



Immunological impact on goats, infected with nematodes

Jatan Dudwe, MM Prakash, S Gaherwal*

Department of Zoology, Government. Holkar (Model, Autonomus) Science College, Indore, Madhya Pradesh, India

Abstract

Present study was carried out to investigate the immunological changes in goats infected with gastrointestinal nematode at Barwani District (M.P). Selected villages were Barwani, Sendhwa, Niwali, Pansemal and Khetia. *Trichuris* and *Haemonchus* nematodes were selected as experimental parasite.

For Immunological study ITH and DTH response were noted in both control and experimental groups. In the present study with the constant dose of somatic antigens, we observed different ITH and DTH response in selected areas. This showed that goats of different area may have different response against somatic antigen. In the experiment animal ITH and DTH response increased as compared to control values. Increase ITH response showed the humeral immunity and increase DTH response showed the cell mediated immunity in the host against parasite and parasitic secretion.

Keywords: immunological, nematodes, goat, rainy, winter and summer season

Introduction

Gastrointestinal nematodes (GINs) remain one of the main constraints to ruminant production, since they can cause reduction in skeletal growth, live –weight gain and in milk yield [1, 2, 3, 4].

Nematode parasite resides in gastro-enteritis put a serious health threat to productivity of goats [5]. Helminthes researchers are of opinion that goats are more susceptible than sheep to gastrointestinal nematodes [6]. Rearing of Pashmina goats is one of the most important activities in Ladakh (India) [7, 8].

Goat meat is one of the most preferred foods. Helminthes were considered as one of the major healths destroys [9, 10]. They are considered responsible for loss of weight, low birth weights, and difficulty in kidding.

Helminthes infections were observed as a major cause of low productivity in livestock, especially in poor world [11]. The anthelmintic resistance in parasitism, particularly in small ruminants, is now-a-days becoming an urgent problem for organic livestock producers to protect their animals from the parasite infection which affects their productivity. [12, 13].

Materials and Method

Experimental animal: Goat (*Capra hircus*).

Experimental parasite: *Trichuris* and *Haemonchus*. These parasites were found very common in the intestine of goat at studied villages. These selected parasites were maintained in the laboratory by serial passage.

Preparation of somatic antigens: Somatic antigens were prepared by using method described by [14]. Somatic antigens of nematodes parasites (*Trichuris* + *Haemonchus*) were prepared by homogenization and lyophilisation. The homogenate were lyophilized and kept at 4 °C.

Immunization of host: An initial dose of 100 µg [15] of the antigenic sample and 0.2 ml of Freund's complete adjuvant (FCA) suspension was injected subcutaneously (SC) in to

host body. The booster dose 100 µg antigenic samples without FCA were given to host at 21 days.

Preparation of Inoculums for Infection: After immunization the 100 viable eggs were fed to each goat. After inoculation, goats were kept in farm, labeled according to the experiments and were fed routinely with the same standard diet.

Collection of Blood: The blood samples were collected from the jugular vein of each surveyed and selected animal with a sterile disposable syringe under the supervision of veterinary doctor and with the farmer's permission or consent. Sample of 5ml of blood was preserved in anticoagulant ethylene diamine tetra acetic acid (EDTA) contained in special vials and kept for immunological studies.

Assay of Hypersensitivity Reactions

Immediate type of hypersensitivity (ITH) skin testing – passive cutaneous anaphylaxis (PCA) [16]

1. Immunoglobulin was injected in a volume of 0.1 ml. The dose was chosen to give maximum and linear relationship. A trial experiment was carried out to select the doses to be injected. The antibodies diluted eight fold (1:8) were chosen for the present study.
2. The selected doses were injected in trader mally with tight fitting 0.5 ml plastic tuberculin syringes fixed with ½ inch 26 G needles. Abdomen and back were selected as the sites which were prepared by removing hairs marked the area. 2-3 injections were given at distinct place as so to not interfere.
3. After 4 hours, 0.1 ml soluble test antigen (volume 1:1000, W/v in normal saline) and a marker dye (1% Evans blue, 20 mg/kg) were injected together intravenously in a small volume.
4. 20 minutes later, animals were killed and then skin opened in such a way so as to free of subcutaneous attachments. Size of lesions was evaluated with a

transparent ruler while the skin was being illuminated from the opposite side with a lamp. Care was taken to avoid either drying or stretching of the skin. The reaction greater than 5 mm in diameter was considered significant.

