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Antibody Response in Broiler Chickens Infected with Different Developmental Stages of *Eimeria tenella*

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Abstract

Antibodies (IgG or IgY) titre values were higher in broilers sera infected with sporulated oocyst and merozoites reaching a peak on day 10 of post primary and secondary infections and day 5 post tertiary infection in sera of broilers (treated and non- treated). At tertiary infection, antibodies increases at day 5, 7, 11 and 14 indicating that antibodies increases in broilers infected with the invasive or zoite stages, (sporozoite and merozoite) of the parasite. There was a significant difference in the antibody output between the sera of the broiler groups ($p < 0.05$).

Keywords: *Antibodies; Sera; Sporulated oocyst; Merozoites; Primary-secondary-tertiary infections; Broilers; Invasive or zoite stages; Parasite*

Abbreviations: IgG: Immunoglobulin G; IgY: Immunoglobulin Y

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Introduction

Coccidiosis caused by protozoans of genus *Eimeria tenella* is a chicken parasitic disease of great economical importance globally characterized by haemorrhage leading to mortality. Conventional disease control strategies depend on vaccination and prophylactic use of anticoccidial drugs. Research has been carried out worldwide to try to elucidate the mechanism of protective immunity against coccidiosis. It was concluded from early studies that cellular immunity is the key to protection against *Eimeria*, whereas humoral immunity plays a very minor role in resistance against infection. By contrast, other studies have pointed towards the ability of antibody to block parasite invasion, development and transmission and to provide passive and maternal immunity against challenge infection.

Herein, recent results demonstrate the ability of antibodies (raised by live immunization or against purified stage-specific *Eimeria* antigens) to inhibit parasite development *in vitro* and *in vivo* and readdress the question of the role of antibody in protection against coccidiosis (Wallach, M., 2010). Enzyme-linked immunosorbent assay has already been used extensively to measure anti-Eimeria antibodies in chickens (Smith., *et al.* 1994). The aim of the study was to determine the antibody response in Broilers infected with different developmental stages of *Eimeria tenella*.

Materials and Methods

Study Area

The experimental settings was at the PETCA building, Anguldi, 5 kilometers from the National Veterinary Research Institute, Vom, Jos Plateau State, Nigeria, where the laboratory work was carried out. The Jos Plateau lies on the pre-cambian from the cambian to jurasic northern Nigeria crystalline complex in central Nigeria. Its average elevation is about 1,250m above mean sea level. The state is bounded on the north and west by Kaduna plains (on the average of 600m above mean sea level) and on the south by Benue plains (on the average of 700m above mean sea level), (PADP, 2002). Geographically, the Jos Plateau is located between latitude 08°24'N and longitude 008°32' and 010°38' east.

The land surface of Jos Plateau consists of plains, hills, depressions and todes of various forms, shapes and sizes. It is a major tourist centre in Nigeria with agriculture as the main occupation of the people. The high altitude confers on the Plateau lower temperature than those encountered elsewhere in Nigeria except the Obudu and Mambilla Plateau. The dry season is determined by the north easterly tropical continental air masses known as harmattan (from October-April) and the wet season is the most tropical maritime air masses from May-September. The average annual rainfall is about 1,100 mm and is evenly distributed. Another element of climate is temperature December and January experience temperatures of below 15°C. During February and March, the temperature rises again about 25°C. Most of the human activities are mining and agriculture involving rearing of chickens in both the rural and urban areas for subsistency and income (PADP, 2002).

Experimental Birds

Four hundred (400) day-old broilers (marshal breed) were purchased from ECWA farms, Jos, brooded and used for the study. The birds were randomly distributed into six different groups of 40 each, in a clean wire cage (n = 40). At two weeks old, each group was again subdivided into two, treated and none treated, of twenty broilers (n = 20) each. The birds were kept in a clean building, and the legs banded or labelled under strict biosecurity measures. Feed (Broiler starter, Grand cereals and oil mills, PLC, Zawan, and Jos-Plateau, Nigeria) and water were provided *adlibitum* the birds were vaccinated with Newcastle disease vaccine (La-Sota) at day 21 and Gomboro disease vaccines at days 14 and 28.

Experimental infection of broilers with infective materials and monitoring

The experimental birds, except the control were orally given primary and secondary challenge infections with the various developmental stages of *Eimeria tenella*, respectively at week 2 and $3^{1/2}$ while at week 5 of age, all birds were infected the sporulated oocyst of the parasite (Table 1). Each group was subdivided into Treated (n = 20) and Non- Treated (n = 20). In each infected group, birds in one of the subdivisions were treated with amprolium 250 WSP^{R} Holland was administered in drinking water at a concentration of 250 mg/1 (0.025%) for a period of 5 days as prescribed by the Manufacturer at the appearance of visible clinical signs.

