Plant Archives Vol. 20, Supplement 2, 2020 pp. 53-57 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

IMMUNIZATION OF *EIMERIA TENELLA* AND *KLEBSIELLA PNEUMONIAE* ANTIGENS EFFECTS ON SOME BLOOD ENZYMES IN LOCAL BREED RABBITS

Basil R.F. Razook*[1], Majid Mohammed Mahmood[1] and Haider Mohammed Ali Al-Rubaie[2]

[1]Unit of Zoonotic Diseases, College of Veterinary Medicine, University of Baghdad, Iraq.

[2]Department of Parasitology, College of Veterinary Medicine, University of Baghdad, Iraq.

*(Corresponding author email: basilraz@yahoo.com)

Abstract

The aim of this study was to investigate the immunization effect of *Eimeria tenella* and *Klebsiella pneumoniae* in some blood enzymes by using 16 local breed rabbits which are divided into 4 equal groups (4 each). The first group immunized with S/C by 1000 ug/ml of sonicated *E. tenella* antigen; the second group immunized by 1000 ug/ml of sonicated *K. pneumoniae* antigen; the third group immunized by 500 ug/ml of both antigens; the forth group was injected with 1 ml S/C phosphate buffer saline (Control). The results were shown that the third group gave high levels of blood enzymes (GGT, AST, ALT, and ALP) followed by the second group and finally the first group compared with control with significant differences ($P<0.05$, $P<0.01$). The conclusion of this study refers to the compared antigens for immunization increase the blood enzymes is the immunized animals and effects by the type of antigen rather than dose of immunization and we think it is the first study that deals with this subject (novel study).

Keywords : *K. pneumoniae*, *E. tenella*, rabbits

Introduction

The coccidia of poultry have been studied extensively, because coccidiosis is a cause of severe losses in chicken and turkey and *Eimeria tenella* is the most economically important of the poultry coccidia and will use it as an example of coccidium of birds and mammals. Transmission takes place from one host to the next by ingestion of resistant form, the oocyst (Marquardt *et al*., 2000). The aim of this study was to investigate the immunization effect of *Eimeria tenella* and *Klebsiella pneumoniae* in some blood enzymes.

Intestinal coccidiosis is a complex of diseases that are of great economic importance in domestic animals and of importance in humans in some circumstances in all parts of the world (Marquardt *et al*., 2000).

Intestinal coccidia are found in all birds and mammals as well as other vertebrates and some invertebrates but not all of them cause medical or economic problems (Marquardt *et al*., 2000).

Klebsiella pneumoniae is rod shape encapsulated gram negative bacterium and belong to the family Enterobacteriaceae. It is a most commonly encountered worldwide as a community-acquired and hospital-acquired pathogen and it is frequently found in the flora of the mouth, skin, intestines, or in natural environments (Guo *et al*., 2012) *K. pneumoniae* infections are usually associated with high mortality rates (Stahlhut *et al*., 2012).

Enzyme activities are vary greatly among tissues and species. It is important to realize that the activity of particular enzyme may be high in one organ or tissue or even specific for that tissue, but if it does not change significantly in the blood when that tissue is damaged, it has little clinical significance. The highest AST activity occurs in heart muscle followed by liver and skeletal muscle. However, increased activity has been associated with hepatocellular damage (hepatic diseases) (Coles, 1986).

K. paumoniae is present in the respiratory tract and feces of about 5% of normal individuals. It causes a small proportion about 1% of bacterial pneumonias. *K. pneumoniae* can produce extensive haemorrhagic necrotizing consolidation of the lung. It prodices urinary tract infection and bacteremia with focal lesions (Brooks *et al*., 2010).

T lymphocytes and their cytokines are essential in the immunity against *Eimeria* infections in both avian and mammalian species (Allen and Fetterer, 2002).

The CD4+ T helper cells and CD8+ cytotoxic T lymphocytes are the major cell subsets of T cells involved in the host that response to *Eimeria* infection (Yun *et al*., 2000).

Al–Samrraee (2017) was found the synergistic immune response interaction between *E. tenella and K. pneumoniae* antigens by increasing the cellular (skin test) and humoral (antibodies' titers) immune response in rabbits. This study was the first one that deal with the effects of sonicated bacterial and parasitic antigens in some blood enzymes in the local breed rabbits.