In vivo method of delayed hypersensitivity (DTH) estimation ^[17]

1. 0.03 ml of soluble test antigen (1:1000 W/v) was injected into planter side of the foot-pad of experimental mice with tip of the 30 G needle (manufactured by Becton, Dickinson and Company, Rutherford, New Jersey, USA), pointing proximally. The joint was extended (at 180 degrees) when the needle was injected into the food-pad.
2. Foot-pad thickness measurement: The DTH response was measured as the increase in foot-pad thickness and compared with the control foot-pad of same mouse injected with suspending fluid. Foot-pad thickness was measured by a caliper (Schnell Taster, Germany). Each measurement was a mean of six readings, triplica each from two different individuals.
3. DTH response was measured at 24th hour after challenge and the reaction greater than 5 mm in

diameter was considered significant.

4. DTH response was confirmed by carrying out histological examination in both foot-pads. Fixation was done in buffered formalin, sectioned and stained with haematoxylin-eosin stains. A positive DTH response was confirmed by observing a strong mononuclear infiltration.

Result and Discussion

Table 1: ITH & DTH response showed by control & experimental group of goat immunized by somatic antigens.

S. No.	Group Name	Dose	Experimental group	
			ITH Response in cm	DTH Response in cm
1.	NINI Control-1	100 µg	–	–
2.	INI Control-2	100 µg	5.2 ±0.59	6.9 ±0.44
3.	Ex-1 (Barwani)	100 µg	14.32 ±0.32	10.8 ±0.22
4.	Ex-2 (Sendhwa)	100 µg	12.46 ±0.28	9.6 ±0.37
5.	Ex-3 (Niwali)	100 µg	11.22 ±0.48	8.8 ±0.66
6.	Ex-4 (Pansemal)	100 µg	9.45 ±0.76	7.9 ±0.72
7.	Ex-5 (Khetia)	100 µg	7.35±0.56	7.2±0.56

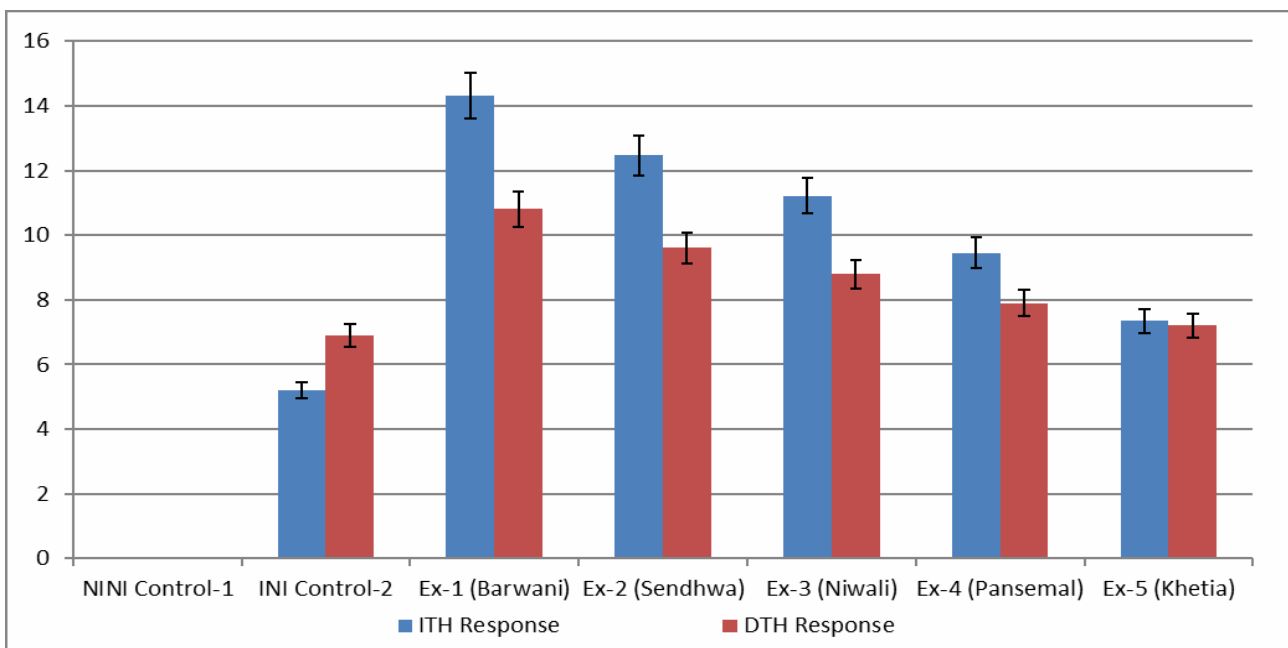


Fig 1: ITH & DTH response showed by control & experimental group of goat immunized by somatic antigens.

