International Journal of Parasitology Research
ISSN: 0975-3702&E-ISSN: 0975-9182, Volume 8, Issue 3, 2016, pp.-187-190.
Available online at http://bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000241

COMPARATIVE PARASITOLOGICAL AND IMMUNOLOGICAL RESPONSE OF RESISTANT GAROLE AND SUSCEPTIBLE SAHABADI SHEEP TO EXPERIMENTALLY INDUCED HAEMONCHOSIS

JAS R.*, BORDOLOI G., DAS S., BRAHMA A., KUMAR D., PANDIT S., BAIDYAAND S. AND GHOSH J.D.

Department of Veterinary Parasitology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata 700 037, India
*Corresponding Author: Email- rumajas@gmail.com

Received: July 08, 2016; Revised: July 27, 201; Accepted: July 28, 2016; Published: August 07, 2016

Abstract- Resistance / resilience of Garole sheep to induced *Haemonchus contortus* infection was explored by comparing the parasitological and immunological indicators with the known susceptible Sahabadi breed of sheep. Ten animals from both the breeds were orally infected with third stage infective larvae (L_3) of *Haemonchus contortus* @ 700 L_3 per kg body weight and six animals of each breed were maintained as respective controls. Pre-patent period of the infection and faecal egg count of all the infected sheep was recorded at 3 days interval from 18 days post infection (DPI) to 42 DPI. No significant difference in pre-patent period was observed. Garole sheep had significantly ($P< 0.05$) lower faecal egg count (FEC) than the Sahabadi sheep up to 24 DPI and after that it was negative. Detection and titration of *H. contortus* specific IgG was performed by indirect enzyme linked immune sorbent assay (ELISA) and the parasite specific serum IgG response as well as titre of IgG was significantly ($P< 0.05$) higher in Garole sheep compared to Sahabadi sheep. The results of the present study indicated that Garole sheep was comparatively more resistant and / or resilient to *H. contortus* than Sahabadi sheep.

Keywords- Garole sheep, *Haemonchus contortus*, Immunological response, Resistance, Resilience

Citation: Jas R., et al., (2016) Comparative Parasitological and Immunological Response of Resistant Garole and Susceptible Sahabadi Sheep to Experimentally Induced Haemonchosis. International Journal of Parasitology Research, ISSN: 0975-3702 & E-ISSN: 0975-9182, Volume 8, Issue 3, pp.-187-190.

 Academic Editor / Reviewer: Dr Ajit Kumar

Introduction

Gastrointestinal nematodes constitute one of the most important limiting factors in small ruminant livestock productivity in both tropical and temperate regions of the world [1] including India[2]. Amongst the nematode parasites *Haemonchus contortus* is known as the most pathogenic and predominant parasite, particularly in sheep and goat [3,4]. Control of nematode infections including haemonchosis has been primarily relied upon the use of anthelmintics. Indiscriminate use of anthelmintics resulted in emergence of resistant strains of the parasite [5]. In India too, anthelmintic resistance has been reported as an emerging challenge to nematode control [6]. Moreover, there is increasing global concern about the chemical residues in animals and animal products. Hence, there is intensive global effort towards reducing the dependence on anthelmintics through alternative control method of gastrointestinal nematodes of sheep [7]. Alternative control strategies for gastrointestinal nematodoses include selection of genetically resistant breeds and the development of effective vaccines [8]. In sheep the ability to acquire immunity and express resistance to gastrointestinal nematodes differs between and within breeds and resistance to parasitic diseases is genetically controlled in animals [9]. The knowledge of the immune mechanisms that play significant role in genetic variation between and within breeds is important for the identification of genetically resistant livestock and immunization against parasitic diseases [10].

Variable degrees of resistance to haemonchosis among sheep breeds have been reported. Some breeds such as Black belly, Gulf Coast Native, St. Croix, Katahdin, Red Maasai, Nali and Polish are highly resistant to haemonchosis [11,12]. There is a common belief that the Garole sheep, the most popular sheep breed in the state of West Bengal, India, is resistant to GI parasitic infections. This has been supported by preliminary studies of Nimbkaret al [13].

