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Immune response of goat (*Capra hircus*) against *Fasciola hepatica*

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Abstract- The immune response of goat against *Fasciola hepatica* was studied in the present investigation. Total 15 goats were examined for this study. The experimental animals were immunized with different somatic antigens. The immune response was observed by PCA and IgE response. The result showed that increased PCA response and increased IgE response found in immunized goats on 60th day post infection in compression to control goats. The maximum PCA reaction and IgE response were observed in goat vaccinated with larval somatic antigens and minimum PCA reaction and IgE response were observed in goat vaccinated with adult somatic antigens. Larval somatic antigens were observed more potent in providing protection as compared to Eggs and adult somatic antigens. The increased in PCA response and IgE response in infected and immunized goat compared to infected and non-immunized goat suggested the involvement of above studied parameters in immune response. This study also proves that larval somatic antigen of *Fasciola hepatica* was effective in imparting immunity in goat.

Keywords-*Fasciola hepatica*, Capra *hircus* (Goat), Somatic Antigens, PCA, IgE response

I. INTRODUCTION

Fascioliasis caused by *Fasciola hepatica* parasite that infects a wide range of mammalian hosts, including domestic ruminants in which fascioliasis is an economically important disease. It is considered less frequent and important than goat, sheep or cattle infection but nonetheless occurs as a major constraint for goat production in many areas of the world. Chronic fascioliasis with high rates of mortality has also been reported (Leathers *et al.,* 1982). Mortality due to clinical processes and the decline in production caused by subclinical processes, in which migration of immature parasites through the liver gives rise to considerable liver damage.

In goat and cattle a development of partial resistance to challenge infection has been established. In other hosts there is no evidence of acquired resistance to primary or secondary infection (Haroun and Hillyer, 1986.) Some mechanisms of immune modulation in *F. hepatica* infection described in different hosts, affecting either antibody activity (Chapman and Mitchell, 1982) or lymphocyte response (Zimmerman *et al.,* 1983).

Fascioliasis is a less important and less frequent infection than other ruminants; however, it is prevalent in different parts of the 14% in India. Natural as well as trial infections found in goats (Reddington *et al.,* 1986). Diagnosis for *Fasciola* spp., Conventional coprological examination through optical microscopy is extensively used (Anderson *et al.,* 1999). However, this fails to detect milder infections or those earlier than 8 weeks of infection (O'Neill *et al.,* 2000). Mostly, fascioliasis appears as chronic disease with low mortality (Leathers *et al.,* 1982). These losses reduced by treating the animals diagnosed at their initial stages of infection through sensitive as well as specific tests e.g. Passive cutaneous anaphylaxis (PCA) and (IgE) enzyme-linked Immuno-sorbent assay (ELISA) (Yavuz *et al.,* 2007).

The aim of present study was to investigate the Immune response against *F. Hepatica* in infected and non-infected and immunized goats with somatic antigen. These findings can be useful for further studies of *F. hepatica* and diagnostic purposes in goats, especially, in areas where fascioliosis is not endemic. Since less previous work has already been carried out on the present parameters this work can be of high priority and prime importance.

II. MATERIALS AND METHODS

Experimental animal: The goats (Capra *hircus*) were used as experimental animal for present study. Total 15 goats were included for this study.

Experimental parasite: *Fasciola hepatica* (Liver fluke) was used as experimental parasite for present study.

Preparation of *F hepatica* somatic antigen: Adult, larval stage and eggs of flukes were obtained from the infected

livers, snail- (*Lymnaeastagnalis*) and infected feacal sample all stages were washed six times in phosphate-buffered saline (PBS), pH 7.3 in order to remove debris. They were then homogenized in a Regular homogenizer with 10 ml of PBS. The homogenate was centrifuged at 13,000 X g for 30 minutes. The supernatant containing soluble antigen, termed liver fluke homogenate (LFH) products was removed into 1ml vials and stored at20°C (Dalton and Heffeman, 1989).