For Immunological study ITH and DTH response were noted both in control and experimental groups. Increase ITH response showed the humeral immunity and increase DTH response showed the cell mediated immunity in the host against parasite and parasitic secretion.

Result of ITH and DTH response obtained in control and experimental group of goats are summarized in Tables- 1 and presented by bar diagram-1

The result of ITH & DTH response in NINI Control-1 (Non-infected non-immunized) was nil but in INI Control-2, ITH response was 5.2 cm and DTH response was 6.9 cm at 100 µg.

ITH response in experimental goat group 1 to 5 (Barwani, Sendhwa, Niwali, Pansemal and Khetia) were 14.32, 12.46, 11.22, 9.45 and 7.35 cm at 100µg concentration respectively.

DTH responses in experimental goat group 1 to 5 (Barwani, Sendhwa, Niwali, Pansemal and Khetia) were 10.8, 9.6, 8.8, 7.9 and 7.2 cm at 100µg concentration respectively.

Maximum ITH and DTH response was observed 14.32 cm and 10.8 cm in experimental group-1 respectively at Barwani. Minimum ITH and DTH response was recorded in experimental group-5 ie Khetia. The values observed were 7.35 cm and 7.2 cm at 100µg concentration respectively.

In the present study with the constant dose of somatic antigens, we observed different ITH and DTH response in selected areas. This showed that goats of different area may have different response against somatic antigen may be due to varied food and hygienic condition etc.

Observation of many scientist using other models shown that helminths can influence vaccine efficacy by modulating

host immune responses in particular when cellular – dependent responses are required [18, 19]. The difference in antibody response in worm infected and none infected groups were also corroborated reported by [20, 21, 22, 23].

This clearly suggests that the difference in antibody response was due to vaccine antigens parasites which may have an impact on immune responses to by stander antigens such as those from infective agents and vaccines. This is in agreement with reports by [24, 25] that helminths have an influence on immune response to disease and vaccine antigens. Increased in ITH reactions showed that the stimulation of reagenic (IgE) response by the antigen. IgE is the antibodies which are involved in anaphylactic reactions. Increased levels of IgE are responsible killing/ expulsion of helminth [26, 27, 28].

Increased DTH response showed the activation of lymphokines. In host body Antigens activated to T- cell. Activated T- cell release a lymphokines following a secondary contact with the same antigens. Lymphokines induce inflammatory reactions and activate and attract macrophages which release mediators for expulsion of killing to parasite/antigen [27, 29].

