BIOCHEMICAL STUDIES AGAINST ASCARIDIASIS INDUCED BY SENSIDIZED BURSAL CELLS

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ABSTRACT

Parasitic helminths comprise a diverse group of metazoan organisms that infect billions of people and their domesticated animals worldwide. It is an unwelcomed invader in hostile territory constantly to contend with enzymes, antibodies, hormones, foreign proteins, protein-split products, tissue defence reactions and predatory phagocytes.Ascaridiasis is caused by Ascaridia galli, the largest internal parasitic nematode or roundworm causing helminthiasis in poultry. It infests the small intestine and can cause ill-thrift and intestinal compaction (enteritis). Immunization has greater impact on the economics of poultry production than all other therapeutic treatment and with the significant advancement in modern medicine, vaccination may become a feasible control alternative (Emery, Mcclure and Wagland, 1993). Present work is to explore potentialities of both conventional and novel approach to the development of vaccines by bursal antigens and to take a more holistic view of the immune response with the object of manipulating the response in such a way that it favours protection in chicks from Ascaridiasis and inhibit any pathological side effects. Data from this study may assist in developing more efficient control measures for A. galli, for combating against economic losses in poultry industry. Studies have been conducted with objectives including Biochemical studies involving various biochemical parameters with respect to sensitized bursal cells with low (300 embryonated eggs of Ascaridia galli) and high dose (1000 embryonated eggs of Ascaridia galli) of infection.

Index Terms: Ascaridia Galli, Ascaridiasis

I. INTRODUCTION

Poultry products are one of the most important protein sources for men throughout the world. Increasingly and specially in urban areas, these poultry products are derived from intensive production, with control of parasitic infections through the use of veterinary medication and good sanitation. In intensive battery systems, control of parasites is also through a high degree of isolation from potential disease causing organisms. The trade volume of poultry products has also increased in line with the rapid growth of global poultry meat and egg production (Windhorst, 2006). Ascaridia galli is a common parasite of poultry and has been reported in chickens, turkeys, guinea fowls, pigeons, ducks and geese (Ruff and Norton, 1997). It has also been reported as a common parasite of pigeons and doves in Zaria (Abdullahi et al., 1992; Oniye et al., 2000; Audu et al., 2004; Gadzama et al., 2005).

Ascaridiasis is caused by Ascaridia galli, the largest internal parasitic nematode or roundworm causing helminthiasis in poultry. It infests the small intestine and can cause ill-thrift and intestinal compaction (enteritis). Droopiness, emaciation and diarrhoea are the common clinical symptoms. The most important clinical sign of A. galli infection is loss of body weight, which increases parallel to worm load (Reid and Carmon, 1958).

Increased feed intake (Gauly et al., 2007), blood loss, reduced body weight, and increased mortality may also occur (Ikeme, 1971). There is a paucity of information regarding development of immunity in chicks against A. galli infection. In view of this, experimental biochemical studies were undertaken with the hope that some serodiagnostic technique may be helpful in the diagnosis of ascaridiasis.

Parasitic infections are known to alter biochemical parameters. Hook worm infection is well documented to cause anaemia in animals as well as humans. The antigens have been reported to cause hypersensitivity reactions. In the present studies, bursal antigens were used for sensitization followed by challenge infections. Therefore, biochemical studies were conducted as per the experimental design to understand whether antigens also modulate biochemical parameters or not. The present studies comprise immunization by conventional vaccines through transfer of sensitized antibody priming cells of bursa against Ascaridia galli, a gastrointestinal nematode of chicks.

II. MATERIAL METHOD AND EXPERIMENTAL DESIGN

Experimental hosts are white leghorn chicks. Experimental parasite is Ascaridia galli. Doses of infection are:-

(i) Low dose - 300 embryonated eggs of Ascaridia galli.

(ii) High dose- 1000 embryonated eggs of Ascaridia galli.

Ascaridia galli were obtained from intestines of infected chicks. The eggs of Ascaridia galli were cultured and incubated for embryonation by Riedal method (1947). These pure embryonated eggs were used for experiments. Counting of eggs was done by dilution method.

Infections were given to the chicks with different doses (300 and 1000) of embryonated eggs of A. galli. Chicks were sacrificed at day 15th day 30th and 45th of infection. Bursa was carefully removed and kept separately in Ringer's solution maintained at 400C, separated from attached expaneous tissues. Bursa was washed three times in Ringer's solution and finally kept in diluted Ringer's solution.