Collection of blood samples and separation of serum: Blood from mice was collected by cardiac puncture under mild ether anesthesia, before incision each mouse were swabbed with 90% alcohol, heart exposed, blood collection from the ventricle by a 2 ml sterilized dry glass syringe fitted with a suitable in cold overnight for clotting after which serum carefully pietted out in to clean sterilized serum collecting tubes and stored at -20^{0}C until required.

Immediate Type Hypersensitivity (PCA): Passive cutaneous anaphylaxis (PCA) was done by Ovary method (Ovary, 1964).
ELISA **Test:** ELISA test was performed by the step suggested by (Voller *et al.,* 1978).

III. Results And Discussion

In the present study we investigated the Immune response in *Fasicola hepatica* infected goats and compare with non-infected goat. The level of immune response was observed by PCA and IgE antibody response in experimental group immunized with different somatic antigens for active immunization. Result of PCA and IgE antibody response in control and vaccinated goats are summarized in table –1 and presented as in figure- 1and 2.

PCA response:
PCA reactions were found to be directly proportional to the quality of antigens. In INI (Infected Non Immunized) group PCA reaction was 8.5cm.

PCA reactions were found to be 9.7 cm in IIESAg, 11.6 cm in IILSAg and 8.4 cm in IIASAg the dose of 100µg concentration on 60th days post infection.

Over all PCA reaction was observed maximum (11.6 cm) in the group IILSAg and minimum (8.4 cm) in the group IIASAg. The PCA was increased in experimental groups as compared to control group.

The maximum PCA reaction was observed in goat vaccinated with larval somatic antigens and minimum PCA reaction was observed in goat vaccinated with adult somatic antigens. Larval somatic antigens were found to be more potent in providing protection as compared to Eggs and adult somatic antigens.

All the values obtained in the various experimental groups were statistically found significant.

The order of PCA response was observed as-

INI <IIASAg<IIESAg<IILSAg

IgE antibody response:
IgE antibody response was found to be directly proportional to the quality of antigens. In NINI group IgE antibody response was140.2 KIU/ml and INI group the IgE antibody response was 145.5 KIU/ml on 60th day post infection.

In experimental groups the IgE antibody response was found to be198.3 KIU/ml in IIESAg, 235.4 KIU/ml in IILSAg and 185.8 KIU/ml in IIASAg at the dose of 100µg concentration on 60th day post infection.

Over all IgE antibody response was observed maximum (235.4 KIU/ml) in the group IILSAg and minimum (185.8 KIU/ml) in the group IIASAg. The IgE antibody response increased in experimental groups as compared to control group.
The maximum IgE antibody response was observed in goat vaccinated with larval somatic antigens and minimum IgE antibody response was observed in goat vaccinated with adult somatic antigens. Larval somatic antigens were found to be more potent in providing protection as compared to larval and adult somatic antigens.

All the values obtained in the various experimental groups were statistically found significant. The data was analysed statically as per the method described by Snedecor and Cochran (Snedecor and Cochran, 1980).

The order of IgE response was observed as-

IILSAg<IIESAg<IIASAg< INI<NINI

Table- 1:PCA and IgE response in infected and vaccinated goat with different somatic antigens of *Fasicola hepatica*.

Group No.	Group Name	Dose	PCA response in cm on 60th day p.i. S.E.M.	IgE Antibody Response on 60th day p.i. (KIU/ml) S.E.M.±
1.	NINI	-	-	140.2
2.	INI	-	8.5 ±0.624	145.5

3.	IIESAg	100µg	9.7	198.3
4.	IILSAg	100µg	11.5	235.4
5.	IIASAg	100µg	8.4	185.8

NINI	Non Infected and Non Immunized
INI	Infected and Non Immunized
IIESAg	Infected and Immunized with Antigen
IILSAg	Infected and Immunized with Larval
IIASAg	Infected and Immunized with Adult
PCA	Passive cutaneous Anaphylaxis
KIU	Kilo International Unit
S.E.M.	Standard Error of Mean
P.I.	Post Infection Days

