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Vol. 5(8), pp. 229-236 August, 2013
DOI 10.5897/JVMAH12.025

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Evaluation of the effectiveness of two medicinal plants *Vernonia amygdalina* and *Leonotis nepetaefolia* on the gastrointestinal parasites of goats in Rwanda: Case study of Huye and Gisagara districts

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Accepted 10 June, 2013

The two most commonly used medicinal plants as traditional healers were identified (*Vernonia amygdalina* and *Leonotis nepetaefolia*) and were tested for their effectiveness as antiparasitic drugs on the gastrointestinal parasites of goats in some sectors of Huye and Gisagara districts of Rwanda. A survey was conducted which revealed that 87.5% of the knowledge of veterinary traditional medicine is transmitted from generation to generation. The combination of *V. amygdalina* and *L. nepetafolia* is used at an average of 87.5% while *V. amygdalina* alone is used on the average of 66.7%. For the goats treated with the combination of *V. amygdalina* and *L. nepetafolia* (group 1), the mean prevalence of faecal shedding of strongyles' eggs was 78%, while for those treated with *L. nepetafolia* alone (group 2) was 77.4% for pregnant goats and 86% for empty ones. For the fecal excretion of coccidial oocysts, the average prevalence was 73% for group 1 and 39% for group 2 whereas it was 13% for nematodirus. This investigation showed no influence of the sex or the age on the fecal egg excretion of Strongyle eggs and coccidia oocysts. The results showed that the single treatments were not effective in reducing the eggs in the faeces while the repeated treatments caused a considerable fall of level of faecal gastro-intestinal egg excretion of strongyle as from day 14.

Key words: Coccidian oocytsts, effectiveness, gastrointestinal helminthes, goats, *Leonotis nepetaefolia*, medicinal plants, Strongyle egg, *Vernonia amygdalina*.

INTRODUCTION

The problem of parasitism has been complicated by the appearance of phenomena characterized by the resistance to the many types of anthelmintics currently in use (Larrat, 1988). This resistance increases in animals that apparently seem to be sound but in reality, that are sick and chronic parasites carriers. All along, the helminthiasis causes losses due to reduction in the animal production and reproduction due to strong morbidities and mortalities as well as an increase in their treatment cost (Beugnet, 2005; Tabuti (2003). Food and Agriculture Organization

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Figure 1. Leaved branch of blooming V. *amygdalina* tree at this stage, the aqueous extracts of this plant are considered more effective.

(FAO), 1992). Various measures have been taken and some remedies are being used to tackle the problem related to the resistant parasitism in domestic animals. The promotion of traditional veterinary medicine in Rwanda can have a significant impact directly on animal husbandry and indirectly on agriculture. This impact should mainly be seen within the framework of putting into place the efficient alternatives to the modern medicine which confronted with several constraints. One example among many is the high cost of drugs for animals which does not correlate with the low income of the stockbreeders and the scarcity of qualified veterinarians (Nyamanga et al., 2008). Among the threatened animals, the goat is menaced while it is the main domestic animal that improves the everyday life of the population in developing countries. Goats can be infected by numerous internal parasites (intestinal protozoa, nematode, etc) and the effect of infection by these gastrointestinal parasites varies according to the parasite involved, the degree of infection, common environmental factors (Terefe et al., 2012).

Some authors reported miscellaneous benefits of the recourse to the medicinal plants in healthcare of animals. The main benefits are the contribution to the fight against many types of diseases at the same time, the relief of the economic and commercial balance and the improvement of the social conditions of the breeders and healers. In addition, the country benefits from it since it leads to the reduction of problems related to synthetic drug residues and to biodiversity protection (Landais and Lhoste, 1990; Pieroni et al., 2004). Within this framework, we carried out an investigation on two used plants mostly used by the traditional healers of two districts of the Southern Province of Rwanda in curing their domestic animals. A survey made to the traditional practitioners revealed that *Vernonia amygdalina* (locally called “Umubirizi”) and *Leonotis nepetaefolia* (locally called “Igicumucumu”) are the most commonly used plants, and these can be considered for antiparasitic activity. So, these were considered in this study.

