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Research Article

THE USE OF FAMACHA©CHART IN EVALUATING THE PREVALENCE OF *HAEMONCHUS CONTORTUS* IN GOATS AND SHEEP AT GOOD HOPE RANCH, SOUTHERN BOTSWANA

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ABSTRACT
The prevalence of haemonchosis was identified on the south east farm of Botswana using a clinical, screening test, FAMACHA©an on-farm diagnostic tool. The overall faecal egg counts revealed that the parasite *Haemonchus contortus* was prevalent in goats and sheep, with sheep showing the highest resistance to the most pronounced anthelmintic at only 70% reduction level. However, faecal egg counts failed to show an association between egg counts and FAMACHA© eye scores at 95% confidence interval ($p>0.200$). The use FAMACHA© was found to be applicable and highly recommended in monitoring anaemia in small ruminants because the system had 100% sensitivity to anaemia on both goats and sheep.

Keywords: *Haemonchus contortus, FAMACHA©*

INTRODUCTION
In 1990, the goat population in Botswana was 2.12 million, of which 70% was owned by local farmers (Mrema and Rannobe, 2000). The population increased in 1993, with approximately more 250 000 sheep recorded and 93% of them belonging to local farmers (Nsoso and Madimabe, 1999). According to Seleka (2001), most of the small-ruminants in Botswana are in the hands of traditional farmers, 97% of goats and 87% of sheep being found in communal lands. About 70% of rural households in Botswana rely on agriculture, with livestock farming being the most important source of their economy (Moreki *et al.*, 2010). Over the years, the above mentioned figures have increased drastically due to the government's initiatives in eradicating poverty, attaining food security and self-sustenance by 2016, through the implementation of policies and self-help projects throughout the country.

The following government initiatives were developed not only to empower the nation but to also improve the agricultural sector in order to diversify Botswana's economy (Nsoso *et al.,* 2004); the Young Farmers Fund (YFF) under the Citizen Entrepreneurship Development Agency (CEDA), the Youth Development Fund (YDF), the National Master Plan for Arable Agriculture and Dairy Development (NAMPAADD) and the Financial Assistance Policy (FAP) which was founded primarily for small-stock (SS) production (Fidzani and Kerapeletswe, 2005).

Young entrepreneurs were now utilizing the government's programmes in large numbers, embarking on small stock farming. According to Inn (2003), small stock farming is a very lucrative business because small stock have high reproduction rates, small body size, shorter generation intervals, easier to de-stock and restock as required (for example, during droughts, can curb loss due to starvation) and minimal capital to establish the farm and manage the animals.

Botswana's climate, as known (or as was known) can be characterized as predominantly sub-tropical. Consequently, the country is largely arid to semi-arid with the rainfall season being in the summer months, October to March. However, Botswana has since recently experienced drastic climate change, observed also in 2012 with soaring temperatures that leave the land very dry. The last rains falling in late March leadto the harvesting season in April. The optimum temperature for the development of eggs and larvae was found to be between 20-35°C (Veglia, 1915). If the weather is too hot, above 35°C and dry with less than 50mm of rain in a month, the eggs will hatch quickly and the larvae will die. If the weather is too cold below 10°C; the eggs will not hatch to develop into L1.

The summer rains, warm temperature and high humidity influences the emergence of *Haemonchus contortus* nematode, which is the most pathogenic and the leading cause of anaemia in small-ruminants (Burke and Miller, 2006; Burke *et al.*, 2007; Chaudary *et al.*, 2007; Gadahi *et al.*, 2009; Kaplan *et al.*, 2004; Kaplan, 2006; Ndamukong *et al.*, 1994; Tasawar *et al.*, 2010; Waller and Chandrawathani, 2005). According to Bath and Van Wyk (2002), as anaemia progresses, the colour in the inner eyelid (ocular mucous membrane) changes from a deep pink of a healthy animal through shades of pink, to a completely white colour in severely parasitized animals, therefore making *H. contortus* the only nematode parasite which can be accurately diagnosed on-farm due to the obvious signs of acute anaemia.

