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IMMUNOGENIC PROPERTIES OF OUTER MEMBRANE PROTEIN OF *CAMPYLOBACTER JEJUNI* IN CHICKS

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SUMMARY

The immune response of chickens to outer membrane proteins (OMP) of *Campylobacter jejuni* (*C. jejuni*) was studied as a step in the search for vaccine candidates. OMP of *C. jejuni* was administered I/M to native breed chicks at one month of age for 4 times weekly intervals. Serum samples were collected for 4 successive weeks and immune response was measured by enzyme linked immunosorbent assay (ELISA). Experimental chicks were challenged with 5×10^8 *C. jejuni* orally after one week of last vaccination. At the end of the experiment, the reisolation of *C. jejuni* was carried out and specimens for histopathological examination were taken. The immunized experimental birds showed increase in titres of specific antibodies in sera than control group. The immunized and challenged experimental birds (group 1) had lower reisolation of *C. jejuni* (16.66%) than unimmunized challenged control group (group 2) (85%), while there was no reisolation in control negative group (group 3). Histopathological examination revealed round cell aggregation and mild to moderate inflammatory changes in liver and intestine of group 1 while more severe degrees of inflammatory changes in group 2. The results indicate that immunization can reduce the level of infection with *C. jejuni* but not completely protection.

INTRODUCTION

Campylobacter jejuni (*C. jejuni*) is a major cause of infectious enteritis in human (Black et al., 1992 and Tauxe, 1992). Poultry have been considered as a reservoir and main source of human campylobacteriosis (Skirrow, 1990) especially during handling and consumption of its products

(Harris et al., 1986). *C. jejuni* causes economic losses in poultry industry, once it is introduced into a poultry flock it spreads rapidly and frequently infect 100% of the flock (Shanker and Sorrell, 1990). *C. jejuni* has been isolated from focal necrotic hepatitis in broiler chickens (Boukraa et al., 1991). It reduces egg production in hens and causes death in embryos (Rabie, 1992).

Some trials for controlling *C. jejuni* infection in chickens were recorded including improvement of farm hygiene (Pearson et al., 1993). Other trials studied immune response of chickens to *C. jejuni* infection as a step in the search for vaccine candidates. Active immunization reduced the level of intestinal infection with *C. jejuni* as formalin inactivated *C. jejuni* whole cells (Rice et al., 1997) formalized antigen with or without immunomodulator (Rabie and Kutkat, 2002). The antigenicity and immunodominance of flagellins during infection are well established (Newell and Nechamkin, 1992 and Widders et al., 1998), cloning of outer membrane protein (OMP) gene from *C. jejuni* (Meinersmann et al., 1997). OMP of *C. jejuni* were extracted of variable molecular weight (Blaser et al., 1984). Dubreuil et al. (1990), Burnens et al. (1995) and Chart et al. (1997), identify, purify and characterize OMP of *C. jejuni.* Zhu et al. (1999), studied response of chicken lymphocytes to *C. jejuni* OMP. Newell and Nachamkin (1992) reported that the major OMP of *C. jejuni* and *C. coli* which are probably porins, are seemed to be immunogenic to some extent during infection.

MATERIALS AND METHODS

Bacteria:

C. jejuni was previously isolated from chicken (Rabie, 1992).

Chickens:

A total of seventy one-day old native breed chicks were procured from a commercial hatchery. They were fed on commercial broiler rations and housed in an insect proof room, with hygienic barrier conditions imposed. At one month age, they were divided into 3 groups. The 1st group (group 1) immunized group were thirty birds and the 2nd group (group 2) kept unimmunized challenged of twenty at a separate isolator (control +ve). The 3rd group (group 3) kept unimmunized and non challenged (control -ve). The 1st group were immunized at weekly intervals for a total 4 immunization (Widders et al., 1996). Antigen was emulsified 1 : 1 in oil adjuvant and approximately 0.1 mg antigen (Widders et al., 1996) delivered in a total volume of a 0.2 ml I/M. All birds were wing bled weekly of a total 4 weeks and the sera were separated and stored at -20°C. After one week of last vaccination, birds of groups 1 and 2 were challenged with 5×10^8 c.f.u *C. jejuni* / bird (Rabie, 1992) and kept under observation for 3 weeks.

