*Indian Journal of Animal Sciences* **82** (7): 679–686, July 2012

# Evaluation of targeted selective treatment strategy in sheep farm of Rajasthan

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Received: 27 July 2011; Accepted: 21 December 2011

## ABSTRACT

The study was undertaken to develop and evaluate a colour chart to identify degree of anaemia in sheep of Rajasthan for management of haemonchosis and maintaining the anthelmintic susceptibility in population of parasites for longer period. Initially each sheep in a flock was monitored at monthly interval for correlating the intensity of *Haemonchus contortus* infection and blood profile (packed cell volume and haemoglobin). At each occasion photographs of conjunctivae were taken and a colour chart was developed (indicating 1–5 degree of conjunctiva colour) to indicate packed cell volume and anaemic status of the sheep. Based on the colour chart, the animals falling in category 1 to 3 were considered as normal while those falling in category 4 and 5 were considered as anaemic. The correlation between faecal egg count and degree of anaemia was positive in wormy season. The colour of conjunctiva was found to have negative correlation with haematological values. In subsequent years the colour chart was applied to all sheep flocks at Sheep Breeding Farm, Fatehpur (Rajasthan). It was observed that monthly proportion of sheep falling in category 4 or 5 were maximum of 17.35% in the month of September, 2008. The system was suitable in detecting the susceptible animals during wormy season. With this approach of targeted selective treatment (TST), the average frequency of anthelmintic drench in flock was 22.93±4.03% per annum compared to > 100% in conventional strategic treatment. In conclusion, though TST approach require some time and labour investment for selecting the animals for treatment but significantly reduced the cost of anthelmintic drenching as well as selection pressure for anthelmintic resistance.

**Key words**: Anaemia, Anthelmintic resistance, Haemonchosis, Sheep, Targeted selective treatment

Based on sheep population (Census 2003), the annual losses due to gastrointestinal nematodes (GIN) alone have been estimated to the tune of ₹ 1191.708 million in sheep flocks of Rajasthan (Singh *et al*. 2011). The rampant emergence of anthelmintic resistance in parasites (Gasbarre *et al*. 2009) with resultant inability to rely upon chemotherapeutic measures poses a significant threat to the sustainability of sheep production. The present situation necessitates the development of novel strategies for management of worms and anthelmintic resistance. The need to conserve the efficacy of currently available drugs and any novel drug to be discovered in future must be balanced with the ability to maintain high levels of animal production and welfare. One approach which has been the focus of recent research and debate is to maintain a parasite population in *refugia* (unexposed to drug), in order to maintain both phenotypic and genotypic susceptibility (Soulsby 2007). The occurrence of over-dispersion phenomenon in faecal egg counts (Sreter *et al*. 1994, Swarnkar *et al*. 2008), within flock variation (Swarnkar *et al*. 2009, Prince *et al*. 2010) and rising importance of *refugia* in management of anthelmintic resistance (van Wyk *et* al. 2006, Singh and Swarnkar 2008) led to evolution of techniques for targeted selective treatment (TST) of the most affected and/or the most anaemic animals to prevent/delay the development of drug resistance (Moors and Gauly 2009). There is a consensus among parasitologists to leave a proportion of the worm population unexposed to treatment to maintain anthelmintic susceptibility and/or to slow the development of resistance (Cringoli *et al*. 2009) and this approach is central within the concept of TST.

There are several methods that have been suggested to target or selectively target for anthelmintic treatments (Besier 2008). Patho-physiological markers such as anaemia and dag score, parasite-based markers such as faecal egg count (FEC) and production indices such as milk production and live weight gain (Bisset *et al*. 2001, van Wyk and Bath 2002, Riley and van Wyk 2009) have been examined as potential markers for TST regimes and in theory, wool production and body condition score could also be included for the same (van Wyk and Bath 2002, Riley and van Wyk 2009). *H. contortus* worm burden has high positive correlation with the level of anaemia (Le Jambre 1995) and using this

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pathognomic clinical sign, FAMACHA© chart was developed by Malan *et al*. (2001) in South Africa. Beside South Africa, this approach has been studied in Brazil (Molenta *et al*. 2004), Germany (Koopmann *et al*. 2006), Ethiopia (Sissay *et al*. 2007), US (Burke *et al.* 2007), Italy (Loria *et al*. 2009) etc and was found suitable only when there was predominance of *H. contortus* (Kaplan *et al*. 2004, Ejlertsen *et al*. 2006). With the application of TST, a reservoir of susceptible worms remains within the population and consequently diluting the resistant alleles (Hoste *et al*. 2002). Gauly *et al*. (2004) questioned the stated practicability of FAMACHA© as it was prepared on the basis of result obtained from Merino or crossbreds. Therefore, the present study was initiated to evaluate the practicability of TST in management of haemonchosis among native breeds of sheep in Rajasthan.