The bursa was teased with forceps and gentle dispersion in a loose fitting glass homogenizer. The cells were separated and suspended into fresh ringer solution, then centrifuged at approximately 3000 rpm for 10 minutes to remove any adherent exogenous material. In addition, the dead cells were disrupted during centrifugation and so the percentage cell viability increases during the washing procedure.

Collected bursal cells were injected subcutaneously (SC) to recipient chicks for biochemical findings.

In present investigation, experiment was conducted in two phases.

PHASE I - Infected and Non-immunized Group

PHASE II- Infected and Immunized Group

Total numbers of groups were six. These are:-

GROUP 1- Control group (Infected and Non-immunized)

This group was further subdivided into three subgroups.

Ca- Control group at day 15 of infection. (4 chicks)

Cb- Control group at day 30 of infection. (4 chicks)

Cc- Control group at day 45 of infection. (4 chicks)

GROUP 2- Infected with 300 embryonated eggs of Ascaridia galli

This group was further subdivided into three subgroups.

(C1)a- Autopsied after day 15 of infection (4 chicks)

(C1)b- Autopsied after day 30 of infection (4 chicks)

(C1)c- Autopsied after day 45 of infection (4 chicks)

GROUP 3- Infected with 1000 embryonated eggs of Ascaridia galli

This group was further subdivided into three subgroups

(C2)a- Autopsied after day 15 of infection (4 chicks)

(C2)b- Autopsied after day 30 of infection (4 chicks)

(C2)c- Autopsied after day 45 of infection (4 chicks)

GROUP 4- Control group (Immunized)

This group was further subdivided into three subgroups.

RCa- Control group at day 15 of immunization (4 chicks)

RCb- Control group at day 30 of immunization (4 chicks)

RCc- Control group at day 45 of immunization (4 chicks)

GROUP 5- Immunized with sensitised bursal cells and challenged with 300 embryonated eggs of Ascaridia galli

This group was again sub-divided into three groups according to the day of autopsying.

(RC1)a – Autopsied after 15 days (4 chicks)

(RC1)b– Autopsied after 30 days (4 chicks)

(RC1)c– Autopsied after 45 days (4 chicks)

GROUP 6- Immunized with sensitised bursal cells and challenged with 1000 embryonated eggs of Ascaridia galli

This group was again sub-divided into three sub-groups according to the day of autopsying

(RC2)a– Autopsied after 15 days (4 chicks)

(RC2)b– Autopsied after 30 days (4 chicks)

(RC2)c– Autopsied after 45 days (4 chicks)

Biochemical parameters including serum Glucose level, Serum protein level, Serum Cholesterol level, Serum Acid Phosphatase level, Serum Alkaline phosphatase level, Serum urea level with respect to sensitized bursal cells plus low and high were studied in both infected and immunized group.

III. RESULT AND DISCUSSION

In present studies, the WLH chicks immunized with bursal antigen revealed protection against ascaridiasis.

During the present investigation, Hypoglycemia (low glucose level) was observed in chicks infected with low and high doses of embryonated eggs of A. galli (Table-1) but combined effect of antigen and experimental ascaridiasis revealed increased level of glucose in comparison to control group(Table-2).

Decline in sugar molecules may be due to greater utilization of sugar due to stress condition in host because of the parasite and it may be because the host's blood sugar are serving as a source to provide carbohydrate to the parasites. It appeared that Ascaridia galli somehow managed to absorb the host’s sugar either directly or indirectly from injured intestinal tissues.

The protein level was found to be decreased during (high and low doses) experimental ascaridiasis (Table-3). The combined effect of immunization and infection revealed a slight increase in total protein level in comparison to control group(Table-4).

Present findings are of opinion that level of total protein increased in immunized group due to changes in the globulin fraction because of increase in secretary antibodies. Hypoproteinemia was frequently observed by Vibe Peterson and Neilson (1979) during their investigation on Strongylus vulgaris infection in horse. Various

changes in the total protein levels are a characteristic feature of many parasitic infections (Von Brand, 1973). He also observed that the variations in the total protein values occur depending on the stage or severity of an infection in the species of host and also the presence of the previous infection, hormonal imbalance and other similar factors.