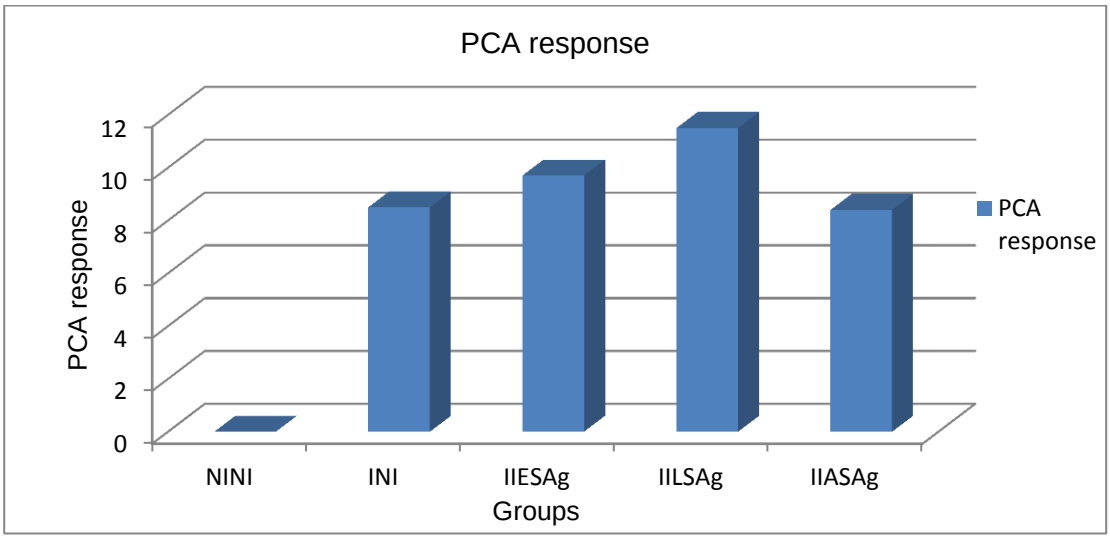

Figure-1: PCA response in infected and vaccinated goat with different somatic antigens of *Fasicola hepatica*.

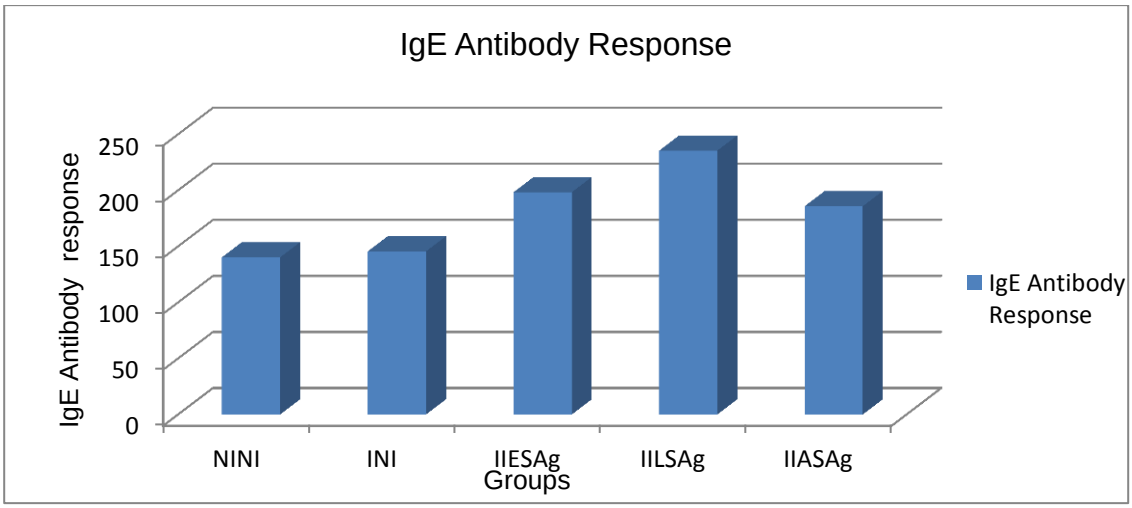

Figure- 2: PCA and IgE response in infected and vaccinated goat with different somatic antigens of *Fasicola hepatica*.

