Indian Journal of Animal Sciences **84** (11): 1177–1180, November 2014/Article
https://doi.org/10.56093/ijans.v84i11.44719

Sensitivity and specificity analysis for targeted selective treatment using eye colour chart in sheep flocks naturally infected with *Haemonchus contortus*

C P SWARNKAR[1] and D SINGH[2]

Central Sheep and Wool Research Institute, Avikanagar, Rajasthan 304 501 India

Received: 13 May 2014; Accepted: 18 July 2014

ABSTRACT

An eye color chart for screening the flock for anaemic sheep was developed and is in use since 2008 in organized farms in arid Rajasthan. In present analysis its accuracy (sensitivity and specificity) was analyzed in identifying anaemic sheep (PCV ≤18 or ≤22) and to quantify the proportion of sheep left untreated when using it for management of *Haemonchus contortus*. A linear trend was observed in proportion of true positives and level of FECs in all the seasons at both the PCV cut-off values. The percentage of correct treatment was maximum (71.3 to 83.4%) with moderate kappa index in individuals with intensity of infection ≥3001 epg. An inverse relation exists between sensitivity and specificity of the TST system. Based on criteria, the overall sensitivity of TST system ranged from 22.9% (criteria as FEC ≤1000 epg and PCV ≤18) to 90.1% (criteria as FEC ≥3001 epg and PCV ≤22). In visually anaemic animals, the sensitivity was lower when PCV cut-off ≤18% was considered positive test results. In highly infected animals, the sensitivity of TST system remained >85% during both monsoon season and winter. The overall specificity varied from 20.0 (criteria as FEC ≥3001 epg and PCV ≤22) to 81.5% (criteria as FEC ≤1,000 epg and PCV ≤18). Thus it appears that in sheep flocks predominantly infected with *H. contortus*, the application of the TST system as a guidance for the administration of anthelmintic drugs can be an important tool in management of GINs in Rajasthan.

Key words: Anaemia, Eye colour chart, *Haemonchus contortus*, Sensitivity, Sheep, Specificity, Targeted selective treatment

The control of infection with gastrointestinal nematodes (GINs) is largely based on preventive or therapeutic use of anthelmintic drugs. The frequent use of anthelmintics particularly during climatic extremes (Swarnkar and Singh 2010) exerted higher selection pressure for resistance in GINs due to nil or low level of *refugia* (Sotomaior *et al.* 2012, Swarnkar and Singh 2012, Swarnkar *et al.* 2012). Thus, the global problem of anthelmintic resistance in GINs, ensures that attention also needs to be given to the sustainability of anthelmintic treatment regimes as well as to their immediate economic benefit (Cringoli *et al.* 2008). The over-dispersion in faecal egg counts in the flocks (Singh and Swarnkar 2010) suggested that targeted and selective treatments of individual animals are the possibilities to reduce frequency of anthelmintic treatment and to maximise GIN populations in *refugia* (Soulsby 2007). There is currently a general agreement to replace the practice of treating the whole flock with targeted selective treatments (TST), where only animals showing clinical symptoms or reduced productivity are the given drugs (Vercruysse *et al.* 2009).

Present address: [1]Scientist (Selection Grade) (swarnkarcp @yahoo.com), [2]Principle Scientist (dherindra.singh56 @gmail.com), Division of Animal Health.

H. contortus worm burden has high positive correlation with the level of anaemia (Le Jambre 1995). Using this pathognomic clinical sign, an eye color chart based on FAMACHA (Malan *et al.* 2001, van Wyk and Bath 2002) principal was developed at Central Sheep and Wool Research Institute, Avikanagar (Rajasthan) and being employed since 2008 in organized farms in arid region (Singh and Swarnkar 2012). The present analysis was done to test the accuracy (sensitivity and specificity) of the eye color chart in identifying anaemic sheep (PCV ≤18 or ≤22) and to quantify the proportion of sheep at farm level left untreated when using the chart for management of *H. contortus*.