Figure 1 shows *V. amygdalina*, commonly called “bitter leaf”. It is a member of the Asteraceae family. In the wild, chimpanzees have been observed to ingest the leaves when suffering from parasitic infections (Huffman and Seifu, 1989; Ademola and Eloff, 2011). In all cases, this medicinal plant, *V. amygdalina*, revealed to exhibit anticancer, anthelminthic and antiparasitic properties (Swee et al., 2010; Gresham et al., 2008; Sweeney et al., 2005; Izevbigie et al., 2004; Kupchan et al., 1969). *L. nepaetifolia* shown by Figure 2, also known as “Lion's ear”, is a species of plant in the Lamiaceae family. *L. nepaetifolia* is known in some Asian countries as “shandilay*”* and the leaves are brewed as a tea for fever, coughs, womb prolapse and malaria (Mendes, 1986; Calixto et al., 1991).

MATERIALS AND METHODS

Field investigation

The experiment was conducted in Save sector of Gisagara district and Ngoma, Gishamvu, Maraba and Mbazi sectors of Huye district. A survey questionnaire was submitted to the traditional healers. The interview was conducted and aimed at collecting the information about their work organization; the medicinal plants they use to treat the verminosis in goats and the minimum doses for respective diseases. Experiments were carried out according to the

Figure 2. Leaved stem and inflorescence of *L. nepaetifolia*. The leaves are generally harvested when the bees arrive in droves for nectar-hunting.

information given about the plants.

Effectiveness evaluation

Sixty-three goats were subjected to the test of the effectiveness of combined extracts of *V. amygdalina* and *L. nepetaefolia* on the strangles while forty-nine goats was also subjected to the test of the effectiveness of the extract of *V. amygdalina* alone. As for the prevalence of faecal eggs shedding, all animals were considered while for the estimation of the rate of eggs reduction in faeces only 45 animals were used for each of the two tests.

Test of prevalence of fecal strongyle eggs and coccidia oocysts and reduction of the eggs/oocyst excretion

The completely experimental designs consisted of four treatments which corresponded to either *V. amygdalina* in single and repeated dose or the combination of the extracts of *L. nepetafolia* in single and repeated dose. The faeces samples were directly taken from the rectum of each goat two days before the treatment (J_2) and then from rectal bolus seven days (J_7), fourteen days (J_{14}), twenty-one days (J_{21}) and twenty-eight days (J_{28}) after treatment. After collection, the samples were brought to veterinary laboratory of the School of Agriculture and Veterinary of Kabutare (EAVK) in Butare for coproscopic examinations. The counting of nematode eggs and the coccidia oocysts was made according to the Mc Master technique (Thienpont et al., 1995). However, for the heavy eggs such as those of the trematodes, it was necessary to use more specific solutions. The test of the effectiveness of different solutions against the strongyles in goats was done based on the reduction percentage of eggs excretion. The protocol for the eggs excretion reduction was done as per World Association for the Advancement of Veterinary Parasitology “WAAVP” (Coles et al., 1992).

Composition of solutions and scheme of treatment and effectiveness evaluation time

Tables 1, 2 and 3 show the composition of the different solutions used in single or repeated treatment, the scheme of treatment and evaluation of the effectiveness of drug solution in goats respectively.

Data analysis

$$\text{Percentage of reduction} = 100\left[1 - \left(\frac{\overline{X}_t}{\overline{X}_c}\right)\right]$$

X_c = arithmetic mean of the excretion per gram of the control(c), untreated goats, between 10 and 14 days after treatment
X_t = arithmetic mean of the excretion per gram of the treated (t) goats, between 10 and 14 days after treatment.

If the percentage reduction is greater at least to 95% and the less confidence limit greater than 90%, the solution is effective. The solution is ineffective if the percentage reduction is less than95% and the confidence limit less than 90%.