The cholesterol level was found to be augmented in infected group of WLH chicks(Table-5). The bursal antigen also mildly augmented the level of cholesterol(Table-6). It may be because hypercholestremia shifts the balance in favour of free radical generation, which led to oxidative tissue damage in host body. Rise in cholesterol level may also be due to the result of enhanced lipid metabolism of the host.

The present investigation revealed a slight rise in serum acid phosphatase activity in A. galli infected group of WLH chicks(Table-7). Combined effect of immunization and experimental ascaridiasis revealed a slight decline in acid phosphatase activity in WLH chicks(Table-8). The decreased acid phosphatase level may be due to disturbance in metabolism of chicks during A. galli infection. Its lower value may be attributed to hypophosphatemia and pernicious anaemia.The present findings are in accordance with Kumar (1983), who reported a rise in the serum acid phosphatase level in albino rats with experimental infection of Bunostomum trigonocephalum.

TABLE 01
BIOCHEMICAL PARAMETERS OF GLUCOSE OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN INFECTED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	287.4	264.8	222.6
		S.D.	± 2.88097	± 1.48324	± 2.19089
		S.E.	± 1.28841	± 0.66332	± 0.9798
2	30	Mean	292.28	253.66	213.906
		S.D.	± 0.42071	± 0.53033	± 0.20293
		S.E.	± 0.18815	± 0.23717	± 0.09075

TABLE 02
BIOCHEMICAL PARAMETERS OF GLUCOSE OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN IMMUNIZED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	291.08	294.1	297.458
		S.D.	± 0.46583	± 0.25495	± 0.48823
		S.E.	± 0.20833	± 0.11402	± 0.21834
2	30	Mean	290.472	296.502	299.582
		S.D.	± 1.28342	± 0.38108	± 0.43637
		S.E.	± 0.57396	± 0.17042	± 0.19515

TABLE 03
BIOCHEMICAL PARAMETERS OF PROTEINS OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN INFECTED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	6.224	6.024	5.68
		S.D.	± 0.13278	± 0.12381	± 0.17889
		S.E.	± 0.05938	± 0.05537	± 0.08
2	30	Mean	6.24	5.922	5.66
		S.D.	± 0.03536	± 0.04207	± 0.35777
		S.E.	± 0.01581	± 0.01881	± 0.16

TABLE 04
BIOCHEMICAL PARAMETERS OF PROTEINS OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN IMMUNIZED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	6.692	7.126	7.228
		S.D.	± 0.21684	± 0.04159	± 0.04817
		S.E.	± 0.09697	± 0.0186	± 0.02154
2	30	Mean	6.712	7.228	7.75
		S.D.	± 0.17079	± 0.24263	± 0.11045
		S.E.	± 0.07638	± 0.10851	± 0.0494

TABLE 05
BIOCHEMICAL PARAMETERS OF CHOLESTEROL OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN INFECTED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	149.5	233.8	272
		S.D.	± 0.4062	± 2.58844	± 0.12247
		S.E.	± 0.18166	± 1.15758	± 0.05477
2	30	Mean	152.08	238.42	261.21
		S.D.	± 0.43243	± 0.32711	± 0.0995
		S.E.	± 0.19339	± 0.14629	± 0.0445

TABLE 06
BIOCHEMICAL PARAMETERS OF CHOLESTEROL OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN IMMUNIZEDGROUP

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	150.044	155.996	161.88
		S.D.	± 0.34056	± 0.07987	± 0.64703
		S.E.	± 0.1523	± 0.03572	± 0.28936
2	30	Mean	152.482	156.396	164.41
		S.D.	± 0.4469	± 0.33923	± 0.44266
		S.E.	± 0.19986	± 0.15171	± 0.19796
		Mean	156.16	157.156	165.478

TABLE 07
BIOCHEMICAL PARAMETERS OF ACID PHOSPHATASE OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN INFECTED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	5.54	7.1	7.4
		S.D.	± 0.01673	± 0.17889	± 0.23022
		S.E.	± 0.00748	± 0.08	± 0.10296
2	30	Mean	5.53	7.44	7.58
		S.D.	± 0.0445	± 0.07855	± 0.09925
		S.E.	± 0.0199	± 0.03513	± 0.04438