At the end of the experiment at 12 weeks of age, all birds were sacrificed. Samples taken from cae-

cum and liver were cultured on semisolid thiol and incubated 48 hours at 42°C, then examined for the presence of *C. jejuni* under phase contrast microscope. Post mortem examination and histopathological examination were carried out on sacrificed birds. Tissue specimens were taken from liver, intestine, fixed in 10% neutral formalin and subjected to paraffin embedding method. Tissue sections of 5μ were then stained by haematoxylin and eosin as routine stain (Bancroft et al., 1996).

Antigen:

After 48 hours of growth on Muller Hinters agar in a microaerophilic atmosphere, cells were harvested and washed twice in phosphate buffered saline, washed cells were suspended in 10 ml of 20 mM Tris buffer and the cell suspension was sonicated for 60 seconds. Intact cells were removed by centrifugation at 6000 xg at 4°C for 30 minutes. The membrane protein was shaken at room temperature for 30 minutes. The preparation was centrifuged at 40,000 xg for 30 minutes at 4°C and the pellet washed three times in tris buffer. Finally, the pellet was suspended in tris buffer (Dubreuil et al., 1988).

ELISA technique (Cawthraw et al., 1994):

Microtiter plates were coated with 100 mg/ml of antigen. Antigen was diluted 1 : 50 carbonate bicarbonate buffer at pH 9.6 and kept overnight at room temperature. The wells were washed with ELISA wash buffer, then incubated with 100 ml chicken sera diluted (1 : 200) in ELISA diluent for 2 hours at 37°C. The wells were washed and incubated with 100 ml antichicken IgG conjugated to peroxidase (Sigma Ltd) diluted 1 : 3000 in ELISA diluent for 30 minutes at 37°C. After washing, the bound peroxidase was detected by incubation with 100 ml of 3, 3`, 5, 5` tetramethyl benzidene substrate at room temperature. The reaction was stopped after 10 minutes by the addition of 50 ml 2M H2So4. The absorbance was read at 450 nm on a microplate reader and the cut point was detected as 0.017 in the same condition.

Electrophoresis for OMP:

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (1970).

RESULTS

Obtained results are shown in tables (1), (2) and (3), figures (1 - 5) and the post mortem lesions recorded were subcapsular haemorrhage in liver, enlargement in gall bladder, pronounced lesions were recorded in group 2 while mild lesions were recorded in group 1 which was immunized while there was no lesion in group 3.

The histopathological lesions of liver of many cases showed centrolateral necrosis (figure 1) and / or focal aggregation of mononuclear cells (figure 3). Examination of different parts of intestinal tract showed that the main lesions were restricted

in the intestinal glands which appeared necrosed and oedematous (figure 2). In many cases round cells aggregation were seen around the intestinal gland (figure 4).

The antibody response to *C. jejuni* in birds vaccinated and challenge was determined in table (1). Increased antibody response to *C. jejuni* was noticed in all birds from groups that were challenged and exposed with *C. jejuni* after vaccinated by OMP clearance of *C. jejuni*. Isolation of *C. jejuni* from the internal organs of birds was determined (table 2).

SDS PAGE: Figure of electrophoretic protein banding pattern for strain of *C. jejuni* indicate the protein bands with KDa 44.316, 50.345, 55.10, 60.304 and 80.012.

Table (1): ELISA titre of *C. jejuni* OMP in chicken serum.

Animal group	Week post immunization				
	0-day pre-immunization (mean±SE)	1st week post-immunization (mean±SE)	2nd week post-immunization (mean±SE)	3rd week post-immunization (mean±SE)	4th week post-immunization (mean±SE)
Immunized group	0.182±0.0122	0.364±0.0128	0.527±0.013	0.667±0.019	0.667±0.012
Control group	0.182±0.122	0.174±0.123	0.176±0.124	0.174±0.123	0.174±0.124

Table (2): The reisolation of *C. jejuni* in sacrificed birds.

Ggroup	The reisolation of *C.jejuni*				% of isolation
	No.of sacrificed birds	Total	Site of isolation		
			Liver	Intestine	
Group 1	30	5	4	1	16.66%
Group 2	20	17	16	12	85%
Group 3	20	0	0	0	0%

Table (3): Molecular weight of OMP C. jejuni.