## MATERIALS AND METHODS

*Formulation of mechanism for targeted selective treatment in flock against* H. contortus: The study was undertaken at Sheep Breeding Farm, Fatehpur (Sikar) situated in arid agro-climate of Rajasthan. A flock of 34 sheep (6–9 month old) naturally infected with gastrointestinal nematodes (predominantly with *H. contortus*) and grazed on naturally contaminated pasture were monitored at monthly interval for color of ocular mucous membrane and on each occasion photographs of individual's conjunctiva were taken from August 2007 to March 2008. Based on the photographs, intensity of infection and haematocrit value a colour chart (consisting of 5 different grades of colour) was prepared and was applied to study its efficacy in adult sheep from July 2008 to March 2011. From July, all the adult sheep were screened at monthly interval for color of ocular mucous membrane, and visually anaemic animals were evaluated for parasitological and haematological parameters.

*Observations:* On the basis of conjunctiva color chart, the sheep were categorized in to five scales (1 to 5) as 1: red (non-anaemic), 2: red-pink (non-anaemic), 3: pink (mild anaemic), 4: pink-white (anaemic) and 5: white (severely anaemic) at monthly interval. For each occasion average eye colour score was ascertained for flock by multiplying the eye colour score with number of animals in flock. Simultaneously each animal was tested for intensity of strongyle infection by faecal egg count (FEC) following modified Mc Master technique (MAFF 1986) and for packed cell volume (PCV) and haemoglobin (Hb) concentration by standard procedure (Schalm *et al*. 1975). Pooled faecal samples were subjected to culture on each occasion to know the proportion of prevalent strongyle species (Soulsby 1965). The observations on peri-parturient rise in FEC in sheep under TST approach were undertaken from December 2008 and lactating ewes were monitored up to March 2009. All the recently lambed and lactating ewes were screened by examining the color of conjunctiva at monthly interval and placed into 5 categories according to color chart. The observations were also recorded for FEC, PCV and Hb in anaemic animals (category 4 and 5) and for only FEC in normal (category 1 to 3) animals. The effect of evaluators on screening of sheep for visual anaemia by color chart was determined through evaluating the same flock of sheep by two independent evaluators. The flock was first screened by local Veterinary Officer (evaluator 1) from SBF, Fatehpur followed by a Scientist (evaluator 2) from CSWRI, Avikanagar. In addition, the impact assessment of TST approach was also studied by comparing the flock performance under TST and targeted treatment (whole flock treatment).

*Statistical analysis*: The data were analyzed by analysis of variance using SPSS ver 15. Pearson's correlation coefficient was determined to know the correlation between FEC, PCV or Hb and status of anaemia (based on eye colour).

## RESULTS AND DISCUSSION

The feasibility of grading the degree of clinical anaemia in conjunctival mucosae was investigated by photographing the ocular mucous membrane and determining the PCV in sheep. A practical full coloured chart with illustration was prepared to assist on-farm evaluations depicting conjunctival mucosae of sheep in the five PCV ranges. The chart show the expected colour of conjunctival mucosae of sheep with PCV of >32% (category 1), 28–32% (category 2), 23–27% (category 3), 18–22% (category 4) and <18% (category 5).

*Formulation of mechanism for targeted selective treatment*

*Magnitude of FEC, PCV and Hb in visually anaemic and normal sheep*: The larval composition on coproculture indicated predominance of *H. contortus*. The mean FEC remained significantly ($P<0.001$) higher (~ 3 times) in visually anaemic group compared to visually normal group during wormy season (August-Nov). However, during non-wormy season (Dec-March) the intensity of strongyle infection remained at acceptable level (< 500 epg) in both the groups (Table 1). The mean PCV ranged from 18.50±0.50% (March) to 27.20±1.24% (August) in visually anaemic group and from 24.67±0.65% (March) to 31.84±0.71% (October) in visually normal group indicating significantly ($P<0.05$) lower values in anaemic group during both wormy and non-wormy season. Similar pattern was observed for haemoglobin (Table 1). The correlation between FEC and degree of anaemia (based on eye colour) was strongly positive in wormy season. The colour of ocular mucous membrane was found to have negative correlation with the PCV and Hb values (Table 2). Based on correlated response, PCV and colour of ocular mucous membrane were considered as suitable determinants to develop mechanism for TST approach in flock. A significantly negative correlation was observed between eye score and PCV and Hb (Sissay *et* al. 2007, Loria *et al*. 2009), while correlation between eye score and FEC was positive (Loria *et al*. 2009).