The present study was an attempt in elucidating the resistance and resilience, if any, of Garole sheep to *H. contortus*. Resistance and resilience was studied by comparing the susceptibility of Garole and Sahabadi sheep to induced haemonchosis in terms of parasitological parameters and detection of parasite specific serum IgG response.

MaterialsandMethods

Animals and experimental design: Based on phenotypic characteristics and consistently lower monthly faecal egg counts (mean EPG = 128) throughout a period of one year, 16 Garole sheep in the age group of 15 to 18 months were selected for the study. Sixteen apparently healthy Sahabadi sheep in the same age group were procured from the local area. These animals were maintained in the concrete floor pens for 3 months before giving experimental infection and fed recommended quantity of concentrate feeds with provision of *ad libitum* clean drinking water. The entire experimental design was approved by the Institutional Animal Ethical Committee. Pre-existing gastro intestinal parasites were eliminated by treatment with Fenbendazole (Panacur®, Intervet) @ 5mg/kg body weight. Thereafter, all possible precautions were taken to prevent extraneous parasitic infections during the course of the experiment. Within breed, the sheep were randomly assigned to 2 groups; infected (n = 10) and uninfected control (n = 6).

The infective third stage larvae (L_3) of *Haemonchus contortus* were procured by culturing [14] the eggs obtained by triturating adult female worms collected from the abomasums of sheep slaughtered at the local abattoir [15]. The L_3 at the dose rate of 700 per kg body weight were orally administered in one helminth free indigenous sheep, after overnight withdrawal of feed, to serve as donor of L_3 for artificial infection. After the patency of the infection the faeces of the donor sheep was cultured and the L_3 were harvested [14]. These L_3 were used for artificial infection of 10 animals from both the breeds as stated earlier.

Parasitological techniques and serum samples: Faecal samples of all the infected sheep were qualitatively examined daily by salt floatation technique [14] from second week post- infection onwards to determine the pre-patent period (PP) of the infection. Quantitative faecal examination for determination of eggs per gram (EPG) of faeces of all the infected sheep was done by Modified McMaster's.

technique [14]on nine occasions at 3 days interval from 18 DPI onwards till the end of the experiment.
Blood samples were collected for serum from all the animals at weekly interval from '0' DPI to 28 DPI following the standard methods and preserved at -20° C for further use.

Preparation of crude somatic antigen of *H. contortus* (CSAg-Hc): Adult *H. contortus* was collected from the abomasums of infected sheep slaughtered at local abattoir. The worms were collected in 0.15M PBS (pH-7.2) and washed for 4-5 times in PBS. Finally, 300 worms were homogenized in 10ml of chilled PBS containing phenyl methyl sulfonyl fluoride (PMSF) @ 25mM and ethylene diamine tetra acetic acid (EDTA) @ 24mM in a glass tissue homogenizer (Remi, RQ 127A, India). The homogenized materials were then sonicated in an Ultra Sonicator (NISSEI, Model-US50, Japan). The sonicated materials were centrifuged at 4^0C at 10000 r.p.m. for 45 minutes. Then the supernatant was collected as crude somatic antigen of *H.contortus* (CSAg-Hc). Protein concentration of CSAg-Hc was estimated by the method of Lowry et al[16] and the protein content of CSAg-Hc was 4.52mg/ml. The antigen was then stored at -20^0C for further use.