MATERIALS AND METHODS

The study was carried out from July 2008 to March 2014 at Sheep Breeding Farm, Fethepur and from July 2011 to October 2012 at Arid Region Campus, Central Sheep and Wool Research Institute, Bikaner. At monthly interval from July to March each year, all the Marwari and crossbred sheep (> 3 months of age, with average number of 207 - 485 / month / year) were categorized for conjunctiva colour using eye colour chart (Singh and Swarnkar 2012) in 5 categories as 1- red (non-anaemic), 2- red-pink (non-anaemic), 3- pink (mild anaemic), 4- pink-white (anaemic) and 5- white (severely anaemic). The faecal and blood samples were collected from sheep of category 4 and 5 and estimated intensity of strongyle infection (faecal egg count, FEC) by modified Mc Master technique (MAFF 1986) and packed cell volume (PCV) by standard procedure (Schalm *et al.* 1975), respectively.

For the calculation of sensitivity and specificity of TST system, FECs were used to check the adequacy of the TST scoring regarding egg excretion with the 3 cut-off values ≤1,000, 1,001–3,000 and ≥3,001 epg. Similarly, anaemia was measured by PCV as the gold standard and 2 cut-off values for PCV (≤18% and ≤22%, respectively) were assigned as no precise value for PCV has been clearly established at which anaemia crosses the threshold of clinical importance. The accuracy of TST system was estimated (Vatta *et al.* 2001, Thrusfield 2005) as follows:

Sensitivity = {True positives / (True positives + False negatives)} × 100
Specificity = {True negatives / (True negatives + False positives)} × 100
PVP = {True positives / (True positives + False positives)} × 100
PVN = {true negatives / (True negatives + False negatives)} × 100

Among anaemic animals, *kappa* (*k*) values were calculated to establish the association between FECs and PCV and agreements were ranked as very good (k= >0.80), good (k= 0.61–0.80), moderate (k=0.41–0.60), fair (k=0.21–0.40) and poor (k=≤0.20) as per Altman *et al.* (2000).

RESULTS AND DISCUSSION

On screening for conjunctiva color, under natural GIN infection (predominantly *H. contortus*), the monthly pattern for frequency distribution (Table 1) of sheep in flock showed an overall proportion of visually anaemic animals to the tune of 2.33% with maximum (2.88%) during monsoon followed by 2.33% during winter and minimum (1.32%) during spring season. Relatively higher numbers of anaemic sheep were identified from July to October. The low frequency of anaemic sheep in present study might be due to fact that flocks were maintained in good plane of nutrition which play a key role in immunity and resilience to GINs (Knox *et al.* 2006).

Table 1. Frequency (%) distribution for different categories of conjunctiva color in sheep

Factor	Number of observations	TST categories				
		1	2	3	4	5
Overall	24,403	4.09	24.94	68.64	2.07	0.26
Seasons						
Monsoon	10,094	4.18	24.58	68.36	2.48	0.4
Winter	7,856	4.05	24.07	69.35	2.13	0.2
Spring	5,453	4.00	26.87	67.82	1.25	0.07
Months						
July	3,201	2.91	27.21	67.38	2.16	0.34
August	3,357	5.03	23.01	69.33	2.29	0.34
September	3,356	4.50	23.71	68.30	2.98	0.51
October	3,398	4.09	23.81	69.63	2.21	0.26
November	2,326	4.51	24.29	68.97	2.06	0.17
December	2,132	3.47	24.25	70.08	2.06	0.14
January	1,570	3.69	23.89	71.4	1.02	0.00
February	1,905	4.25	29.29	65.36	1.00	00.1
March	1,978	3.99	26.90	67.34	1.67	00.1

Monsoon: July-September; winter: October-December; spring: January-March.