Table 1. Mean (±SE) FEC, PCV and Hb in sheep naturally infected with strongyle worms and grouped according to colour of ocular mucous membrane

| Month | FEC (epg) | | PCV (%) | | Hb (g%) | |
|---|---|---|---|---|---|---|
| | Anaemic | Normal | Anaemic | Normal | Anaemic | Normal |
| Aug | 2486.7±393.3 | 855.6±175.8 | 27.20±1.24 | 31.32±0.72 | 8.80 ±0.35 | 10.12±0.17 |
| Oct | 2553.8±472.2 | 890.5±257.2 | 25.08±0.75 | 31.84±0.71 | 8.88±0.24 | 10.37±0.29 |
| Nov | 1238.9±265.0 | 587.5±158.1 | 23.24±1.11 | 27.56±0.70 | 7.41±0.34 | 8.69±0.16 |
| Dec | 462.5 ±164.6 | 221.7±53.3 | 21.63±1.05 | 27.78±0.56 | 6.63±0.46 | 8.78±0.14 |
| Jan | 557.1±220.9 | 188.0±51.7 | 18.71±1.39 | 26.16±0.77 | 7.34±0.48 | 9.23±0.28 |
| Feb | 71.4±35.9 | 150.0±48.2 | 21.43±0.99 | 27.64±0.57 | 7.71±0.34 | 9.34±0.24 |
| Mar | 100.0±100.0 | 188.5±53.6 | 18.50±0.50 | 24.67±0.67 | 7.25±0.25 | 8.04±0.13 |

Table 2. Pearson's correlation coefficient between visual anaemia and FEC/PCV/Hb in sheep

| | Month | | | | | | | Overall |
|---|---|---|---|---|---|---|---|---|
| | Aug | Oct | Nov | Dec | Jan | Feb | Mar | |
| Anaemia v/s FEC | 0.585 | 0.512 | 0.379 | 0.323 | 0.411 | - 0.168 | - 0.087 | 0.444 |
| | (0.001) | (0.002) | (0.030) | (0.077) | (0.019) | (0.384) | (0.658) | (0.001) |
| Anaemia v/s PCV | - 0.471 | - 0.760 | - 0.505 | - 0.710 | - 0.639 | - 0.718 | - 0.482 | - 0.452 |
| | (0.005) | (0.001) | (0.003) | (0.001) | (0.001) | (0.001) | (0.130) | (0.001) |
| Anaemia v/s Hb | - 0.546 | - 0.557 | - 0.511 | - 0.744 | - 0.510 | - 0.549 | - 0.336 | - 0.424 |
| | (0.001) | (0.001) | (0.002) | (0.001) | (0.003) | (0.002) | (0.093) | (0.001) |

Figures in parenthesis indicate P - values for correlation.

*Comparison of extent of anaemia based on visual inspection of eye and actual PCV value*: Out of 243 observations, a total of 28.4% of the estimates lies out of the corresponding categories. However, 21.4% of the mistakes were not serious as PCV values of the animals were not low enough (> 22%) to merit drenching (Table 3) and in these, anaemia was less severe than estimated compared to 7.00% in which the anaemia was more severe and rated as normal based on colour of ocular mucous membrane.

It is a known phenomenon that during the course of fetal haemonchosis, the colour of the conjunctiva of sheep changes from red (of healthy sheep) through sheds of pink to practically white as a result of a progressively worsening anaemia in infected ones. Thus, in order to bridge the gap between ability to identify anaemic animals more accurately on visual inspection and to obtain similarity among results with different observers, a colour chart was prepared from the available photographs by categorizing colour of ocular mucous membrane of sheep in to 5 groups based on PCV value (Fig. 1).