Assay of immediate type of hypersensitivity (ITH) reactions was performed by skin testing for passive cutaneous anaphylaxis (PCA). Result of PCA reactions in goat infected and vaccinated goats with various somatic antigens are summarized in tables -1 and figures-1 and 2.

Immediate type hypersensitivity is an allergic reaction induced by specific antigen provoked by re-exposure to the same antigen mediated by specific IgE antibodies, and produced by the cellular release of histamine and other vasoactive mediators, resulting in an immediate local or system i.e. reaction IgE antibodies that constitutively express high affinity surface receptors for the Fc component of IgE. Binding and cross linking of the allergen to surface receptor and bound IgE triggers the immediate release from cytoplasmic granules of mast cells and basophils performed vasoactive mediators of immediate hyperseitivity and also released the biochemically active mediators (Roitt *et al.,* 1993).

In the present investigation the PCA reactions were directly proportional to the quality of antigens. In IILSAg group PCA reaction was observed maximum (11.5cm) in the group IIESAg and minimum (8.4cm) in the group IIASAg at the dose of 100µg concentration on 60^{th} days post infection. The PCA reaction was increased in experimental groups as compared to control group. All the values obtained in the various experimental groups were statically found significant.

Increased in PCA reactions indicate the stimulation of reaginic (IgE) response by the antigen as these are the only type of antibodies which are involved in anaphylactic reactions. Increased levels of IgE are responsible killing/ expulsion of helminth parasites conferring protection to the host is well known (Hagan *et al.,* 1991; Urban *et al.,* 1991; Gaherwal and Prakash 2009; Yazdanbakhsh *et al.,* 2002). The results PCA reaction of the present study also correlates with those of the observations of the above mentioned authors. Results of the present study indicate that the host may acquire immune response even after oral larval-infections.

In the present investigation IgE antibody response was found to be directly proportional to the quality of antigens. In NINI group the IgE antibody response was 140.2KIU/ml and INI group the IgE antibody response was 145.5 KIU/ml on 60^{th} days post infection.

In experimental group IgE antibody response was observed maximum (235.4 KIU/ml) in the group IILSAgand minimum (185.8KIU/ml) in the group IIASAg . The IgE antibody response increased in experimental group as compared to control group.

The maximum IgE antibody response observed in goat vaccinated with Larval somatic antigen and minimum IgE antibody response was observed in goats vaccinated with Adult somatic antigens. Larval somatic antigens were found to be more potent in providing protection as compared to eggs and adult somatic antigens. All the values obtained in the various experimental groups were statistically found significant.

IgE antibodies are known to play a central role in mediating type I hypersensitivity reactions. The production of IgE tends to increase during parasite infections, but the ultimate effects of IgE vary considerably, depending on the host-parasite relationships. Hyper immune allergic reactions have been closely associated with IgE production (Hagan *et al.*, 1991).

The role of IgE antibodies in killing/ expulsion of allergen (Jarrett and Miller, 1982) and helminthes parasites (Ogilvie and Jones, 1971) are well known. The same result obtained in the present investigation. Thus the results of present study supported by above mentioned researchers.

CONCLUSION

The maximum PCA reaction was observed in goats vaccinated with larval somatic antigens and minimum PCA reaction was observed in goats vaccinated with adult somatic antigens. Larval somatic antigens were more potent in providing protection as compared to eggs and adult somatic antigens. The maximum IgE antibody response was observed in goats vaccinated with larval somatic antigens and minimum IgE antibody response was observed in goats vaccinated with adult somatic antigens. Larval somatic antigens were more potent in providing protection as compared to egg and adult somatic antigens.

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