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SNUFFLES DISEASE IN RABBITS: 2- IMMUNOLOGICAL STUDY

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ABSTRACT
Snuffles disease is a major problem in rabbits, for the importance of this disease, this study aimed to estimate the levels of humoral and cell mediated immune responses against *Pasteurella multocida* infection in rabbits. The infective dose was 10^2 CFU/ml, injected I/P into group of rabbits (27) whereas other group (7) kept as control. Humoral Immune response estimated at the 7th, 14th and 21st days post infection using passive hemagglutination test. The mean value of antibodies was 469.3±42.66 at 21st day post infection, more than those at 7th and 14th days (170.6±26.98 and 213.3± 26.98) respectively in infected group and negative antibodies titers in control group. Cellular Immunity estimated at 27th day post infection using DTH-skin test and found the mean thickness of indurations for crude whole bacterial cell sonicated Ag (WBCS Ag) was 1.17±0.09 mm more than those for 1/10 and 1/100 diluted WBCS Ag at 48hrs, whereas mean thickness of indurations for crude WBCS Ag was 0.91±0.09 at 24hrs and also more than those of 1/10 and 1/100 WBCS Ag comparable to thickness of skin in control group. *Pasteurella multocida* infection induces both humoral and cell mediated immune responses in rabbits.

KEYWORDS: snuffles disease in rabbit's immunological study.

INTRODUCTION

Snuffles disease is a common problem in rabbits occurs during the stress conditions such as shipping, mating, experimental handling and environmental disturbances[1]. Rabbits are harboring the causative agent (*Pasteurella multocida)* in upper respiratory tract and following the stress the *P. multocida* replicates rapidly and causes diseases such as pneumonia, otitis and rhinitis[2,3]. *P. multocida* have several antigenic structures such as capsule, fimbria, lipopolysaccharide (LPS), outer-membrane proteins (OMB) which play a role in pathogenesis of the disease, also have a role in immune response against the causative agent[4]. For the importance of this disease in rabbits, this study aimed to study some immunological parameters associated with experimental infection of rabbits with the *P. multocida.*

MATERIALS & METHODS

A strain of *P. multocida* was reidentified[5] and the LP50 was 10^7 CFU/ml and lethal infective dose was 10^2 CFU/ml[6]. Preparation of whole bacterial cell sonicated Ag (WBCS Ag): prepared according to[7] with some modification, after culturing of *P. multocida* on blood agar, harvesting with pbs, washing three times with pbs and pellets were suspended in pbs and sonicated by ultrasonicated for 30 minutes and the sonicated suspension was centrifuged and supernatant filtered through Millipore filter, the total protein were 21.8mg/ml. Experimental infection: two groups of rabbits were taken 1st group control injected with 1ml of pbs, 2nd group infective group injected I/P with 10^2 CFU/ml of *p. multocida* suspension, at the 7th, 14th and 21st day post infection the passive hemagglutination test was done according to[8] and at the day 27th post infection, skin test (DTH test) was done for CMI [9].

RESULTS & DISCUSSION

1. Humoral Immunity

The passive hemagglutination test (PHA)

The results showed differences between the infected group and non infected (control) (Table-1). The mean values of Abs at 21st day post infection were 469.3±42.66 more than those at the 7th and 14th day (170.6±26.98 and 213.3± 26.98) respectively in infected group with *p. multocida.*

TABLE 1: Antibody titers of infected rabbits with *P. multocida*

7th day	14th day	21st day	control
128	256	512	0
128	128	512	0
256	256	512	0
128	256	256	0
256	256	512	0
128	128	512	0
170.6±26.98	213.3±26.98	469.3±42.66	0
M±SE	M±SE	M±SE	

The mean of the antibody titers of 6 animals, M: Mean, SE: Standard error

The development of humoral immune response after the infection by *P. multocida* play important role in decrease the bacterial numbers from the tissue in advance days of the experiment which was more evident in this experiment [10,11] described that the antibodies play important role in clearing the bacteria from the body and inhibit the rapid spread of the organisms to blood stream and other reticuloendothelial tissues. The LPS of bacteria induce substantial B-cell response leading to copious plasm cell production which release Abs into the blood stream which more evident in this study[12,13] found that the outer membrane protein gave a high titer of Abs against the *P. multocida* during the 7 – 21 day, similarly[14] referred to the outer membrane proteins of *P. multocida* stimulate a good immune response against experimental pneumonic pasteurellosis, however *P. multocida* infection enhance extensive humoral immune response detected in this study by passive hemagglutination test at the 7th, 14th and 21st days post infection.

2. Cell Mediated Immunity

DTH-skin test

The results revealed that there is differences between infected group and control group in thickness of indurate areas the mean thickness (indurations) of reactive skin at 48hr. of infected group was 1.17±0.09mm for crude whole bacterial sonicated Ag (WBCS) and more than that recorded for 1/10 and 1/100 diluted WBCS Ag, whereas, mean induration thickness for crude WBCS Ag were 0.91±0.09mm at 24hrs and also more than for 1/10 and 1/100 diluted crude WBCS Ag (Table-2). The whole indurations area thickness for control group was negative.

TABLE 2: The means and standard error of different skin thickness (mm) at 27th day post *P. multocida* infection

Animal	24 hrs				48 hrs				Control
	crude	1/10	1/100	PBS	crude	1/10	1/100	PBS	
1	1	0.4	0.3	0	1.3	0.6	0.5	0	0
2	0.6	0.2	0.1	0	0.8	0.4	0.3	0	0
3	0.6	0.2	0.1	0	0.9	0.4	0.3	0	0
4	0.9	0.5	0.3	0	1.1	0.7	0.5	0	0
5	1.3	0.9	0.6	0	1.5	1.1	0.8	0	0
6	1	0.4	0.2	0	1.3	0.6	0.4	0	0
7	1	0.4	0.3	0	1.3	0.6	0.5	0	0
M ± SE	0.91±0.09	0.43±0.09	0.27±0.04	0	1.17±0.09	0.62± 0.08	0.47± 0.06	0	0

The mean of skin thickness of 7 animals, M: Mean, SE: Standard error

The results of the skin test in this study agreed with [4] who studied the immunological activity of lipopolysaccharide - protein complex of *P. multocida* in laboratory animals and they found a good skin reaction areas (indurations) in the animals (mice) had prior exposure to the Ags of *P. multocida* and they explained that this skin reaction response absolutely depended on presence of memory cells modulate Th1 response which secrete interferon-gamma (INF-γ) a potent stimulator for macrophages to migrate into skin reaction area (induration) in addition to the effect of 1L1 and TNF-α, β secreted by macrophages to stimulate lymphocytes migration into indurated skin area[15]. The ability and activity of DTH-skin reactions depend also on Th1 cells recognize the Ag together with 1L1 secretion by macrophages which enhances proliferation and differentiation of other T cells into Th1 cells which secrete 1L2 a chemo tactic factor for attraction of macrophages around area of activated T cells[16]. Also the INF-γ secretion by Th1 cells enhancing the cytolytic activity of accumulated macrophages leading to induration of the skin area[17].

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