A linear trend was observed in proportion of true positives and level of FECs in all the seasons at both the PCV cut-off values. On classification of visually anaemic animals as per magnitude of intensity of strongyle infection, it was observed that percentage of correct treatment was maximum (71.3 to 83.4%) in individuals with intensity of infection ≥3,001 epg and it remained >65% in all the seasons (Table 2). In the present study, the kappa index indicated poor (criteria as FEC ≤1,000 epg) to moderate (criteria as FEC ≥3,001 epg) agreement between FECs and PCV in visually anaemic sheep. The proportions of false negatives were <10% with both PCV cut-off in sheep possessing FEC ≥3,001 epg (Table 2). A higher percentage of false negative and especially false positive scores with a considerably lower kappa value indicated that the TST scores were less accurate in animal with FECs ≤1,000 epg compared to FECs ≥3,001 epg. Therefore the lower accuracy for FECs ≤1,000 epg group did not lead to a higher risk of nematode related losses, but meant that more treatments than considered necessary were given. Ejlertsen *et al.* (2006) observed 0.7–1.6% false negative and 9.7–21.4% false positive scorings with anaemia chart in goats in Kenya.

An inverse relation was observed between sensitivity and specificity of the TST system (Table 3). The overall sensitivity of TST system ranged from 22.9% (criteria as FEC ≤1,000 epg and PCV ≤18) to 90.1% (criteria as FEC ≥3,001 epg and PCV ≤22). In highly infected animals, the sensitivity of TST system remained >85% during both monsoon season and winter. In visually anaemic animals, the sensitivity was lower when PCV cut-off ≤18% was considered positive test results. Under these conditions, however, the specificity of the results was higher. A high sensitivity of a clinical test is more important than high specificity (Sotomaior *et al.* 2012). When selecting the optimal cut-off for a positive test result there is normally a trade-off between minimizing the number of false-positive and the number of false-negative results. In TST system, a false-negative result means that an animal is in danger of dying because it is not treated, while a false-positive result means that an animal was treated without being in danger of dying. A low number of false-negative results are therefore preferred over a low number of false-positive results, since the first is much more acceptable (van Wyk *et al.* 2006). This is seen from the perspective that the aim of reducing the proportion of treated animals most likely still will be fulfilled compared to the practice of treating entire flocks at the same time and the cost of treatment is relatively low (Singh and Swarnkar 2012).

The overall specificity varied from 20.0% (criteria as FEC ≥3001 epg and PCV ≤22) to 81.5% (criteria as FEC ≤1,000 epg and PCV ≤18). Similarly, on perusal of earlier studies on FAMACHA© system, different percentage of

Table 2. Results (%) of TST based on different criteria of evaluation in anaemic sheep

Criteria (cut-off)		Season (n)	False negative	False positive	True negative	True positive	Correct treatment
FEC (epg)	PCV (%)						
≤1000	≤18	Overall (267)	24.0	12.7	56.2	7.1	63.3
		Monsoon (131)	20.6	13	61.1	5.3	66.4
(k value = 0.000)		Winter (97)	28.9	7.2	55.7	8.2	63.9
		Spring (39)	23.1	25.6	41	10.3	51.3
	≤22	Overall (267)	52.4	5.6	22.1	19.9	42.0
		Monsoon (131)	48.9	8.4	22.9	19.8	42.7
		Winter (97)	60.8	3.1	21.6	14.4	36.1
		Spring (39)	43.6	2.6	20.5	33.3	53.8
1001-3000	≤18	Overall (77)	31.2	13	36.4	19.4	55.8
		Monsoon (39)	20.5	12.8	43.6	23.1	66.7
(k value = 0.324)		Winter (27)	48.1	11.1	29.6	11.1	40.7
		Spring (11)	27.3	18.2	27.3	27.3	54.6
	≤22	Overall (77)	57.1	6.5	10.4	26.0	36.4
		Monsoon (39)	53.8	7.7	10.3	28.2	38.5
		Winter (27)	70.4	3.7	7.4	18.5	25.9
		Spring (11)	36.4	9.1	18.2	36.4	54.6
≥3001	≤18	Overall (157)	8.3	20.4	5.7	65.6	71.3
		Monsoon (98)	9.2	22.4	5.1	63.3	68.4
(k value = 0.554)		Winter (56)	5.4	17.9	7.1	69.6	76.8
		Spring (3)	33.3	0.0	0.0	66.7	66.7
	≤22	Overall (157)	8.9	7.6	1.9	81.5	83.4
		Monsoon (98)	11.2	9.2	3.1	76.5	79.6
		Winter (56)	3.6	5.4	0.0	91.1	91.1
		Spring (3)	33.3	0.0	0.0	66.7	66.7

(Correct treatment = true negative + true positive).