*Impact of TST on drench frequency*: Based on colour of ocular mucous membrane and PCV values, in the year 2007–08, out of 34 animals, only 3 (8.8%) were drenched with anthelmintic during wormy season. However, in non-wormy season a grater proportion of animals (20–40%) fall in anaemic categories (4 and 5) but the intensity of infection (< 500 epg) indicated that this decline in PCV was not attributable to haemonchosis and hence animals were not drenched during that period.

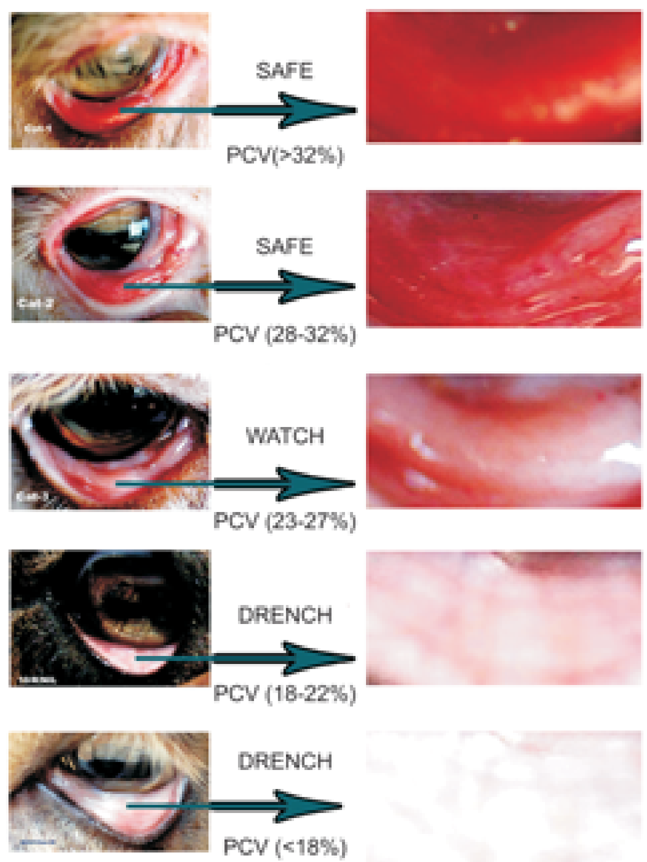


Fig. 1. Colour chart for visual screening of sheep in different level of anaemia due to haemonchosis.

Table 3. Success rates for visual assessment of anaemia in sheep

| | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Overall |
|---|---|---|---|---|---|---|---|---|---|
| No. of observations | 33 | 28 | 31 | 33 | 31 | 32 | 29 | 26 | 243 |
| Correct grouping (%) | 63.64 | 42.86 | 67.74 | 63.64 | 90.32 | 81.25 | 93.10 | 69.23 | 71.60 |
| Too high estimate (%) (Normal v/s anaemic) | 0.00 | 3.57 | 0.00 | 3.03 | 3.23 | 18.75 | 0.00 | 30.77 | 7.00 |
| Too low estimate (%) (Anaemic v/s normal) | 36.36 | 53.57 | 32.26 | 33.33 | 6.45 | 0.00 | 6.90 | 0.00 | 21.40 |

*B. Implementation of TST in sheep flock*

*Status of anaemia on the basis of conjunctiva colour in adult sheep during 2008-11*: As depicted in Fig. 2, the average monthly eye colour score for flock (on 5 point scale) during the period of 3 years ranged between 2.07 (March 2011) and 3.01 (September 2008). Moors and Gauly (2009) observed that mean FAMACHA score in sheep flocks ranged between 1.91±0.94 to 2.25±1.01. The results of screening on the basis of colour chart (Fig. 1) exhibited that monthly proportion of sheep falling in category 4 or 5 (anaemic) varied from nil (Jan to March 2011) to 17.35% (Sep 2008). In the present study, relatively increase in proportion of visually anaemic sheep was started after mid monsoon and reached to peak up to end of monsoon (Fig. 3). In subsequent seasons due to unfavourable climatic conditions for translation of exogenous stages of strongyle worms, the intensity of infection remained quite low and unable to produce anaemia (Swarnkar and Singh 2011).