To obtain serum, blood samples were collected from the experimental birds using the method described by Talebi and Mulcahy (1995). Briefly, 1 ml of blood sample was obtained from the wing vein of each bird using 20 gauge needle (Becton Dickson co., Plymouth, UK) into a 2 ml vacutainer. Samples were obtained on days 2, 4, 6, 8, and 10 after primary and secondary infections, and on days 5, 7, 11, 14, 17, 20 and 24 after tertiary infection (Rose and Hasketh, 1982). The blood which had been allowed to clot for 1 hour at room temperature, was left over night at 40C and then centrifuge at 800g for 5 minutes. The serum samples were thereafter heated at 56°C for 30 minutes to inactivate the compliment before storage at -20°C. All sera were analyzed with the developed ELISA Triplicate

Group Treatment and No. of birds		Infection type/ Age of bird		
		I°/wk 2	2°/wk 3 1⁄2	3°/wk 2 challenge with virulent *E. tenella*
I	T (n = 20)	10^5 USO	10^5 USO	10^5 SO
	NT (n = 20)	10^5 USO	10^5 USO	10^5 SO
II	T (n = 20)	10^5 SO	10^5 SO	10^5 SO
	N T(n = 20)	10^5 SO	10^5 SO	10^5 SO
III	T (n = 20)	10^5 SCZ	10^5 SCZ	10^5 SO
	NT (n = 20)	10^5 SCZ	10^5 SCZ	10^5 SO
Iv	T (n = 20)	10^5 MRZ	10^5 MRZ	10^5 SO
	NT (n = 20)	10^5 MRZ	10^5 MRZ	10^5 SO
V	T (n = 20)	10^5 GMT	10^5 GMT	10^5 SO
	NT (n = 20)	10^5 GMT	10^5 GMT	10^5 SO
VI		0	0	0
KEY; USO-Unsporulated oocyst			1°- primary infection	
	SO- Sporulated oocyst		2°- Secondary infection	
	SCZ- Schizoites		3°- Tertiary infection WK-Week T –Treated NT – Non treated	
	MRZ- Merozoites			
	GMT- Gametocytes			

Table 1: *Experimental infection of broilers with developmental stages of Eimeria tenella.*

Enzyme-linked immunosorbent assay

Hay Dottom Nune certified microtiter plates (Roskilde Denmark) were coated with 50 µ 1 of soluble *E. tenella* antigen (sporozoites from characterized sporulated *Eimeria tenella* oocysts)/web at a concentration of 5 µ g/ml carbonate buffer (pH 9.6) for 1 hour at 39°C. The plates were rinsed five times with saline/tween (S/T), and treated with 75 µ l of PBS containing 3% BSA, 1% rabbit serum and 0.05% sodium azide for 1 hr at room temperature to block non-specific adsorption. The plates were washed five times with saline/tween (S/T). A 50 µ l test serum sample, diluted 1: 1000 in PBS-T (including 1% rabbit serum and 0.05% sodium azide) was added to each well and incubated for 2 hours at room temperature.

The plates were washed 5 times with S/T and 50 µ l of 1:1000 dilution of rabbit anti chicken immunoglobulin peroxidase (Pelfreeze Rogers, Arkansas) in PBS-T was added. After 2 hours incubation, plates were washed five times with S/T and freshly prepared substrate solution (2 mM OPD 6.15 Mm H_2O_2 in 0.1M citrate buffer pH 6.0) was added per well. The enzyme-substrate reaction was stopped after 30 minutes by addition of 100 µ l to each well of 2N H_2SO_4. Absorbance were measured at 492 nM (A_{492}) in a Biotele ELISA Reader (Ref S1118170, Multiskan Ex, USA). All serum samples from the experiment were analysed on a single day.

Enzyme linked immunospot assay

The spleen was crushed by pressing on fine mesh Petri dishes containing RPMI-1640 (Sigma, Aldrich Cheme, and GmbH, Germany). The suspension was then passed through nylon cell strainer (70 µm; Becton, Dickson, Lincoln Park, NJ). The filtrate was centrifuged at 250g for 10 minutes at 4°C and the sediment collected. Lysis buffer (1 ml/spleen) was added for erythrocyte lysis and placed on ice for 2 minutes. The suspension was passed through the cell strainer again and was centrifuged again 250g for 10 minutes at 4°C to collect

the sediment. The cell suspension (10 µl) was mixed with same amount of trypan blue (Sigma, Aldrich Cheme GmbH, and Germany) and the number of cells was counted in a haemocytometer. The centrifuged ion was adjusted to 10^6 cells/100 µl with RPMI-1640. Nitro cellulose – microlitre plates (96 wells, Millipode multiscreen MAHA) were used in the ELISPOT assay. Individual wells of the plate were filled with 100 µl of goat anti-chick 1g (H+L) – UNLB (primary antibody) at a final concentration of 2 µl/ml and were allowed to stand overnight at 4°C in a humid chamber. Unadsorbed antibodies were removed by three successive washings with PBS. Wells were immediately filled with 100 µl RPMI – 1640 to saturate the remaining finding sites and incubated at 37°C for 2 hrs.