Table 3. Quality of TST system judged by FECs with three cut-offs and by PCV with two cut-offs in visually anaemic sheep naturally infected with GIN (predominantly with *H. contortus*)

Parameters	Cut-off criteria					
	FEC ≤1000 epg		FEC 1001-3000 epg		FEC ≥3001 epg	
	PCV ≤18	PCV ≤22	PCV ≤18	PCV ≤22	PCV ≤18	PCV ≤22
Sensitivity (%)						
Overall	22.9	27.5	38.5	31.3	88.8	90.1
Monsoon	20.6	28.9	52.9	33.3	87.3	87.2
Winter	22.2	19.2	18.8	20.3	92.9	96.2
Spring	30.8	43.3	50.0	50.0	66.7	66.7
Specificity (%)						
Overall	81.5	79.7	73.7	61.5	22	20
Monsoon	82.5	73.2	77.3	57.1	18.5	25
Winter	88.5	87.5	72.7	66.7	28.6	0
Spring	61.5	87.5	72.7	66.7	0	0
PVP (%)						
Overall	35.8	77.9	60.0	80.0	76.3	91.4
Monsoon	29.2	70.3	64.3	78.6	73.8	89.3
Winter	53.3	82.4	50.0	83.3	79.6	94.4
Spring	28.6	92.9	60.0	80.0	100	100
PVN (%)						
Overall	70.1	29.6	53.8	15.4	40.9	17.6
Monsoon	74.8	31.9	68.0	16	35.7	21.4
Winter	65.9	26.3	38.1	9.5	57.1	0.0
Spring	64.0	32.0	50.0	33.3	0.0	0.0

sensitivity (70–100%) and specificity were found depending on the criteria employed, management system, evaluator's experience and prevalence of anaemic animals in flock (Reynecke *et al*. 2011, Sotomaior *et al*. 2012). The sensitivity increased with both PCV cut-off as the intensity of infection increases, indicating that it may reach 100% and the number of false negatives could drop to zero if a PCV of ≤18 or ≤22% with intensity of infection >3000 is considered as the cut-off level in anaemic animals are considered positive. Using FAMACHA category and PCV as criteria, Kaplan *et al.* (2004) reported higher sensitivity with PCV ≤15% with FAMACHA categories 3–5 and recommended these animals for routine treatment. In the present study, the number of false positive was relatively higher when PCV cut-off was ≤18% in all the infection levels, further indicating that by keeping PCV cut-off ≤18%, 7–8% animals were additionally drenched compared to PCV cut-off ≤22%, thereby reducing chance of any further complication in flock.

From the present study it appeared that in sheep flocks predominantly infected with *H. contortus*, the application of the TST system as a guidance for the administration of anthelmintic drugs can be an important tool in management of GINs in Rajasthan. Here, a simple checking of the conjunctiva color according to the TST system could prevent heavy infections and even from production and mortality losses in addition to significant reduction in use of anthelmintics.

ACKNOWLEDGEMENTS

The study was supported by ICAR, New Delhi under All India Network Programme on Gastrointestinal Parasitism. The facilities provided by Director, CSWRI, Avikanagar, Assistant Director and Dr S K Bisaria, Senior Veterinary Officer, Sheep Breeding Farm are thankfully acknowledged.

REFERENCES

Altman D G, Machin D, Bryant T N and Gardner M J. 2000. *Statistics with Confidence*. 2nd edn, p. 240. BMJ Books, London

Cringoli G, Veneziano V, Jackson F, Vercruysse J, Greer A W, Fedele V, Mezzino L and Rinaldi L. 2008. Effects of strategic anthelmintic treatments on the milk production of dairy sheep naturally infected by gastrointestinal strongyles. *Veterinary Parasitology* **156**: 340–45.

Ejlertsen M, Githigia S M, Otieno R O and Thamsborg S M. 2006. Accuracy of an anaemia scoring chart applied on goats in sub humid Kenya and its potential for control of *Haemonchus contortus* infections. *Veterinary Parasitology* **141**: 291–301.