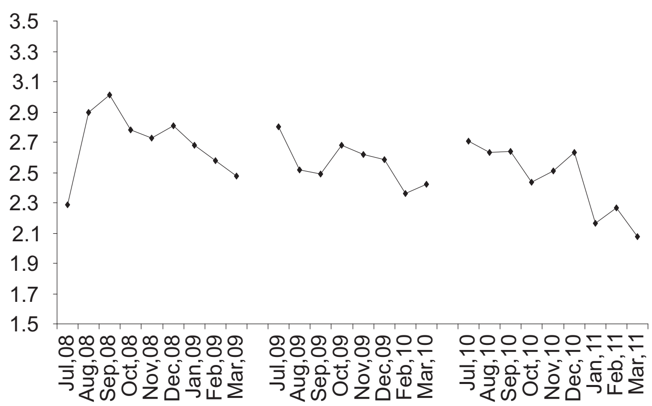


Fig. 2. Monthly mean eye colour score for sheep flocks maintained on TST for worm management.

Further more, it was interesting to observe that lambing and lactation stress failed to increase the FEC as well as proportion of anaemic animals in flock due to unsuitable climatic conditions which is in agreement with the reported absence of typical peri-parturient rise in faecal egg counts in sheep from Rajasthan (Swarnkar *et al*. 1998, 2008). The animals found anaemic were given extra nutritional care till they recovered as adjudged by colour of conjunctiva. In the year 2010–11, due to en-mass culling during Nov-Dec, the remaining sheep had the opportunity to graze on pasture with less stocking density, resulted in better nutritional management of animals and dilution of pasture infectivity leading to absence of anaemia as well as reduced intensity of strongyle infection (Swarnkar and Singh 2005).

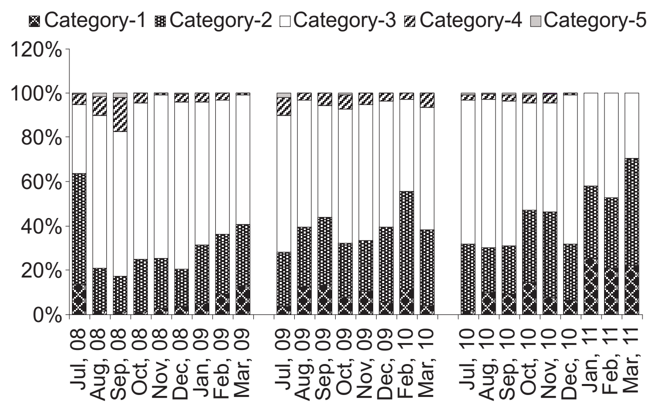


Fig. 3. Frequency distribution (%) of adult sheep in flocks according to conjunctiva colour.

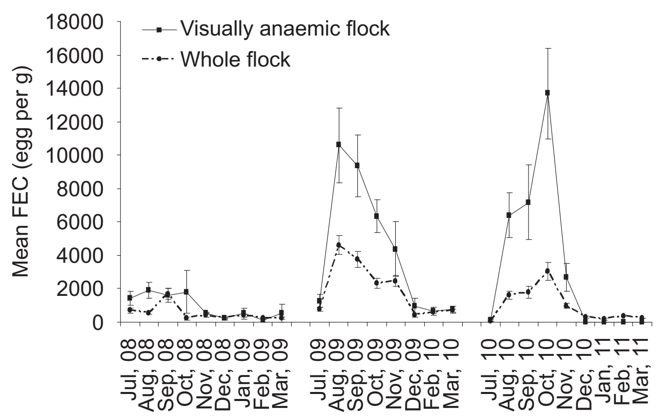


Fig. 4. Mean (±SE) intensity of strongyle infection in anaemic sheep naturally infected with strongyle worms and screened on the basis colour of conjunctiva.

*Magnitude of FEC in visually anaemic adult sheep*: The mean FEC for animals categorized as anaemic on the basis of colour chart (category 4–5) ranged from nil (Dec, 10) to 13681.0±2704.9 (Oct, 10). On comparison of intensity of strongyle infection between whole flock and visually anaemic flock, it was observed that magnitude of FEC remained significantly higher in anaemic group during wormy season (July to November) while during other months it remained almost comparable (Fig. 4).

*Haematological profile of visually anaemic adult sheep*: As depicted in Fig. 5, the mean PCV values in anaemic animals ranged from 14.48±1.12% (October, 10) to 24.75±1.96% (July, 10). Similarly mean Hb concentration varied from 5.24±0.26 (Oct, 10) to 8.75±0.25 g% (March, 09). Further it was observed that anthelmintic treatment along with better nutritional care through reduced stocking pressure of anaemic animals contributed in restoration of the haematological values.