Detection and titration of *H. contortus* specific IgG by indirect ELISA: The indirect enzyme linked immunosorbent assay (ELISA) using CSAg-Hc was standardized for detection and titration of *H. contortus* specific IgG in the sera samples of experimental sheep. The optimal concentration/dilution of the ELISA reagents including the concentration of coating antigen, dilution of the positive and the negative reference sera as well as the anti-sheep IgG-horse radish peroxidase (HRPO) conjugate (Bangalore GeNei, Bangalore, India) and the optimal test conditions were determined by chequer board dilution assay. The dilution of the antigen showing the highest difference between the optical density of the positive sera and that of the corresponding dilution of the negative sera was considered optimum and used in the test. Pooled sera samples collected four weeks after the patency of the *Haemonchus* infection in the experimental sheep were used as positive control. The pooled sera collected from the Garole sheep maintained under parasite free conditions for three months were considered as negative control.

Detection of *H. contortus* specific IgG by indirect ELISA: The indirect ELISA for detection and titration of serum IgG against *H. contortus* in the experimentally infected sheep was performed according to the method of Voleret al [17] with some modifications.
Flat-bottom 96-well micro-ELISA plates (Nunc, Maxisorp) were coated with 50µl (equivalent to 4µg of antigenic protein) of CSAg-Hc in 0.05M carbonate-bicarbonate buffer, pH 9.6 and incubated overnight at 4^0C. Then 250µl of blocking buffer (2% bovine serum albumin in PBS) were added after washing with PBS 0.05% Tween 20 and incubated at 37^0C for 2hrs. After washing 50 µl of the diluted (1:200) test sera were added in duplicate wells keeping the appropriate controls (positive, negative and conjugate) and incubated for 3hrs at 37^0C. Then 50 µl of rabbit anti-sheep IgG- HRPO (Bangalore GeNei, India), diluted in blocking buffer (1:1000), was added after washing and the plate was incubated at 37^0C for 2 hrs. Thereafter 200 µl of freshly prepared substrate chromogen solution containing Ortho-phenylenediamine dihydrochloride (OPD tablets, Sigma, U.S.A.) and hydrogen peroxide (H_2O_2) was added after washing and incubated at 37^0C for 15 minutes. After development of colour the reaction was then stopped by adding 50 µl of 2.5M sulphuric acid (H_2SO_4) to all the wells. The optical density (O.D.) of the wells was measured at 492nm in the ELISA reader (Multiskan EX, ELISA Reader, Thermo, Japan).

Titration of the anti-*H. contortus* IgG in the sheep sera: Two fold serial dilution of the test serum, starting with 1in 100 up to 1 in 12800, was made in the wells of the ELISA plates and the assay, as standardized, was performed. All the test samples including the negative control were tested in duplicate wells and their mean O.D. values were calculated. The sera samples collected on 0, 7, 14, 21 and 28 DPI were used in this study. The mean O.D. values of the serially diluted sera of the infected sheep of both the breeds and those of the corresponding dilutions of the negative sheep sera, as measured at 492 nm, were plotted on the Y axis and the serum dilutions were plotted on the X axis and a graph was prepared. Three lines, one for the infected Garole sheep, one for infected Sahabadi sheep and the third for the pooled negative control sera, were thus obtained. The IgG titre of the infected sheep sera was determined following the guidelines of OIE manual, (2003). Thus the highest dilution of the test sera just below the dilution at which the O.D. value of the test sera nearest to that of the negative control sera was taken as the titre of the anti-*H. contortus* IgG.

Statistical Analysis: All the parameters for each group on different post-infection days were compared (Analyze-Compare Means). Then they were analyzed separately i.e. between groups and between post-infection days by Duncan method (One- way- ANOVA) and the significance (p- value) was recorded at 5 % ($p<0.05$) level and 1 % ($p<0.01$) level. The complete statistical analyses were done with the help of Statistical Package for Social Scientist (SPSS), Windows Version 10.0.