Kaplan R M, Burke J M, Terrill T H, Miller J E, Getz W R, Mobini S, Valencia E, Williams M J, Williamson L H, Larsen M and Vatta A F. 2004. Validation of the FAMACHA eye color chart for detecting clinical anaemia in sheep and goats on farms in the southern United States. *Veterinary Parasitology* **123**: 105–20.

Knox M R, Torres-Acosta J F J and Aguilar-Caballero A J. 2006. Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastrointestinal nematodes. *Veterinary Parasitology* **139**: 385–93.

Le Jambre L F. 1995. Relationship of blood loss to worm numbers, biomass and egg production in *Haemonchus contortus* infected sheep. *International Journal for Parasitology* **25**: 269–73.

MAFF. 1986. *Manual of Veterinary Parasitological Techniques.* Vol. 18. Ministry of Agriculture, Fisheries and Food, London.

Malan F S, Van Wyk J A and Wessels C D. 2001. Clinical evaluation of anaemia in sheep: early trials. Onderstepoort Journal of Veterinary Research **68**: 165–74.

Reynecke D P, van Wyk J A, Gummow B, Dorny P and Boomker J. 2011. Validation of the FAMACHA© eye colour chart using sensitivity / specificity analysis on two South African sheep farms. *Veterinary Parasitology* **177**: 203–11.

Schalm O W, Jain N C and Carroll E J. 1975. *Veterinary Haematology*. 3rd edn, pp 15–81. Lea and Febiger, Philladelphia, USA.

Singh D and Swarnkar C P. 2010. Harnessing the advantages of over-dispersion in faecal egg counts through targeted selective treatment and breeding for diseases resistance. In: XX National Congress of Indian Association for the Advancement of Veterinary Parasitology, CCS HAU, Hisar, Haryana, 18 - 20 February.

Singh D and Swarnkar C P. 2012. Evaluation of targeted selective treatment strategy in sheep farm of Rajasthan. *Indian Journal of Animal Sciences* **82**: 679–86.

Sotomaior C S, Rosalinski-Moraes F, de Costa A R B, Maria D, Monteiro A L G and van Wyk J A. 2012. Sensitivity and specificity of the FAMACHA© system in Suffolk sheep and cross Boer goats. *Veterinary Parasitology* **190**: 114–19.

Soulsby L. 2007. New concepts in strongyle control and anthelmintic resistance: the role of refugia. *Veterinary Journal* **174**: 6–7.

Swarnkar C P and Singh D. 2010. Questionnaire Survey on sheep husbandry and worm management practices adopted by farmers in Rajasthan. *Indian Journal of Small Ruminants* **16**: 199–209.

Swarnkar C P and Singh D. 2012. Seasonal variation in efficacy of anthelmintics and prevalence of anthelmintic resistance in gastrointestinal nematodes of sheep in Rajasthan *Indian Journal of Animal Sciences* **82**: 451–56.

Swarnkar C P, Singh D and Kumar S. 2012. Prevalence of benzimidazole resistant β-tubulin alleles in *Haemonchus contortus* larvae from sheep of Rajasthan. *Indian Journal of Animal Sciences* **82**: 1477–81.

Thrusfield M V. 2005. *Veterinary Epidemiology*. 3rd edn., Blackwell, Cambridge.

Van Wyk J A and Bath G F. 2002. The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Veterinary Research* **33**: 509–29.

van Wyk J A, Hoste H, Kaplan R M and Besier R B. 2006. Targeted selective treatment for worm management - how do we sell rational programs to farmers? *Veterinary Parasitology* **139**: 336–46.

Vatta A F, Letty B A, van der Linde M J, van Wijk E F, Hansen J W and Krecek R C. 2001. Testing for clinical anaemia caused by *Haemonchus* spp. in goats farmed under resource-poor conditions in South Africa using an eye colour chart developed for sheep. *Veterinary Parasitology* **99**: 1–14.

Vercruysse J, Jackson F, Besier B and Pomroy B. 2009. Novel solutions for the sustainable control of nematodes in ruminants (PARASOL). *Veterinary Parasitology* **164**: 1–2.