A study carried out by Kaminsky *et al.*, (2008) indicated that the prevalence of *H. contortus* was more than 74% in both sheep and goats, and Kaplan *et al.*, (2004) confirmed that *H. contortus* was the primary and most predominant parasite recovered in the faecal cultures of small ruminants. Veglia (1915) indicated that the sun is the chief agent for the destruction of *H. contortus* eggs and larvae, under natural conditions of open grasslands in Southern Africa, and mortality increases with increased dryness of the soil and atmosphere.

The generation interval for *H. contortus* is 4-5 weeks. This is because, it takes at least a week for the egg to hatch and develop to infective larvae and then at least three weeks to develop to maturity and lay eggs after ingestion by small ruminants (Veglia, 1915). Therefore, deworming every 4-6 weeks is precisely what causes dewormer resistance, because each generation of worms is selected for dewormer resistance **(**Southern Consortium for Small Ruminant Parasite Control, 2006)**.** Moreover, there has been a rapid increase in anthelmintic resistance in most strains of *H. contortus*already, such that total failure of modern, broad spectrum dewormers is a reality in most regions in the tropics and sub-tropics (Waller, 2004).

The Howick strain of *H. contortus* has been characterized in Mpumalanga South Africa, and has shown to have multiple resistance to compounds from all five groups of anthelmintics; the benzimidazoles, levamisole-morantel, macro-cyclic lactones, organophosphorus, and the salicylanilides-substituted phenols (Van Wyk *et al.*, 1997). Consequently, where chemotherapy for the worm can no longer be used, more than 20% annual mortalities of animals in a kraal can be experienced (Waller, 2004).

MATERIALS AND METHODS

The small ruminants' population at the ranch comprised of 73 adult sheep and 39 adult goats (males and females). The study sample consisted of 15 sheep and 15 goats, all of which were adults irrespective of gender.

The 1st faecal samples were collected and analysed for faecal egg counts before treatment of the small stock with the drug Ecomectin (1% Ivermectin). The faecal egg count reduction tests were then carried out 10 days on the second faecal samples after treatment with the drug Ecomectin (1% Ivermectin). A 30 day period was allowed to elapse before the administration of the second drug, Virbac (1.92% Albendazole; 3.94% Closantel).The FAMACHA© chart which is an on-farm diagnosis for acute Haemonchosis was used to score the eye colours of the 30 animals in the test group. Small ruminants which had FAMACHA©eye scores of < 3 or ≥ 3 were dewormed. However, pregnant or lactating ewes and does (regardless of whether their eye scores were < 3 or≥ 3) and weaners, were not included in this study because their immunity is already compromised and they have diverse nutritional needs.

To perform faecal egg count reduction tests, faecal samples were collected before the small ruminants were treated 10 days after with an anthelmintic. Two grams of faeces were weighed and placed into a Duran bottle, into which 28ml of dH_2O was added to make a total volume of 30ml. Glass beads were added to assist in the crushing of the soaked faeces. After thoroughly mixing, the solution was strained through a sieve and the liquid collected into a 15ml centrifuge tube. To concentrate the eggs, the liquid was centrifuged at 1500 rpm for 3 minutes; after which the supernatant was discarded and a floatation medium ofsaturated sugar solution; was added to the level where the supernatant was.

The solution was mixed and enough liquid was drawn to fill half of the McMaster slides which were allowed to stand for 3 minutes to allow the eggs to float to the surface of the floatation medium and to

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come into contact with the upper glass of the counting chamber. Helminth eggs were viewed under low magnification; and those that appeared even on the grid lines in both halves of the slides were counted. The faecal egg counts were carried out using the McMaster's technique (sensitivity = 50 EPG; Gordon and Whitlock, 1939).