All survey data and excretion per gram were encoded on an Excel spreadsheet. This spread sheet was used to produce tables and calculate the arithmetic means and standard deviations. Analysis of variance performed with statistical package for social

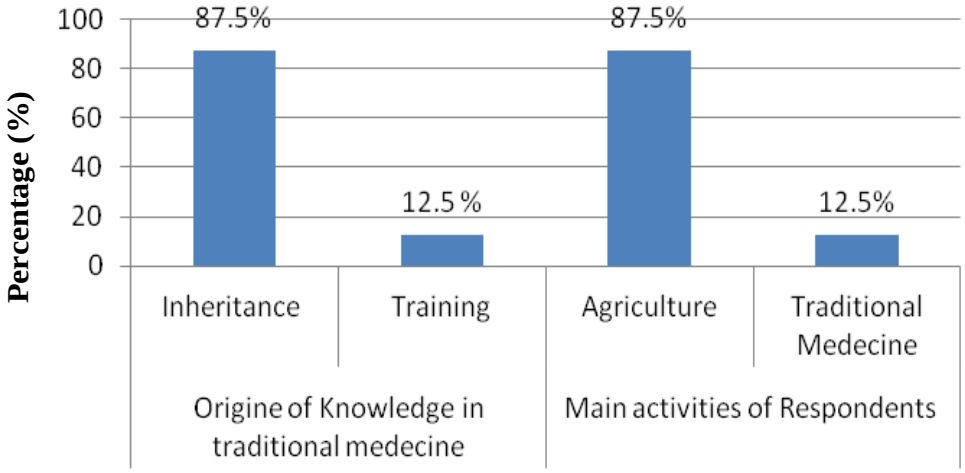

Figure 3. The origin of knowledge of interviewed persons in traditional veterinary medicine. In this survey, the traditional veterinary healers listed about 33 plants with animal heliminthes remedial properties and targeted the two plants (*V. amygdalina* and *L. nepetaefolia*) to be frequently and efficiently used.

sciences (SPSS 14.0) software was used to compare the prevalence of fecal shedding of eggs and the value of excretion per gram of goats groups. The values of excretion per gram underwent logarithmic transformation before being analyzed statistically. The software FECR4 (Cameron, 2004) was used to calculate the rate of reduction of excretion per gram. The results of these tests to reduce excretion per gram based on the criteria were used to decide the effectiveness of the treatment used. Then, the number of eggs excreted per gram of faeces is given by the formula Y*100, with "y" represents the number of eggs counted in the two cavities of the cell.

RESULTS

Data from field investigation

The survey results (Figure 3) show that major part of interviewed people are farmers and their traditional medicine knowledge was inherited by their parents while only a small fraction of them got their knowledge from trainings accorded by the stakeholders. The diagram below shows interviewees' statistics with regards the origin of their knowledge in traditional medicine and their main activities.

Prevalence of fecal strongylous eggs and coccidia oocysts in goats

Goats treated with the combined V. amygdalina and L. nepetaefolia (group 1)

This investigation in Ngoma, Maraba and Save sectors showed that the comparison between the average prevalence of strongyle eggs excretion is quite high (78%), coccidia oocysts 73%, nematodirus 13%. There is a significant difference between the observations in Maraba and Ngoma sectors but for the event of nematodirus ($p = 0.025$) as well as the case of oocysts ($p = 0.039$), there was a significant difference between the results in Maraba and save sectors ($p = 0.008$). Age wise, there was no significant difference between the prevalence of eggs excretion of strongyle and coccidia oocysts obtained. As for the races of goats subjected to this investigation (14 goats of crossed race and 49 goats of local race), the prevalence of the egg excretion of strongyles for the cross bred was 100% while for local breed goats, the prevalence was 71% ($p = 0.04$). It was the same case for the prevalence of the infestation by nematodirus ($p = 0.003$). However, for the coccidia oocysts, there was no significant difference ($p > 0.05$).

Goats treated with V. amygdalina alone (group 2)

The average prevalence of strongyles eggs and coccidia oocysts was 77.4 and 39%, respectively depending on the physiological state. The average prevalence of fecal shedding of strongyle eggs was 71% for pregnant goats and 86% for void goats. For oocysts of coccidia prevalence was slightly higher in pregnant goats compared to non-pregnant ones (41 against 36%). There was no significant difference between the two groups ($p > 0.05$).

Table 1. Composition of the different solutions used in single or repeated treatment.