The medium was discarded and the plates were dried with absorbent paper incubation of Ig-secreting cells. A 100 µl cell suspension containing 10^6 cells was dispensed into each well in duplicate and they were incubated undisturbed at 37°C for 4 hours. The plates were rinsed twice by immersion in PBS containing 0.05% Tween 20 (PBST) for 2-3 minutes. The wash buffer was removed from the plates and the outer surfaces of the plates were dried carefully. A 100 µl of PBST containing Goat anti-chick IgG-AP (1000-fold dilution) were added to each well and the plates incubated at 4°C over night. The plates were then rinsed three times by immersion in PBST and dried. Each well was then filled with 100 µl BCIP/NBT solution; prepared by adding 66µl of NBT (containing 50 µl/ml nitroblue tetrazolium in 70% N, N-dimethylformamide) in alkaline phosphate buffer (containing 5.8 g Nacl, 0.1 g $Mgcl_2$ 12.1g Tris). The plates were thoroughly washed with running tap water and air-dried for 24 hours. Blue spots showing fuzzy borders were considered positive for immunoglobulin G (IgG).

Results

Antibodies (IgG or IgY) titre values were higher in sera from broilers infected with sporulated oocyst and merozoites reaching a peak on day 10 of post primary and secondary infections and day 5 post tertiary infection in both broilers treated and non-treated, (Figure 1, 2 and 3). The antibodies values were relatively low in broilers infected with unsporulated oocysts, schizonts and gametocytes at primary and secondary infection in both treated and non treated broilers at day 10 (Figure 1 and 2). At tertiary infection, antibodies increases at day 5, 7, 11 and 14 (Figure 3) respectively. Generally, antibodies levels of sera from the infected broilers with the different developmental stages of the parasite, treated and non treated increased post inoculation and after reaching peak levels, they began to decline (Figure 3). The control birds show no antibodies in the sera. The study demonstrates a non significant difference in the antibody titre values of the treated and non-treated sera of the infected broilers groups (II and IV), $p < 0.05$.

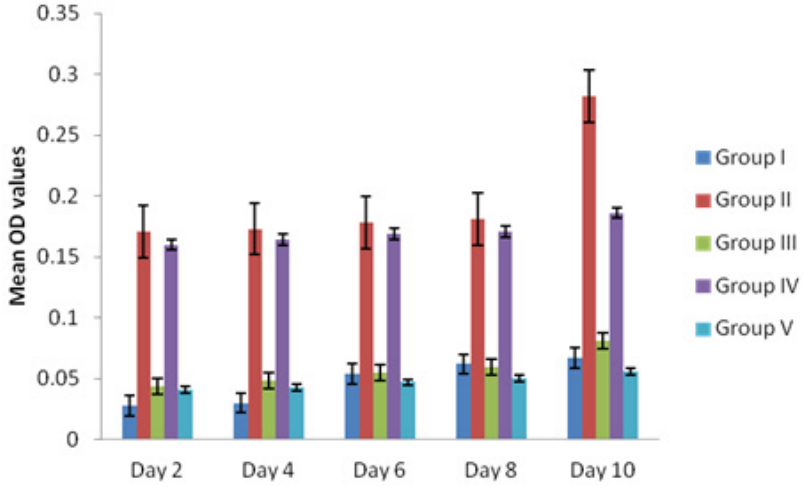

Figure 1: *Antibodies level in sera of the experimentally infected broilers with the different stages (unsporulated oocyst, sporulated oocyst, schizonts, merozoites and gametocytes) of Eimeria tenella at optical density (O.D) or absorbance of 492 nm) at primary infection.*