The macrophages, CD8+ cytotoxic and natural killer cells that elicit the inflammatory response to eliminate the antigen (Jayapal, 2007).

IgG, IgA, and IgM play a vital role in the binding of foreign antigens and the presence of these antibodies molecules on a microbial or parasitic surface can cause clumping or agglutination and IgG and IgM are activate the complement system (Tizard, 1992).

Ghazal *et al*. (2016) referred to the damaging effect of single and multi-walled carbon nanotubes in liver texture in the New Zealand white rabbit, which lead to an increase in the ALT and AST.

Another study to Alzien (2016) described the deleterious effect of alcohol administration for a long term could cause an elevated levels of liver enzymes (ALT, AST, ALP).

Materials and Methods

Materials

1) *Eimeria tenella* oocysts were collected from a natural infected ceca of broiler chicken according to (Red and Long, 1978, Conway and Mackenzie, 2007) and sonicated to prepare an antigen (Mitov *et al*., 1992)
2) *K. pneumoniae* : the isolates were yielded from the Zoonotic Diseases Unit, College of Veterinary Medicine, University of Baghdad, killed whole cell sonicated antigen was prepared according to (Mitov *et al*., 1992), the total protein of both antigens was measured by using the Biuret method (Henry *et al*., 1974).
3) Animals : sixteen local breed rabbits of both sexes about 1.5 to 2 Kg were used, which divided into four groups (4 each) as follows:
   a. The first group was given 1000 ug/ml S/C of sonicated *E. tenella* oocysts antigen(SETO).
   b. The second group was given 1000 ug/ml S/C of sonicated *K. pneumoniae* (SKP).
   c. The third group was given both antigens (SETO 500 pg/ml and SKP 500 ug/ml S/C.
   d. The fourth group as control which given 1 ml of phosphate buffer saline (pH= 7.2) S/C.
4) Booster dose of each antigen was given at the same doses to all first three groups (1, 2, and 3) after 14 days from the first dose.
5) Blood samples were collected every 14 days for three intervals by heart puncture and sera were isolated and stored in deep freeze (-20 ºC) until use (Weiss and Wardrop, 2010). Enzyme concentrations were measured condition to mannitic are produce of RANOX company for AST (AS147) and ALT (AL146), and GGT and ALP by using Reflotron.
6) Statistical analysis : the data were compared by using T test and $P<0.05$ was considered as significant (Al-Morani, 1986).

Results

Concentration of serum enzymes after 14 days serum enzymes were differed in their concentrations in different immunized groups with significant ($P<0.01$) differences. In the third group that immunized by both antigens *E. tenella* and *K. pneumoniae* showed high enzymes concentrations (GGT, AST, ALT, and ALP) 7-64±0.24, 57.82±3.27, 50.87±0.72 and 47.05±2.61 respectively followed by the second group that immunized by *K. pneumoniae* 7.53±0.22, 43.43±1.88, 48.73±0.40 and 24.45±0.68 respectively and finally the third group that immunized by *E. tenella* that showed 6.04±0.00, 31.45±0.45, 32.53±0.93 and 19.25±0.62 respectively compared to the control group (6.04±0.00, 21.41±0.69, 42.86±0.76 and 14.65±0.13 respectively (Table 1).

Blood enzymes concentrations after 28 days of immunization blood enzymes (GGT, AST, ALT, and ALP) were showed an elevation in the their concentrations after 28 days except the first group (*E. tenella*) that gave a decrease concentration in GGT and ALT compared to the control group but with significant ($P<0.05$) differences and the third group that immunized with both *E. tenella* and *K. pneumoniae* was showed highest blood enzymes concentration followed by the second group (*K. pneumoniae*) compared to the control group with significant ($P<0.01$) differences (Table 2).

The blood enzymes concentrations after 42 days of immunization are shown in table (3). It showed that the enzymes (GGT and ALT concentrations) in the first group (*E. tenella*) decreased compared to the control group but increased significantly in AST and none significantly ($P>0.05$) in ALP, while the immunised group second (*K. pneumoniae*) and third group (*E. tenella* and *K. pneumoniae*) were showed significant differences ($P<0.01$) compared to the control group.

Discussion

All of the intestinal species of coccidia have much the same pattern of transmission and development in the host (Marquardt *et al*., 2000).