Types of measurements	***Mixture of V. amygdalina* and *L. nepetaefolia***	***V. amygdalina***
	Solution 1	**Solution 2**
Single dose per 15 animals		
Average quantity *V. amygdalina* (kg)	2.5	22.5
Average quantity *L. nepetaefolia* (kg)	1.7	-
Average quantity of water (L)	6	7.5
Single dose (L)	0.33	0.33
Repeated dose per 15 animals during 3 days		
Traditional healers	67	91
Average quantity *V. amygdalina* (kg)	1.81	56.5
Average quantity *L. nepetaefolia* (kg)	1.63	-
Average quantity water (L)	6	22.5
Daily dose (L)	0.33	0.33
Average time (days)	**3**	**3**
Average time of treatment (days)	1	1

The “solution 1” consists of a mixture of *V. amygdalina* and *L. nepetafolia*. The “Solution 2” consists of a solution of *V. amygdalina* alone.

Table 2. Scheme of treatment and evaluation of the effectiveness of drug solutions in goats.

Groups of animals	Batch according to the type of treatment	Numbers of animals by batch	Administered solutions	Time of treatment
Group 1 (treated with the mixture of *V. amygdalina* and *L. nepetaefolia*)	Batch of control	15	Nil	No treatment
	Batch treated with a single dose	15	Solution 1 containing *V. amygdalina and L. nepetaefolia*	1 day
	Batch treated with a repeated dose	15	Solution 2 containing *V amygdalina* and *L. nepetaefolia*	3 days
Group 2 (treated with *V. amygdalina*)	Batch of control	15	Did not receive anything	No treatment
	Batch treated with a single dose	15	Solution 1 containing *Vernonia amygdalina*	1 day
	Batch treated with a repeated dose	15	Solution 2, which contain *Vernonia amygdalina*	3 days

Table 3. Results of the test of reduction of eggs per gram for the group treated with both *V. amygdalina* and *L. nepetaefolia*. Case of single treatment.

Batches	Single treatment				Control			
Day of analysis	J_7	J_{14}	J_{21}	J_{28}	J_7	J_{14}	J_{21}	J_{28}
Number of animals	14	14	14	13	14	15	15	15
Arithmetic mean	264	943	700	662	1.479	840	1.260	1.480
Variance (eggs per gram)	25.549	-	1,555.385	939.231	2,772.582	548.286	749.714	687.429
Reduction (%)	82	-12	44	55				
Variance of reduction	0.12	0.21	0.26	0.19				
Lower limit within confidence 95%	91	0	81	82				
Upper limit within confidence 95%	63	57	0	0				

Table 4. Results of the test of reduction of eggs per gram for the group treated with *V. amygdalina and L. nepetaefolia*. Case of repeated treatment.

Batches	Single treatment				Control			
Day of analysis	J_7	J_{14}	J_{21}	J_{28}	J_7	J_{14}	J_{21}	J_{28}
Number of animals	12	13	15	13	13	15	15	15
Arithmetic mean	42	46	13	8	954	840	1.260	1.480
Variance (eggs per gram)	4.470	4.359	1.238	769	1,532.692	548.286	749.714	687.429
Reduction (%)	96	95	99	99				
Variance (reduction)	0.34	0.21	0.50	1.02				
Lower limit within confidence 95%	85	98	100	100				
Upper limit within confidence 95%	99	86	95	96				

Reduction level of the eggs excretion per gram of feaces

For all these treated animals, the analysis of the faeces was carried out in 7, 14, 21 and 28 days of the treatment while the reduction of excretion per gram was used to evaluate the effectiveness degree of the solutions.

Reduction level of the excretion per gram in goats of group 1

The Tables 3 and 4 used in the subsection show the results of the test of eggs reduction per gram for the group with both *V. amygdalina* and *L. nepetaefolia* in the case of single treatment and the case of repeated treatment respectively. The single treatment on day 7, the percentage of reduction was 82% from the following days until day 28 while the percentage of reduction was 55%. Consequently, the single treatment has not been effective in this investigation. For the repeated treatment, the action started on 14th day with 95% and on 28th day was 99% of reduction.