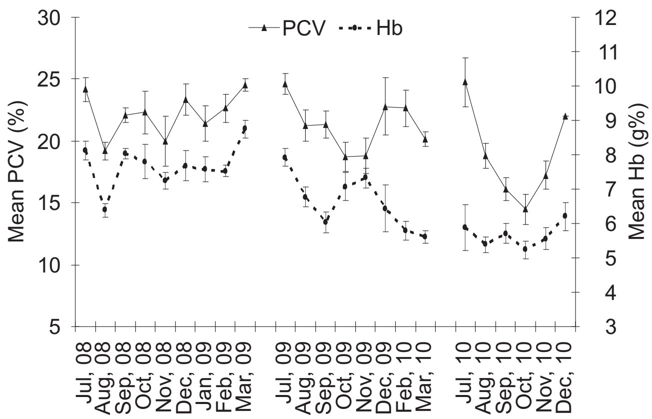


Fig. 5. Haematological values in adult sheep naturally infected with strongyle worms and screened on the basis colour of conjunctiva.

*Observations on peri-parturient rise in FEC in sheep under TST approach*: The magnitude of FEC revealed that there was absence of peri-parturient rise in FEC in sheep flocks of arid region. Similar observations were earlier reported from farm and field flocks of both arid and semi-arid agro-climate of Rajasthan (Singh *et al*. 1997, Swarnkar *et al*. 1998, 2008). The mean FEC in anaemic group ranged from 660.0±448.2 (dry stage) to 125.0±75.0 (3–7 week of lactation) compared to 334.6±99.4 (dry stage) and 218.8±53.4 (3–7 week of lactation) in visually normal ewes (Table 4). The PCV and Hb values in anaemic group remained within normal limit. The study suggested that there is no need of anthelmintic treatment in lactating ewes even if they appear anaemic.

*Effect of evaluators on status of anaemic proportion in flock based on conjunctiva colour*: It has been observed that difference among two evaluators in detecting the anaemic animals in sheep flocks (based on eye colour chart) was higher at initial (July) phase which became marginalized in succeeding months (Table 5). The study demonstrated application feasibility of eye colour chart by different evaluator. Wolstenholme *et al*. (2004) emphasized that only animals that suffer from parasite should be treated, while animals that tolerate infection should not be treated. Thus TST involves application of curative treatment through individual assessment without causing loss in productivity or economic damage (Miller and Waller 2004). The evaluation of colour of ocular mucosae can be influenced by conditions such as vasoconstriction due to stress following

Table 4. Mean (±SE) parasitological and haematological observations for sheep in different stages of lactation and maintained under TST approach

| Stage of lactation | Anaemic sheep (conjunctiva colour category, 4–5) | | | | Normal sheep (conjunctiva colour category, 1–3) | | | |
|---|---|---|---|---|---|---|---|---|
| | No. | FEC (epg) | PCV (%) | Hb (g%) | No. | FEC (epg) | PCV (%) | Hb (g%) |
| Dry | 6 | 660.0±448.2 | 20.20±1.68 | 7.04±0.32 | 26 | 334.6±99.4 | - | - |
| 1–3 wk | 6 | 333.3±138.2 | 23.83±1.19 | 7.95±0.36 | 41 | 265.9±34.3 | - | - |
| 3–7 wk | 4 | 125.0±75.0 | 21.50±1.32 | 7.63±0.55 | 16 | 218.8±53.4 | - | - |
| 7–12 wk | 6 | 183.3±183.3 | 24.17±0.79 | 8.05±0.28 | 37 | 264.9±64.1 | - | - |

Table 5. Comparative proportion (%) of sheep based on conjunctiva colour by two evaluators

| Month | Evaluator | No. screened | Category | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | Non- anaemic (1 to 3) | 4 | 5 | Anaemic (4 to 5) |
| Jul | 12 | 214 | 3.273.27 | 16.8221.50 | 56.5466.82 | 76.6491.59 | 21.966.54 | 1.401.89 | 23.368.41 |
| Aug | 12 | 259 | 16.6016.69 | 25.1025.10 | 54.4454.83 | 96.1496.52 | 3.863.09 | 0.000.39 | 3.863.48 |
| Sep | 12 | 211 | 20.3820.38 | 36.5036.50 | 39.3439.81 | 96.2196.68 | 3.793.32 | 0.000.00 | 3.793.32 |
| Oct | 12 | 212 | 8.498.49 | 26.8926.89 | 57.3559.43 | 92.9294.81 | 7.084.72 | 0.000.47 | 7.085.19 |
| Nov | 12 | 205 | 10.7310.73 | 18.5418.54 | 61.9564.39 | 91.2293.36 | 8.295.85 | 0.490.49 | 8.786.34 |
| Dec | 12 | 179 | 3.913.91 | 32.4032.40 | 57.5459.22 | 93.8595.52 | 6.154.47 | 0.000.00 | 6.154.47 |
| Feb | 12 | 183 | 10.3810.38 | 51.9151.91 | 35.5235.52 | 97.8197.81 | 2.192.19 | 0.000.00 | 2.192.19 |
| Mar | 12 | 187 | 1.071.07 | 35.8335.83 | 58.8259.89 | 95.7296.79 | 4.282.67 | 0.000.54 | 4.283.21 |