Results and Discussion

Pre-patent Period (PP) and faecal egg count (FEC): The mean PP of *H. contortus* infection in Garole sheep was 17.8 ± 0.34 days whereas in Sahabadi sheep it was 16.4 ± 0.6 days. However, the difference in PP between the two breeds of sheep was not statistically significant. Susceptibility/ resistance to *H. contortus* infection were assessed in terms of pre-patent period of the infection and EPG of the infected animals. Pre-patent period of the infection in sheep is 2-3 weeks [18]. In the present study, the pre-patent period of the infection in both Garole and Sahabadi sheep was well within the previously established range. However, it was comparatively longer in Garole sheep. The difference, although not significant, could be attributed to the breed difference in the ability to inhibit the intra-abomasal development of the parasite. Thus Garole sheep indicated a greater ability in this regard.
The mean FEC in Garole sheep was significantly ($P< 0.01$) lower compared to Sahabadi sheep up to 24 DPI. After 24 DPI all the infected Garole sheep became negative for the infection both on quantitative and qualitative faecal examination [Fig-1]. On the contrary, the Sahabadi sheep recorded consistently higher EPG till the end of the experiment on 42 DPI, although the increase was not statistically significant. Faecal egg count is generally considered as a useful indicator of resistance/ immune response to nematode infections [19]. Garole sheep in the present study had significantly lower EPG indicating thereby a greater degree of resistance or stronger immune response to the infection. Breed difference in EPG in primary infection is variable; some studies have revealed significant differences which were attributed primarily to the acquired immune response [20].

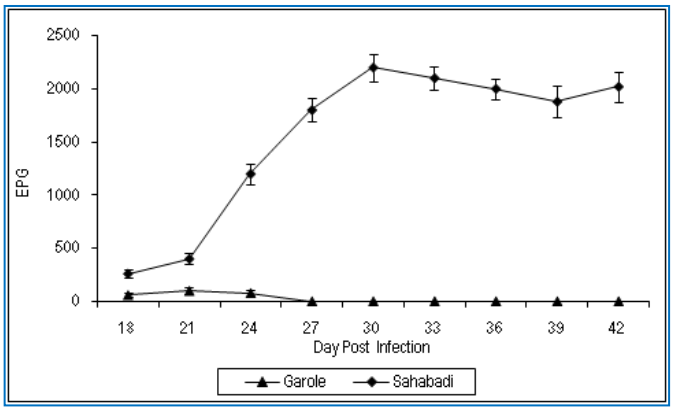

Fig-1 Mean ± SE of faecal egg output (EPG) in *Haemonchus contortus* infected Garole and Sahabadi sheep

Low worm count at necropsy is accounted as an important indicator of resistance to *H. contortus*in sheep [19,21]. Lower FEC and worm burden was also recorded by Altaif and Dargie [22] in Scottish Blackface lamb, known for its resistance to *H. contortus* after primary infection. Resistance to helminth is the characteristic of having no / fewer parasites, which is indirectly measured as FEC, whereas

resilience is the ability of the host to completely or partially combat the effects of the infection on productivity [23].Hence, parasitological parameters, especially the FEC was sufficient indication of the presence of *H. contortus* resistance in Garole sheep. Association of genetic markers with this resistance is a primary prerequisite before *Haemonchus*- resistance in Garole sheep could be exploited in breeding programme for development of *Haemonchus* resistant breed.

Serum anti *H. contortus* IgG response: Comparison of serum IgG response in terms of O.D. value, at the fixed dilution of 1 in 200, in the infected Garole and Sahabadi sheep compared to the negative control serum indicates that both the groups of infected sheep had significantly ($P<0.05$) higher concentration of *H. contortus* specific IgG during the early phase of the infection till 28 DPI [Fig-2]. However, the *H. Contortus* specific serum IgG response was significantly ($P< 0.05$) higher in Garole sheep on 21 DPI compared to Sahabadi sheep.