The sporozoites seek a proper host cell, most often an enterocyte but sometimes an endothelial cell. Each species of coccidium seek a preferred type of cell in a particular location of the intestines (Marquardt *et al*., 2000).

Upon infection with coccidia and termination of the life cycle, the host becomes solidly immunized. The greater part of immunologic studies have been done on *Eimeria* spp. of chickens mostly *E. tenella* (Marquardt *et al*., 2000).

It has long been known that antibodies are present in the blood of animals after infection with *E. tenella* functional immunity to the coccidia lies mainly in cell-mediated immunity (CMI) (Marquardt *et al*., 2000)

Immunity to coccidia is characterized by the following: 1- completely sterile. 2- not permanent. 3- species specific. 4- principally CMI (Marquardt *et al*., 2000).

Encapsulated strain of *K. pneumoniae* have been shown to suppress the pulmonary inflammatory response by decreasing the production pro-inflammatory cytokines TNF alpha, Interferon gamma and interleukin 6 (IL-6), while increasing the production of the anti-inflammatory cytokines (Yoshida *et al*., 2001).

Lipopolysaccharides (LPS) is known to play a role in bacterial pathogenesis and is the causative agent of septic shock (Caroff *et al*., 2002).

In lungs of rabbits injected with *K. pneumoniae* antigen, accumulation of inflammatory cells infiltration in the interstitial tissue; liver containing numerous neutrophils and macrophages, proliferation of lymphocytes and macrophages (Razook, 2018)

Fimbriae are non-flagellar filaments projections on the bacterial cell surface, these fimbriae are thought to play an important role during the early stages of bacterial adhesion to the host cells (Schroll *et al*., 2010).

After recognition antigen of *K. pneumoniae* that stimulate innate immune responses against this bacterial antigens and increased neutrophil activation (Schurr *et al*., 2005).

Combination between antigens stimulates and improves the cellular and humoral responses (Sadeq, 2018).

After infection with *K. pneumoniae* can show increases in cytokines and chemokines including interferon gamma, tumor necrosis factor and interleukins (6, 7, 12, 1β and 10)

during *K. pneumoniae* infection result in increase mortality and bacterial burden in lungs (Moore *et al*., 2002).

Exposure of the mucosal immune system to bacterial antigens is likely to promote an inflammatory response to produce of antibodies against *K. pneumoniae*, although directed against *Klebsiella* spp. (Castinel *et al*., 2008).

The current study showed the synergistic effect between these 2 antigens killed molecule sonicated antigens of *E. coli* O157 and Klebocin of *K. pneumoniae* which stimulates CMI (Sadeq, 2018).

T1 cell secreted interferon gamma, tumor necrosis factor beta and IL-2 which activates macrophages and responsible for CMI and phagocyte dependent protective response (Tizard, 2013).

Klebocin give good immunity against infection due to its toxicity to another bacteria and inhibited the microbial growth (Sadeq, 2018).

Alterations in serum activity due to malfunctioning of the liver occur as a result of three processes : 1) an elevation of enzymes due to disruption of hepatic cells as a result of necrosis or as a consequence of altered membrane permeability. Included in this group are the enzymes alanine aminotransferase (ALT), formerly known as glutamic pyruvic transaminase (GPT) aspartate aminotransferase (AST) formerly called glutamic oxaloacetic transaminase (GOT), arginase, glutamic dehydrogenase (AGD), iditol dehydrogenase (ID) and lactic dehydrogenase (LDH). 2) A decrease in concentration in the serum resulting from impaired synthesis by the liver (choline esterase). 3) An elevation in enzyme levels due to cholestasis. The enzymes affected include alkaline phosphatase (ALP), gamma-glutamyl transferase or GGT gamma glutamyl transferase, leucine amino peptidase (LAP), aminotransferase function to catalyse transfer of an amino group from an amino acid to a keto acid. The two clinically important amino transferases are alanine aminotransferase and aspartate aminotransferase. These enzymes have a wide distribution in animal tissue and are present in small quantities in the serum of all animals as a consequence of normal tissue destruction and subsequent enzyme release. These enzymes have their principal functions and greatest concentration within the cell, increases observed in serum reflect cellular abnormalities (Coles, 1986).

ALT is increased in serum when cellular degeneration or destruction occurs in liver diseases (Coles, 1986).