Data Analysis and Statistical Analysis

The EPG were calculated using the following equation:

$$\text{No. of EPG of faeces} = \frac{\text{No. of eggs counted} \times 30\text{ml (total volume)}}{0.3\text{ml (vol. of counting chamber)} \times 2\text{g}}$$

The shortcut conversion is: Total No. of eggs counted on both sides of slide × 50

To validate FMCH, the following equations were examined:

Sensitivity = (true positives / (true positives + false negatives)) × 100

Specificity = (true negatives/ (true negatives + false positives)) × 100

Predictive value of a negative = (true negatives/ (true -ves+ false -ves)) × 100

Predictive value of a +ve= (true +ves/ (true positives + false positives)) × 100

Spearman's rank correlation coefficients (r_s) were calculated to measure the association between FAMACHA© eye scores and fecal egg counts. Each small ruminant was analysed separately using the following equations:

At $\alpha = 0.05 \longrightarrow H_o$ rejected in a two-tailed test if $r_s > r_s^*$ or $r_s^* \geq r_s$,

r_s^* (p = 0.4429), n = 15

$$r_s = \frac{\sum x^2 + \sum y^2 - \sum d_i^2}{2\sqrt{(\sum x^2 \sum y^2)}}$$

To break the ties between the FMCH scores and FEC ranks which were large relative to the total observations, the following equations were used:

$T = t^3 – t / 12$ where t = the no. of observations that are tied for some particular rank

Therefore; $\sum x^2 = [(n^3 – n/12) - \sum T_x]$ and $\sum y^2 = [(n^3 – n/12) - \sum T_y]$

RESULTS

The prevalence of anaemia in sheep and goats and the justification of FAMACHA© as a screening test (Table 1 and Tables 4-7 below) were based on previous studies which proved that: for sheep, FMCH eye scores of 3 = 550 FECs or more, 4 = 1050 FECs or more and 5 = 2050 FECs or more and for goats, FMCH eye scores of 3 = 500 FECs or more, 4 = 550 FECs or more and 5 = 1000 FECs or more. In addition, a high prevalence of anaemia in goats is supported by the high sensitivity of FMCH in goats than in sheep before treatmentwith 93% and 83% in goats and sheep respectively (Table 4 and Table 6). However, the FMCH system proved to be highly specific in sheep than in goats after treatment, 22% and 0% in sheep and goats respectively, with a predictive positive values of 6.7% and 22% in sheep and goats respectively (Table 5 and Table 7).

Table 1: Prevalence (%) of Anaemia pre- and post-treatment with anthelmintics for both Sheep and Goats

Animal	Dewormers			
	Ecomectin (1% Ivermectin)		Virbac (1.92% Albendazole; 3.94% Closantel)	
	Before (%)	After (%)	Before (%)	After (%)
Sheep	67	60	40	18
Goat	73	80	93	7

Based on individuals with > 2000 EPG as a measure indicative value of the presence of Hc.

Table 2: Mean FEC (EPG) with minimum (min) and maximum (max) egg counts, pre- and post-treatment with anthelmintic

ANTHELMINTIC	SHEEP		GOAT	
	Mean FEC-pre (min/max)	Mean FEC-post (min/max)	Mean FEC-pre (min/max)	Mean FEC-post (min/max)
Ecomectin (1% Ivermectin)	2940 (300/8150)	1183 (0/6300)	993 (250/2800)	723 (0/1450)
Virbac (1.92% Albendazole; 3.94% Closantel)	1787 (0/9800)	395 (0/2800)	1683 (400/4800)	103 (0/650)

EPG > 500 indicate clinical anaemia with FMCH score=3.

Table 2 indicates a 75% worm infestation (>2000 EPG) in the flock and herd before treatment and after treatment, and in both scenarios the clinical level of EPG for *Haemonchus contortus*, which is 2000+, is outrun by the animals. The small stockhad either developed resistance (less than 50%) or not responding to Ivermectin containing an anthelmintic as evidenced in Table 3. However, Virbac is able to cause close to 80% worm reduction in a heavily infested animal. Table 8 and Table 9 show sufficiently insignificant ($p>0.200$) correlations between FMCH scores and FEC before and after deworming the animals. However, there is an indication of a positive association after treatment, with sheep showing a higher correlation than goats.

Table 3: Reduction level (%) revealing resistance to anthelmintics in Sheep and Goats

SPECIES	FECAL EGG COUNT REDUCTIONS (%)	
	Ecomectin (1% Ivermectin)	Virbac (1.92% Albendazole; 3.94% Closantel)
Sheep	44.5	79
Goat	-10.7	95

Ecomectin was not effective to protect the SS against *Haemonchus contortus*; A reduction level<50% means it is forever futile to be used on the SRs again.