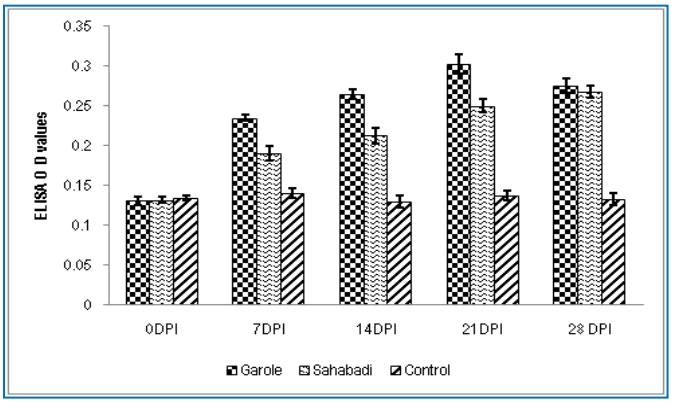

Fig-2 Serum IgG response (Mean ± SE of ELISA$_{492nm}$OD values) in *H. contortus* infected Garole and Sahabadi sheep

Pattern of *H. contortus* specific serum IgG titre: It is evident from [Fig-3] that the initial titre of IgG on 7 DPI was higher in Sahabadi sheep and subsequently the titre was higher in Garole sheep on 14 DPI [Fig-4] and 21 DPI [Fig-5] and on 28 DPI the serum IgG titre of Garole and Sahabadi was very close to each other [Fig-6]. This pattern of IgG titre suggested that the late pre-patent stage of the parasite elicited stronger IgG response in Garole sheep in comparison with Sahabadi sheep. The titration of serum IgG was not done beyond 28 DPI, since the parasites were eliminated after 24 DPI.

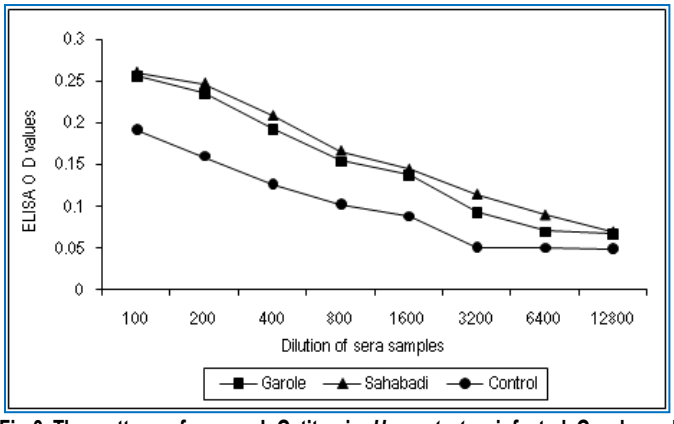

Fig-3 The pattern of serum IgG titre in *H. contortus* infected Garole and Sahabadi sheep on 7 DPI

Expulsion of the worms is a clear indication of rejection of the adult worms along with the immature ones, if any, after 24 DPI in Garole sheep. Association of the mast cells and globule leucocytes with rejection of challenge larvae of *H. contortus* is a well-recognized phenomenon in sheep [24,25]. Parasite specific IgA, IgG1 and IgE in the abomasal mucosa are also involved in the response against gastrointestinal nematodes including *H. contortus* [24, 26, 27]. However, involvement of such mechanisms in expulsion of existing adult worms, as occurred in the present study, needs to be explored. Nonetheless, early and elevated serum IgG response in Garole sheep, if considered as an indicator of stimulated immune response to the infection, it was clear that Garole sheep was resistant to *H. contortus* and this resistance might presumably be attributed to a higher degree of immune response to the infection in Garole sheep compared with the Sahabadi sheep.

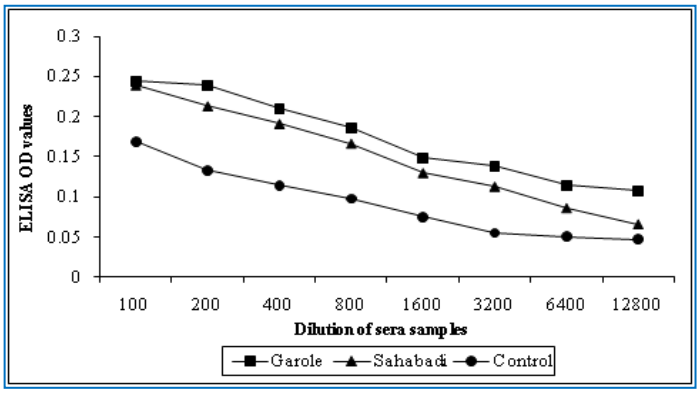

Fig-4 The pattern of serum IgG titre in *H. contortus* infected Garole and Sahabadi sheep on 14 DPI