Reference

1. Waller PJ. Sustainable helminth control of ruminants in developing countries. *Vet Parasitol*,1997;71:195-207.
2. Rinaldi L, Veneziano V, Cringoli G. Dairy Goat Production and the Importance of Gastrointestinal Strongyle Parasitism, *Transactions of the Royal Society of Tropical Medicine and Hygiene*,2007;101(8):745- 746.
3. Van Houtert MF, Sykes AR. Implication of nutrition for the ability of ruminants to withstand gastrointestinal nematode infection. *Int j Parasitol*,2010;26:1151-1167.
4. Mavrot F, Hertzberg H, Torgerson P. Effect of gastrointestinal nematode infection on sheep performance: a systematic review and meta-analysis. *Parasit*,2015;8:557.
5. Nwosu CO, Madu PP, Richards WS. Prevalence and seasonal changes in the population of gastrointestinal nematodes of small ruminants in the semi-arid zone of north- eastern Nigeria. *Vet. Parasitol*,2007;144:118- 124.
6. Jallow OA, McGregor BA, Anderson N, Holmes, J.H.G. Intake of trichostrongylus larvae by goats and sheep grazing together. *Aust. Vet. J*,1994;71:361-364.
7. Carmichael IH. Helminthiasis in domestic and wild ruminants in Botswana- preliminary investigations. *Trop. Anim. Hlth. Prod*,1972;4:175-181.
8. Akerejola OO, Schillhorn van Veen TW, Njoku CO. Ovine and Caprine diseases in Nigeria: A review of economic losses. *Bull. Anim. Hlth. Prod. Afr*,1979;27:65-70.
9. Ijaz M, Khan MS, Avais M, Ashraf K, Ali M, Saima. Infection rate and chemotherapy of various helminths in goats in and around Lahore. *Pakistan Vet. J*,2008;28:167-170.
10. Amadi ANC, Avoaja DA, Essien EJ. Epidemiology of Helminth Parasites of West African Dwarf Goat (*Capra hircus*) in Umuariaga in Ikwuano L.G.A. Abia State. *J. Appl. Sci. Environ. Manage*,2012;4:359-362.
11. Githiori JB, Athanasiadou S, Thamsborg SM. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Vet Parasitol*,2006;139(4):308-20.
12. Thamsborg SM, Roepstorff A. Parasites and Organic Livestock Parasite Problems In Organic Livestock Production Systems and Options For Control, Running Head: The Royal Veterinary and Agricultural University, Copenhagen, Denmark, 2014.
13. Lindgren K, Boch *et al.* Roepstorff A Animal health and welfare in production systems for organic fattening pigs *Org. Agr*,2014;4:135-147.
14. Artis D, Potten CS, Else KJ, Finkelman FD, Grecnis RK. Host intestinal epithelial cell hyper- proliferation during chronic infection is regulated by interferon. *Exp. Parasite*,1999;92:144-158.
15. Lowry OH, Rosenboun NH, Farr AL, Pandial RJ. Protein measurement with the folinphenol reagent. *J. of biological chemistry*,1951;193:265-275.
16. Ovary Z. Passive cutaneous-anaphylaxis in: immunological method. (Ed. Ackroyd, J.F.) Oxford, Blackwell, 1964, 259-283.
17. Talwar GP. A hand book of practical immunology. Vikas publishing house Pvt. Ltd., New Delhi, 1983.
18. Stelekati E, Wherry EJ. Chronic bystander infections and immunity to unrelated antigens. *Cell Host Microbe*,2012;18:12(4):458-469.
19. Stelekati E, Shin H, Doering AT. Bystander chronic infection negatively impacts development of CD8+ T cell memory. *Immunity*,2014;15:40(5):801-813.
20. Elias D, Akuffo H, Thors H, Pawlowski A. and Britton, S. Low dose chronic S. Mansoni infection increases susceptibility to mycobacterium bovis BCG infection in mice. *Clin. Exp. Immunol*,2005;139(3):398-404.
21. Van Riet E, Hartgers FC, Yazdanbakhsh M. Chronic helminthes infection induces immunomodulation: consequences and Mechanisms”, *Immunobiology*,2007;212:475-490.
22. Urban JF Jr, Steenhard NR. Infection with parasitic nematodes confounds vaccination efficacy. *Vet. Parasitol*,2007;148(1):14-20.
23. Steenhard NR, Jungersen G, Kokotovic B, Beshah E, Dawson HD, Urban JF *et al.* Ascarissuum infection negatively affects the response to a Mycoplasma hypopneumoniae vaccination and subsequent challenge infection in pigs. *Vaccine*,2009;27(37):5161-9.
24. Salgame P, Yap GS, Gause WC. Effect of helminthes induced immunity on infections with microbial pathogens *Nat. Immunol*,2013;14(11):1118-1126.
25. Chatterjee S, Nutman TB. Helminth-Induced Immune Regulation: Implications for Immune Responses to Tuberculosis. *Public library of science*, 2015.
26. Urban, Joseph F, Katona Jr, Paul WE, Finkelman FD. Interleukin 4 is important in protective immunity to a gastrointestinal nematode infection in mice. *Proceeding of the National Academy of Sciences*,1991;88(13):5513-5517.
27. Gaherwal S, Kaskehikar P, Verma S, Solanki A. Hapersensitivity response in infected and vaccinated mice: *Hymonolepis diminuta*. *Indian research communication*,2008;2(1):79-31.
28. Gaherwal S, Prakash MM. Blood cell counts associated Immunity in *Trichuris muris*. *J Cell Tiss Res*,2009;9(1):1763-1766.
29. Roitt I, Brostoff J, Male D. Immunology. Third Edition: Published by Mosby – year Book Europe. Limited ISBN0,1993;5:397-44765.
30. Shrestha R, Ghimire R, Bhattarai N. Study of farmer’s attitude and consent towards consumption of goat milk and milk product in eastern Chitwan, Nepal. *International Journal of Veterinary Sciences and Animal Husbandry*. 2020;5(3):17-20.