AST is present in all tissues of the body; it is not an organ-specific test and consequently may be utilised to detect destruction in a wide variety of tissues. This enzyme appears in extremely high concentrations in muscle both skeletal and cardiac, it is of value in confirming a diagnosis of muscular degeneration. AST levels may be increased with liver diseases in all species but can not be considered to be a specific test for liver damage (Coles, 1986).

Alkaline phosphatase (AP) is widely distributed in body, and is found in high concentrations in bone (Osteoblasts), intestinal mucosa, renal tubule cells, liver and placenta. These tissues has a distinctly different isoenzymes of AP. Isoenzyme whose synthesis is induced by corticosteroids and possibly by other drugs (Coles, 1986).

AP activity in normal cattle and sheep presents such a wide range of values that its use as an indicator of liver insufficiency or obstructive icterus (Coles, 1986).

Acute hepatocellular necrosis results in minimal increases in SAP, whereas the ALT and ID levels are dramatically increased in a comparable disease. Treatment with corticosteroids or adrenocorticotrophic hormone (ACTH) is increases AP (Coles, 1986).

AP determinations may be useful in the diagnosis of obstructive and degenerative hepatic diseases (Coles, 1986).

Gamm-glutamyl transferase (GGT) is an enzyme found in the cytosol associated with cell membranes. The enzyme is present in several organs but serum activity of this enzyme is almost exclusively results from the GGT of hepatic origin (Coles, 1986).

In glucocorticoid-induced hepatopathy the GGT increase in serum activity was slower than that of 5) SAP or ALT.

AL-bdeery and AL-Zubaidi (2014) noted that liver enzymes (ALP, ALT, AST and GGT) significantly increased in the female New Zealand white rabbits ($P<0.01$) and this was attributed to the pregnancy status of the animals.

AL-Zorri (2009) mentioned that diabetic rabbits showed increased levels of liver enzyme (GOT and GPT) at $P<0.05$.

Table 1 : The blood enzymes concentrations after 14 days of immunization by *E. tenella* and *K. pneumoniae* in rabbits.

Enzymes	*E. tenella*	*K. pneumoniae*	*E. tenella* and *K. pneumoniae*	Control
GGT (ug/L)	6.04±0.00 A**	7.53±0.22 B	7.64±0.24 B	6.04±0.00 A
AST (ug/L)	31.45±0.44 A**	43.43±1.88 B	57.82±3.27 B	21.41±0.69 C
ALT (ug/L)	32.53±0.93 A**	48.73±0.40 B	50.87±0.74 B	42.86±0.76 C
ALP (ug/L)	19.25±0.62 A**	24.45±0.68 B	47.05±2.61 C	14.65±0.13 D

The different horizontal letters refer to the significant ($P<0.01$) difference between groups.

Table 2 : The blood enzyme concentrations of the 28 days post immunization by *E. tenella* and *K. pneumoniae* in rabbits

Enzymes	*E. tenella*	*K. pneumoniae*	*E. tenella* and *K. pneumoniae*	Control
GGT (ug/L)	6.12±0.00 A	7.64±0.20 B	7.69±0.04 B	6.49±0.18 C
AST (ug/L)	33.50±0.64 A	45.93±1.64 B	61.76±5.42 C	22.89±0.99 D
ALT (ug/L)	34.15±1.28 A**	48.71±0.64 B	51.36±0.20 C	45.57±0.31 D
ALP (ug/L)	20.08±0.20 A**	24.83±1.16 B	45.31±2.64 C	15.86±0.66 D

The different horizontal letters refer to the significant (P< 0.05 and P<0.01) difference between groups.

Table 3 : The blood enzyme concentrations of the 42 days post immunization by *E. tenella* and *K. pneumoniae* in rabbits

Enzymes	*E. tenella*	*K. pneumoniae*	*E. tenella* and *K. pneumoniae*	Control
GGT (ug/L)	6.18±0.19 A**	7.67±0.00 B	7.91±0.90 AB	6.49±0.17 AC
AST (ug/L)	36.08±1.09 A**	47.60±1.36 B	64.46±1.06 C	24.22±0.63 D
ALT (ug/L)	36.34±0.57 A**	50.37±0.36 B	53.01±0.11 C	43.67±0.25 D
ALP (ug/L)	21.33±0.93 AC	25.67±1.41 A	45.71±1.10 B**	18.42±0.63 AC

The different horizontal letters refer to the significant (P< 0.05 and P<0.01) difference between groups.

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