Table 4: Validation of FAMACHA© based on eye scores and FEC, before treatment of Sheep with Virbac (1.92% Albendazole; 3.94% Closantel)

		TRUE STATUS		
		Anaemic	Not anaemic	Total
FAMACHA©	Positive	5	9	14
	Negative	1	0	1
	Total	6	9	15

Sensitivity=5/6 × 100=83%; Predictive value of +ve=5/14 ×100=36%

Table 5: Validation of FAMACHA© based on eye scores and FEC, after treatment of Sheep with Virbac (1.92% Albendazole; 3.94% Closantel)

		TRUE STATUS		
		Anaemic	Not anaemic	Total
FAMACHA©	Positive	2	7	9
	Negative	0	2	2
	Total	2	9	11

Sensitivity=2/2 × 100=100%; Predictive value of +ve=2/9 ×100=22%; Specificity=2/9 × 100=22%; Predictive value of –ve=2/2 ×100=100%

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Table 6: Validation of FAMACHA© based on eye scores and FEC, before treatment of Goats with Virbac (1.92% Albendazole; 3.94% Closantel)

		TRUE STATUS		
		Anaemic	Not anaemic	Total
	Positive	14	0	14
FAMACHA©	Negative	1	0	1
	Total	15	0	15

Sensitivity=14/15 × 100=93%; Predictive value of +ve=14/14 ×100=22%; Specificity=all diseased with FMCH scores > 4

Table 7: Validation of FAMACHA© based on eye scores and FEC, after treatment of Goats with Virbac (1.92% Albendazole; 3.94% Closantel)

		TRUE STATUS		
FAMACHA©		Anaemic	Not anaemic	Total
	Positive	1	14	15
	Negative	0	0	0
	Total	1	14	11

Sensitivity=1/1 × 100=100%; Predictive value of +ve=1/15 ×100=6.7%; r_s= 0.0745 indicating some positive correlation of FMCH scores and FECs

Table 8: Spearman's rank correlation coefficients (r_s) of FAMACHA© eye scores and corresponding FEC of Sheep pre- and post-treatment with Virbac (1.92% Albendazole; 3.94% Closantel) at α=0.05

	(r_s)	**Conclusion**	**p-value**
Before	-0.0521	H_o not rejected	p>0.200
After	0.414	H_o not rejected	p>0.200

Table 9: Spearman's rank correlation coefficients (r_s) of FAMACHA© eye scores and corresponding FEC of Goats pre- and post-treatment with Virbac (1.92% Albendazole; 3.94% Closantel) at α=0.05

	(r_s)	**Conclusion**	**p-value**
Before	-0.364	H_o not rejected	p>0.200
After	0.0745	H_o not rejected	p>0.200

DISCUSSION AND CONCLUSION

The study commenced on 21st of February 2013 until 2nd of April 2013. A pilot study on the FEC at the ranch was performed in one of the days of January 2013. During the time period of January to March, the rainfall recorded at the ranch was as follows: January; 12th=8mm, 13th=29mm, 14th=3mm, 23rd=35mm; February; 4th=11mm, 25th=6mm; March; 11th=15.5mm, 15th=28mm and 28th=26mm. These records illustrate the relatively low rainfall experienced at the ranch. Temperature, however, escalated to an average maximum of 38°C at the study site in Good Hope. Therefore, the weather conditions for the period of January to March 2013 had been significantly unfavourable to the development of *Haemonchus contortus*. Veglia (1915) confirms that the high temperatures experienced in the veld during the summer time undoubtedly helps to kill a large number of larvae, but at the same time many of these larvae are killed before the maximum temperature is even reached.