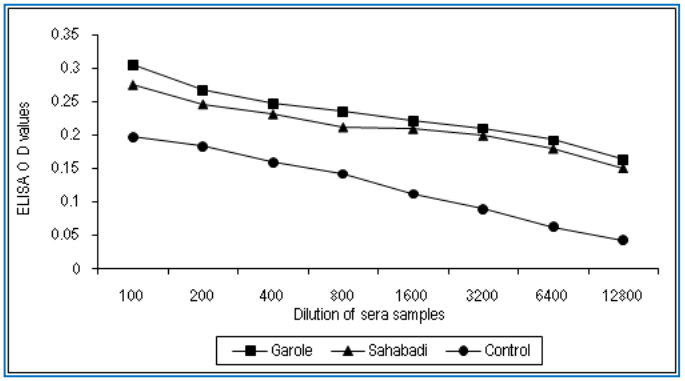

Fig-5 The pattern of serum IgG titre in *H. contortus* infected Garole and Sahabadi sheep on 21 DPI

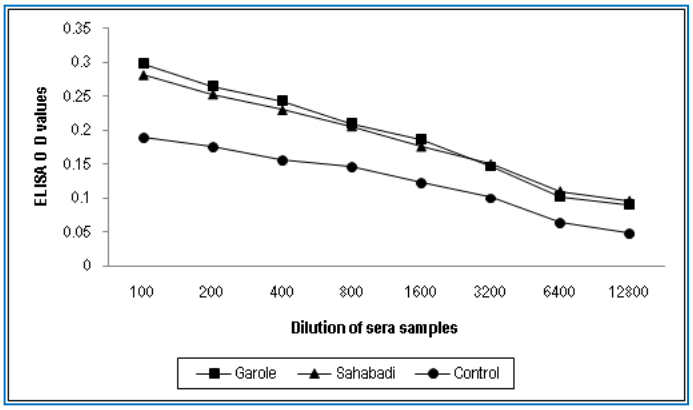

Fig-6 The pattern of serum IgG titre in *H. contortus* infected Garole and Sahabadi sheep on 28 DPI

Response against GI nematodes involves production of parasite specific IgA, IgG1 and IgE [25, 28]. Significant serum IgG response to *H. contortus* in Garole sheep in this study indicated that this could be used as an immunological marker of resistance or susceptibility to this infection. Bambouet al [29] concluded that genetic segregation in resistant and susceptible animals was not related to humoral immune response. However, cellular immune response to nematodes in the GI mucosa is genetically controlled and could be a valuable marker of resistance and susceptibility to *H. Contortus* [29].

Conclusion

The results of the present study concluded that the Garole sheep is resistant /resilient to *H. contortus* infection as evident from the lower negative impact of the infection on parasitological indicators and the serum IgG response compared to Sahabadi sheep. The outcome of the present study has opened up the areas of future investigation on the Genetic markers associated with resistance / resilience in Garole sheep.

Acknowledgements

The authors thankfully acknowledge the financial assistance of the Indian Council of Agricultural Research, New Delhi in conducting this study under the research project entitled "All India Network Programme on Gastrointestinal Parasitism".