Reduction level of egg per gram in goats of group 2

In this subsection, the Tables 5 and 6 show the test of reduction eggs per gram for the goats treated with *V. amygdalina* alone in the case of single treatment and the case of repeated treatment respectively. Also in this case, the single treatment has not been effective to the goats of group 1. There was a reduction in the excretion of eggs from day 7 until day 28. For the case of repeated treatment, the reduction of the excretion of eggs per gram was fast and progressive from day 7 (88%) to day 28 (98%). During this test, the treatment was effective as from the 14th day.

DISCUSSION

In this study, we noticed that 87.5% of knowledge of the questioned traditional healers of animals were transmitted from generation to generations, frequently from parents to children. This situation was also reported by McCorkle and Martin (1998) and Nyamanga et al. (2008). According to them, around 80% of traditional

Table 5. Test of reduction eggs per gram for the goats treated with *Vernonia amygdalina* alone. Case of single treatment

Batches	Single treatment				Control			
Day of analysis	J_7	J_{14}	J_{21}	J_{28}	J_7	J_{14}	J_{21}	J_{28}
Number of animals	15	15	15	15	15	15	15	15
Arithmetic mean	80	173	147	160	500	607	740	687
Value (egg per gram)	14.571	37.810	19.810	19.714	118.571	112.095	109.714	114.095
Reduction (%)	84	71	80	77	-	-	-	-
Variance (reduction)	0.18	0.10	0.07	007	-	-	-	-
Lower limit within confidence 95%	93	85	65	60	-	-	-	-
Upper limit within confidence 95%	61	44	89	86	-	-	-	-

Table 6. Results of the test of reduction of eggs per gram in goats treated with *Vernonia amygdalina* alone. Case of repeated treatment.

Batches	Single treatment				Control			
Day of analysis	J_7	J_{14}	J_{21}	J_{28}	J_7	J_{14}	J_{21}	J_{28}
Number of animals	15	15	15	15	15	15	15	15
Arithmetic mean	60	27	27	13	520	15.607	740	687
Value (egg per gram)	12.571	2.095	2.095	1.238	81.714	112.095	109.714	114.095
Reduction (%)	88	96	96	98	-	-	-	-
Variance (reduction)	0.25	0.22	0.21	0.48	-	-	-	-
Lower limit within confidence 95%	96	98	99	92	-	-	-	-
Upper limit within confidence 95%	67	88	91	100	-	-	-	-

medicine knowledge come from father to their sons while around 18% of knowledge of traditional medicine come from the trainings.

With regards to plants used by traditional healers for animal verminosis treatment, 87.5% of questioned traditional healers from the aforementioned locations use the combination of *V. amygdalina* and *L. nepetafolia* while 66.7% of them use *V. amygdalina* alone. Our observations were similar to the observations earlier recorded by Wan Yong Ho et al. (2010) and Nfi et al. (1999). They observed that *V. amygdalina* alone was able to control the animal verminosis on the average rate of 52.4%. Alawa et al. (2000, 2003) hypothesized that the traditional antihelmintic claim of this plant extract may be contributed by the cleansing of gastric and intestines through increase of smooth muscle motility. The survey results showed that the posology is imprecise because there was no defined quantity.

As far as the rhythm of frequency is concerned it was also reported that it depends on the nature of remedy and the seriousness of diseases (Larrat, 1988). The animals of group 1 have shown a mean prevalence of fecal shedding of strongyle eggs of 78% while those of group 2 have shown the average prevalence of 77.4% for pregnant goats and 86% for empty females. These results differ from the prevalence (49.5%) reported by Terefe et al. (2012) but similar to those found by Gashururu (2006) and Hitimana (2009). As for the results of the test of the reduction of the eggs per gram in faeces, the evaluation of the effectiveness of the medicine containing *L. nepetaefolia* and or *V. amygdalina* was made in the image of other previous authors (Beugnet, 2005; Githiori, 2004). The solutions in single treatment (UT1 and UT2) were administered only once as indicated by certain traditional

healers. By single or repeated treatment of *V. amygdalina* and associated with other plants, the solutions were given in three successive days according to the previous reports of Niang (1996) and Kabore et al. (2007).

ACKNOWLEDGEMENTS

The authors are greatly indebted to the authorities of the Institute of Scientific and Technological Research (IRST). Thanks are also due to the VSF (Veterinarians Without Borders) and the School of Agriculture and Veterinary of Kabutare for their logistic support.

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