Nevertheless, some eggs were seen late in March. This is because, moisture had accumulated from the January and February rains, with the maximum rainfall recorded at the ranch being 35mm on the 23rd of January; and this contributed to the larvae reaching maturity and eggs being produced.The absence of worm eggs in faecal matter does not always mean no or few worms. This is because the generation interval of *Haemonchus contortus* is 4-5 weeks, therefore eggs of parasite worms appear depending on when a goat or sheep consumed the effective larvae as there has to be an elapse of 4-5weeks in order to be able to view the *Haemonchus contortus* eggs (Southern Consortium for Small Ruminant Parasite Control 2006). Table 8 and 9 indicated a positive correlation between FECs and eye scores. Furthermore, the correlation coefficients calculated indicated that there was no sufficient evidence to conclude on the association between FECs and eye scores ($p>0.200$). Conversely, for the negative correlation seen for pre-treatment of sheep and goats in Table 8 and Table9, there may be some instances where the inner membrane layer of the small ruminants appears redder than it normally would be and therefore, the FMCH tool would diagnose an animal as not anaemic even though it really were anaemic; a situation seen in (Table 4 and Table 6). Such instances may be due to hot and/or dusty conditions which irritate the eyes**,** fevers**,** infectious eye diseases and diseases associated with blood circulatory failures **(**Southern Consortium for Small Ruminant Parasite Control, 2006). Moreover, the negative correlations do not imply that there is no relationship existing between the two variables, FECs and eye scores.Other factors such as nutrition, weight, stocking rates, soil conditions, vegetation analysis and repetitive trials of research on the SRs and the study area, could also be considered to reveal whether FEC and FMCH scores were related or not.

Table 1 indicates that goats generally had a higher prevalence of *Haemonchus contortus* than sheep. These findings are particularly interesting because it is generally stated that sheep would have more *Haemonchus contortus* parasites since they are strictly grazers unlike the goats which browse and occasionally feed on grass. The study has also shown that FMCH tool is very applicable to goatsin identifying anaemic conditions by observing their eye membranes.

Previous studies by Burke *et al.,* (2007), Kaplan *et al.*, (2004) and Scheuerle *et al.*, (2010) on the validation of FMCH on the basis of FECs and the study of the prevalence of *Haemonchus contortus* using FECs showed that for sheep, FMCH eye scores were 3 = 550 FECs or more, 4 = 1050 FECs or more and 5 = 2050 FECs or more; and for goats, FMCH eye scores were 3 = 500 FECs or more, 4 = 550 FECs or more and 5 = 1000 FECs or more. This still emphasised the fact that sheep are more prone to infection by *Haemonchus contortus.* Additionally, FECs have been documented to display many limitations as a means of studying prevalence of parasite infections in animals (Amarante, 2000). Other causes of anaemia to be aware of include; hookworms, liver flukes, external parasites, blood parasites, bacterial and viral infections as well as nutritional deficiencies. However, the top most cause of anemia is *Haemonchus contortus* (Southern Consortium of Small Ruminant Parasite Control, 2006). Therefore, the drug-resistance *Haemonchus contortus* should be a concern to farmers, especially, as seen from Table 3 that, Ecomectin has become futile as a dewormer to the herd and flock of Good Hope ranch. If there was no resistance to the dewormer, 100% faecal egg count reduction would be observed. If less than 95% faecal egg count reduction exists, it means that resistance to the dewormeris starting to develop. The FMCH tool assists in identifying only those animals which require anthelmintic treatment.

Salicylanilide-Closantel, used in this study (Tables 1-9), is said to be effective against *Haemonchus contortus* and gives a protection from re-infection for 60 days. The sheep resistance to Virbac, as seen in Table 3, therefore poses a concern that resistance is starting to develop.

Furthermore, the study suggests that more investigations especially in the pharamaceutical industry should be done on goats because even though the newly discovered AADs (amino-acetonitrile derivatives) dewormers were confirmed to be effective against *Haemonchus contortus*, they were only tested on sheep (Kaminsky *et al.*, 2008). From this study, the *Haemonchus contortus* egg was discovered from the goats, and both goats and sheep coexist together in many farms.

Further work is also needed in the validation of FAMACHA© as a tool of prime importance in combating mortality losses due to *Haemonchus contortus* in order to assist and educate those involved with subsistence farming of small ruminant livestock.

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