Table 6. Comparative performance for targeted treatment and TST approaches for worm control in organized sheep farm of arid Rajasthan

| | Targeted treatment | TST |
|---|---|---|
| Total number of sheep | 5263 | 1081 |
| Lambing (%) | 65.08 | 79.78 |
| Morbidity (%) | 34.52 | 20.48 |
| Mortality (%) | 7.94 | 6.63 |
| Disposal (%) | 16.14 | 9.19 |
| Annual GFY (kg/head) | 1.265 | 1.877 |
| No. of anthelmintic doses used/year | 8466 | 165 |
| Cost of anthelmintic intervention (₹) | 50796.0 (@ 6.00/dose) | 990.0 (@ 6.00/dose) |
| Cost of anthelmintic/annum (₹) | 9.65/head | 0.92/head |

the catch of the animals, local inflammation etc (Loria *et al.* 2009).

*Impact of TST on flock performance*: The estimated comparative performance of TST and en-mass targeted treatment exhibited that over the period of 3 years, the frequency of anthelmintic treatment under TST was 29.31% in 2008–09 which reduced to 23.99% in 2009–10 and further to 15.50% in 2010–11 with an average of 22.93±4.03% per annum. During the period of 3 years no adverse effect of TST were observed on flock performance (Table 6). The cost of anthelmintic intervention in flock was only 10% of that incurred with targeted treatment particularly due to reduced use of anthelmintics. With the use of FAMACHA© method, Molento *et al*. (2009) in Brazil, drenched anthelmintic to 9.5±3.3% of ewes in flocks in a span of one year. Gaba *et al.* (2010) observed 90% reduction in the number of anthelmintic treatments with TST compared to mass treatment. Similarly 40–60% reduction in drench frequency was recorded by Cringoli *et al.* (2009) and Gallidis *et al*. (2009). Further, it was found that TST 20% and TST 30% of the flock were efficient both at maintaining anthelmintic resistance at low level and controlling nematode parasite infections. With TST approach, van Wyk (2001) observed reduction in selection pressure on the parasite populations when compared with preventive treatment. During the 5 years of study, Molento *et al*. (2009) observed no significant difference in body weight, fertility and mortality and recorded 75.3% to 89.4% reduction in losses with TST compared with suppressive treatments. The cost of anthelmintics was reduced by 58% (38–96%) with the application of FAMACHA© method (Bath and van Wyk 2001).

Although farmers and veterinarians are naturally more concerned with the short-term economic, production and parasitological benefits accruing from prophylactic anthelmintic treatments, it is important that attention has to be given on the sustainability of anthelmintic treatment regimes. The acceptance by producers of TST strategies and their implementation may require a high level of input and education to the farming community. It is crucial that practitioners/farmers and advisors are made aware not only of the cost benefits, and potential disadvantages, accruing from a TST approach but also those associated with extreme multi-drug resistance (Kenyon *et al*. 2009). The use of TST thus requires some time investment for selecting the animals for treatment and the acceptance that a reduction in the number of treatments is necessary, based either on the fact that levels of resistance in gastrointestinal nematodes will decrease due to reduced selective pressure (Gaba *et al*. 2006) or that limited use of anthelmintics is more environment friendly and accords with organic production regulations.

## ACKNOWLEDGEMENTS

The authors are grateful to the Director, CSWRI, Avikanagar for permitting and providing necessary facilities to carry out this research programme. The valuable help from Officers and staff of Sheep Breeding Farm, Fatehpur, Rajasthan during the entire course of present study is also dully acknowledged. The financial support received from ICAR, New Delhi to carry out this study under "All India Network Programme on Gastrointestinal Parasitism is gratefully acknowledged.

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