TABLE 08
BIOCHEMICAL PARAMETERS OF ACID PHOSPHATASE OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN IMMUNIZED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	5.248	5.308	5.18
		S.D.	± 0.04147	± 0.2251	± 0.05874
		S.E.	± 0.01855	± 0.10067	± 0.02627
2	30	Mean	5.254	5.068	5.144
		S.D.	± 0.43021	± 0.22219	± 0.20107
		S.E.	± 0.1924	± 0.09937	± 0.08992

TABLE 09
BIOCHEMICAL PARAMETERS OF ALKALINE PHOSPHATASE OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN INFECTED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	17.16	14.838	13.166
		S.D.	± 0.32863	± 0.42399	± 0.17199
		S.E.	± 0.14697	± 0.18962	± 0.07692
2	30	Mean	16.872	14.988	13.014
		S.D.	± 0.07981	± 0.07855	± 0.10597
		S.E.	± 0.03569	± 0.03513	± 0.04739

TABLE 10
BIOCHEMICAL PARAMETERS OF ALKALINE PHOSPHATASE OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN IMMUNIZED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	17.59	17.28	17.6
		S.D.	± 0.57035	± 0.50695	± 0.60828
		S.E.	± 0.25507	± 0.22672	± 0.27203
2	30	Mean	15.97	16.122	16.464
		S.D.	± 0.10368	± 0.14167	± 0.09813
		S.E.	± 0.04637	± 0.06336	± 0.04389

TABLE 11
BIOCHEMICAL PARAMETERS OF UREA OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN INFECTED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	6.12	8.3	9.9
		S.D.	± 0.20494	± 0.2	± 0.18708
		S.E.	± 0.09165	± 0.08944	± 0.08367
2	30	Mean	6.4	8.02	9.62
		S.D.	± 0.30822	± 0.58907	± 0.46043
		S.E.	± 0.13784	± 0.26344	± 0.20591

TABLE 12
BIOCHEMICAL PARAMETERS OF UREA OF WHITE LEG HORN CHICKS INFECTED WITH THE LOW AND HIGH DOSES OF EMBRYONATED EGGS OF *ASCARIDIA GALLI* IN IMMUNIZED GROUP.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	6.4	6.206	6.296
		S.D.	± 0.25495	± 0.04278	± 0.04336
		S.E.	± 0.11402	± 0.01913	± 0.01939
2	30	Mean	6.28	6.226	6.382
		S.D.	± 0.40866	± 0.17897	± 0.32568
		S.E.	± 0.18276	± 0.08004	± 0.14565

In the present studies the serum alkaline phosphatase was found decreased during experimental ascaridiasis in WLH chicks(Table-9) and after treatment of infected group with bursal antigen, a slight rise was observed in comparison to control group(Table-10). Increase in alkaline phosphatase may be due to metabolic products and endotoxins secreted by the parasites and metabolites which act as antigen or immunogens affecting the synthesis of alkaline phosphatase in host's body.

Rani (1986) reported decreased alkaline phosphatase level in WLH chicks with light and heavy doses of A. galli infection due to inhibition of enzymes during infection. Braret al., (1991) found increased level of serum alkaline phosphatase in desert sheep infected with Haemonchuscontortus.

The present investigation revealed a significant elevation in serum urea level in WLH chicks infected with A. galli eggs(Table-11). Combined effect of treatment with antigen and experimental ascaridiasis revealed that the serum urea level slightly decreased in comparison to control group(Table-12). Elevation in level of serum urea in infected group could be attributed to nephritis and nephrotoxicity caused by the ingestion of toxic substances, intestinal obstruction or by infection caused by parasite in host's body. Heavy infections may also be supposed to lead to intestinal obstruction, ultimately leading to increased serum urea level. El-Abdinet al., (1975) also reported that the level of urea increased slightly after treatment with anthelmintic in comparison to the control group.

The rise in serum urea level in the present investigation in infected group may also be attributed to enhanced nitrogen metabolism brought about by the round worm A. galli leading to a high rate of urea production.

IV. CONCLUSION

On the basis of various experimental evidences, the above studies proved beyond doubt that bursal antigen and infected eggs induced biochemical modulations in experimental WLH chicks and hence bursal antigen could be adopted as conventional vaccine against Ascaridiasis.

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