References

[1] Perry B.D., Randolph T.F., Mc. Dermott J.J., Sones K.R. and Thornton P.K. (2002) Investing in Animal Health Research to Alleviate Poverty. International Livestock Research Institute (ILRI), Nairobi, Kenya.
[2] Jas R. and Ghosh J.D. (2009) *Indian Journal of Animal Science*, 79(8), 3-5.
[3] Ghosh J.D., Jas R. and Bordoloi G. (2012) *Indian Journal of Animal Science*, 82(8), 818- 821.
[4] Jas R. and Ghosh J.D. (2007) *Environment & Ecology*, 25S(4), 1142 -1145.
[5] Waller P. J. (1997) *Veterinary Parasitology*, 72, 391- 412.
[6] Yadav C.L., Kumar R., Uppal R.P. and Verma S.P. (1995) *Veterinary Parasitology*, 60(3-4), 355- 360.
[7] Beh K.J., Hulme D.J., Callaghan M.J., Leish Z., Lenane I., Windon R.G. and Maddox J.F. (2002) *Animal Genetics*, 33, 97-106.
[8] Newton S.E. and Munn E.A. (1999) *Parasitology Today*, 15, 116-122.
[9] Stear M.J., Strain S.A.J. and Bishop S.C. (1999) *International Journal Parasitology*, 29, 51 - 56.
[10] Gill H.S., Altmann K., Cross M.L. and Husband A.J. (2000) *Immunology*, 99, 458 - 463.
[11] Aumont G., Gruner L. and Hostache G. (2003) *Veterinary Parasitology*, 116, 139- 150.
[12] Burke J.M. and Miller J.E. (2004) *Small Ruminant Research*, 54, 43 - 51.
[13] Nimbkar C., Ghalasi P.M., Swan A. A., Walkden-Brown S. W. and Kahn L. P. (2003) *Animal Science*,76, 503 - 515.
[14] Soulsby E.J.L. (1982) Helminths, arthropods and protozoa of domesticated animals.The English Language Book Society and Bailliere Tindall, London.
[15] Johnson D.A., Behnke J.M. and Coles G.C. (2004) *Parasitololgy*, 129(1), 115 -126.
[16] Lowry O.H., Rosebrough N.J., Farr A.B. and Randall R.J. (1951) *Journal of Biological Chemistry*,193, 265.
[17] Voller A., Bidwell D.E. and Bartlelt A. (1976) *Bulletin of WHO*, 53, 55 - 65.
[18] Urquhart G.M. (1996) Veterinary Parasitology, Blackwell Science
[19] Mugambi J., Audho J. and Baker R. (2005) *Veterinary Parasitology*, 127, 263 - 275.
[20] Courtney C.H., Parker C.F., Mc. Clure K.E. and Herd R.P. (1985) *International Journal for Parasitology*,15, 239 - 243.
[21] Woolaston R.R. and Piper L.R. (1996) *Animal Science*, 62, 451- 460.
[22] Altaif K.I. and Dargie J.D. (1978) *Parasitology*, 77, 161 - 175.
[23] Woolaston R.R. and Baker R.L. (1996) *International Journal for Parasitology*, 26, 845 - 855.
[24] Amarante A.F.T., Bricarello P.A., Huntley J.F., Mazzolin L.P. and Gomes J.C. (2005) *Veterinary Parasitology*,128, 99 -107.
[25] Kemp J.M., Robinson A.N., Meeusen ElsN. T. and Piedrafita D.M. (2009) *International Journal for Parasitology*, 39(14), 1589 -1594.
[26] Strain S.A.J. and Stear M.J. (2001) *Parasite Immunology*, 23, 527- 531.
[27] Harrison G.B.L., Pulford H.D., Hein W.R., Barber T.K., Shaw R.J., Mc. Neill M., Wakefield S.T.J. and Shoemaker C.B. (2003) *Parasite Immunology*, 25, 45 - 53.
[28] Shaw R.J., Gatehouse T.K. and Mc. Neill M.M. (1998) *International Journal for Parasitology*, 28, 293- 302.
[29] Bambou J.C., Chevrotiere Claudia De La, Varo H., Arquet R., Kooyman F. N.J. and Mandonet N. (2008) *Veterinary Parasitology*, 158, 311 - 318.