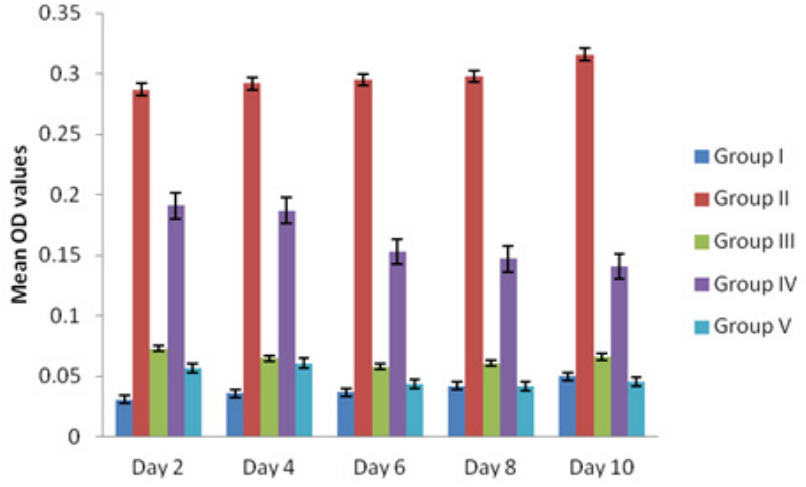

Figure 2: *Antibodies level in sera of the experimentally infected broilers with the different stages (unsporulated oocyst, sporulated oocyst, schizonts, merozoites and gametocytes) of Eimeria tenella at optical density (O.D) or absorbance of 492 nm) at secondary infection.*

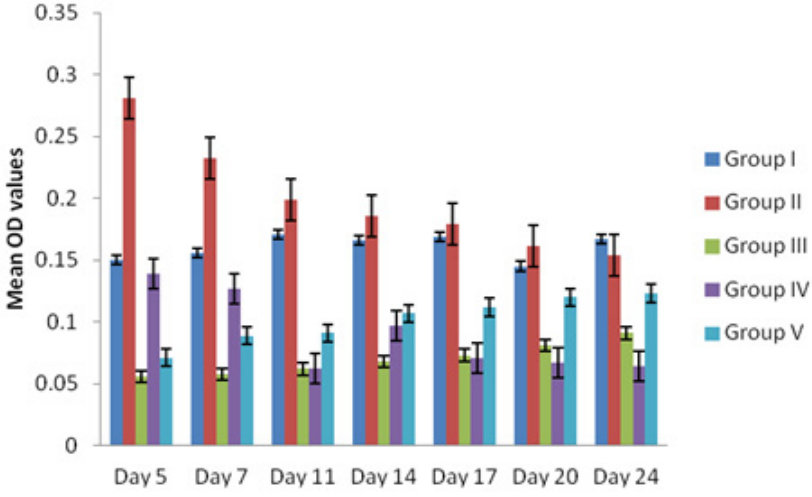

Figure 3: *Antibodies level in sera of the experimentally infected broilers with the different stages (unsporulated oocyst, sporulated oocyst, schizonts, merozoites and gametocytes) of Eimeria tenella at optical density (O.D) or absorbance of 492 nm) at tertiary infection.*

Discussion

Serum antibody levels increased rapidly on day 10 in the broilers at primary and secondary infections and day 5 at tertiary infection. This varied from the reports Bumstead., *et al*. (1995) who recorded a peak of humoral immune response between day 14 and 21 post coccidial infection in birds. This may be due to differences in the immunogenic potential of the isolate, age, environment and genetic background of the birds. Antibodies remain significantly high in broilers infected with sporulated oocyst (sporozoite) and merozoite at the end of each infection period, suggesting that the level of antibodies appears to be related to the severity of the developmental stage of the parasite.

This is in concordance with the reports of Constantinoiu., *et al*. (2007) who reported high antibodies levels persistence in commercial flock after natural exposure to Eimeria or following infection with live vaccine. The present study revealed that there was no significant difference in the antibody titre values in the treated and non treated broilers. This is consistent with the results of Kiani and Farhang (2008), but is inconsistent with the reports of Kurkure., *et al*. (2006) who stated that chicks treated with coxynil showed higher antibody titre values than those maintained on feed without coxynil. There are still debates on antibodies inducing protective immunity. Dalloul and Lillehoj, (2005) stated that antibodies play a minor role as cell mediated immunity (CMI). Gilbert., *et al*. (1998), Talebi and Mulcahy (1995), reported that the levels of serum antibodies following infection do not correlate with protection or oocyst output and antibody levels in chickens. This variation may be due to the age, dose and strain of the parasite as well as the genetic background of the broilers. However, the study agrees with the findings of Rose (1987) who showed that antibody could have deleterious effects, including agglutination, lysis, neutralization of infectivity and morphological changes on various developmental stages of Eimeria if they come in close contact with the parasite. The first subunit vaccine (CoxAbic^{R}) is based on transfer of protective antibodies from immunized hens to embryo (Belli., *et al*. 2004), indicating that antibodies do play an important role in immunity.

Conclusion

The following can be concluded from the results obtained:

1. The sporozoites and merozoites showed strong infectivity and elicited stronger antibody titre values in infected broilers with sporulated oocysts and merozoites in infected birds at primary-secondary-tertiary infections, indicating that they might be potential vaccines candidates against avian coccidiosis.
2. The immunoglobulins were IgG or IgY

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