E., Himonas, C. and Coles, G.C., 2001. Drought and flock isolation may enhance the development of anthelmintic resistance in nematodes. Veterinary Parasitology, 97: 253 – 259. DOI: 10.1016/S0304-4017(01)00435-6

Reason, C.J.C., Hachigonta, S. and Phaladi, R.F., 2005. Interannual variability in rainy season characteristics over the Limpopo region of Southern Africa, South Africa. International Journal of Climatology, 25:1835-1853. doi:10.1002/joc.1228, http://dx.doi.org/10.1002/joc.1228.

Sargison, N.D., 2011. Pharmaceutical Control of Endoparasitic Helminth Infections in Sheep. Veterinary Clinics of North America: Food and Animal practice, 27: 139-156.

Sargison, N.D., 2016. Keys to solving problems in small ruminants: Anthelmintic resistance as a threat to sustainable nematode control. Small Ruminant Research, 142:11-15.

Sargison, N.D., Jackson, F., Bartley, D.J., Wilson, D.J., Stenhouse, L.J. and Penny, C.D., 2007. Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. Veterinary Parasitology, 145:65-76.

Sangster, N.C. and Gill J., 1999. Pharmacology of anthelmintic resistance. Parasitology Today, 15:141-146.

Silvestre, A. and Humbert, J.F., 2002. Diversity of benzimidazole resistance alleles in populations of small ruminants parasites. *International Journal of Parasitology*, 32: 921-928.

Smith, G., Grenfell, B.T., Isham, V. and Cornell, S.,1999. Anthelmintic resistance revisited: under-dosing, chemoprophylactic strategies, and mating probabilities. International Journal Parasitology 29: 77-91.

Taylor, M.A., Learmount, J., Lunn, E., Morgan, C. and Craig, B.H., 2009. Multiple resistance to anthelmintics in sheep nematodes and comparison of methods used for their detection. Small Ruminants Research 86:67-70. DOI: 10.1016/j.smallrumres.2009.09.020.

Tennant, W.J. and Hewitson, B., 2002. Intra-seasonal rainfall characteristics and their importance to the seasonal prediction problem. International Journal of Climatology, 22:1033-1048. doi:10.1002/joc.778, http://dx.doi.org/10.1002/joc.778.

Thomas, D.S.G., Twyman, C., Osbahr, H. and Hewiston, B., 2005. Adapting to climate change and variability in Limpopo.

Torres-Acosta, J.F.J., Mendoza-De-Gives, P., Aguilar-Caballero, A.J. and Cué Llar-Ordaz, J.A., 2012. Anthelmintic resistance in sheep farms: update of the situation in the American continent. Veterinary Parasitology, 189:89-96. DOI:10.1016/j.vetpar.2012.03.037.

Traversa, D., Paoletti, B., Otranto, D. and Miller, J., 2007. First report of multiple drug resistance in *trichostrongyles* affecting sheep under field conditions in Italy. Parasitology Research, 101:1713-1716. DOI: 10.1007/s00436-008-0934-3.

Tsotetsi, A.M, Njiro, S., Katsande, T.C., Mayo, G. and Baloyi, F., 2013. Prevalence of gastrointestinal helminths and anthelmintic resistance on small-scale farms in Gauteng Province, South Africa. Tropical Animal Health and Production, 45(3):751-761.

Vatta, A.F. and Lindberg, A.L.E., 2006. Managing anthelmintic resistance in small ruminant livestock of resource-poor farmers in South Africa Review article.

Van Wyk, J.A., Malan, F.S. and Bath, G.F., 1997. Rampant anthelmintic resistance in sheep in South Africa – what are the options?

Van Wyk, J.A., Stetson, M.O., Van Der Merwe, J.S, Vorster R.J. and Viljoen, P.G. 1999. Anthelmintic resiatance in South Africa: Surveys indicate an extremely serious situation in sheep and goat farming. Onderstepoort Journal of Veterinary Research, 66:273-284.

Waller, P.J.,1994. The development of anthelmintic resistance in ruminant livestock. Acta Tropica, 56: 233-243.

Wolstenholme, A.J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G. and Sangster, N.C., 2004. Drug resistance in veterinary helminths. Trends in Parasitology, 20:469-476.