Lane bands	Lane 1 (mol.w.) Marker	Lane 2 (mol.w.) Sample
1	97	80.612
2	66	60.304
3	46	55.10
4	29.40	50.345
5	14	44.316

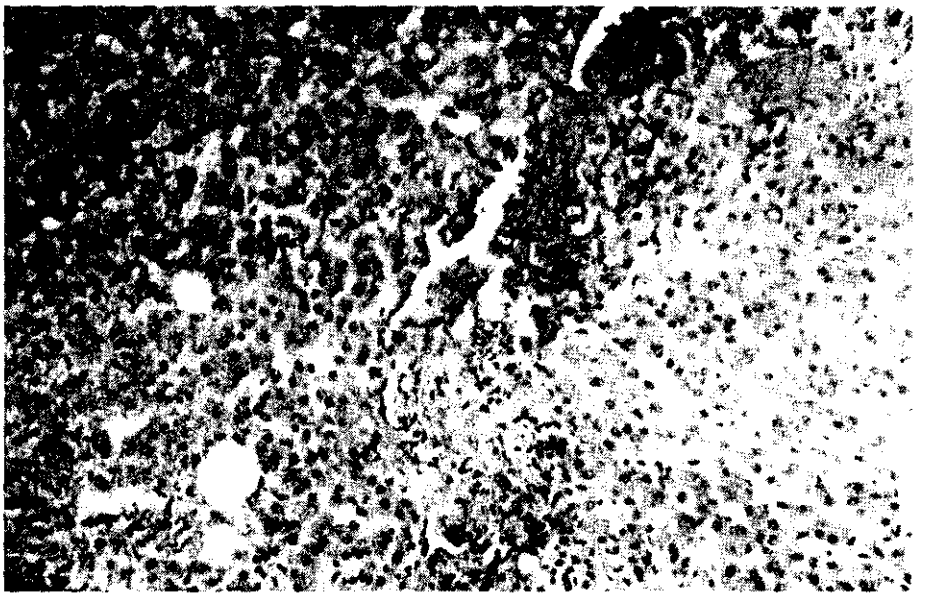

Fig. (1): Liver showing centrolobular necrosis. H & E stain, X 250.

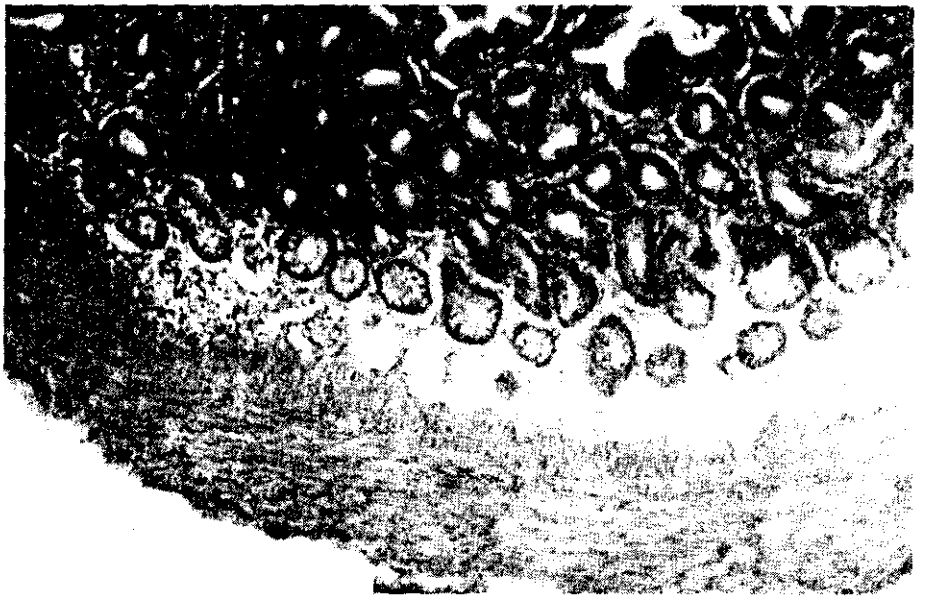

Fig. (2): Intestine showing periglandular oedema of intestinal gland associated with necrosis of some. H & E stain, X 125.

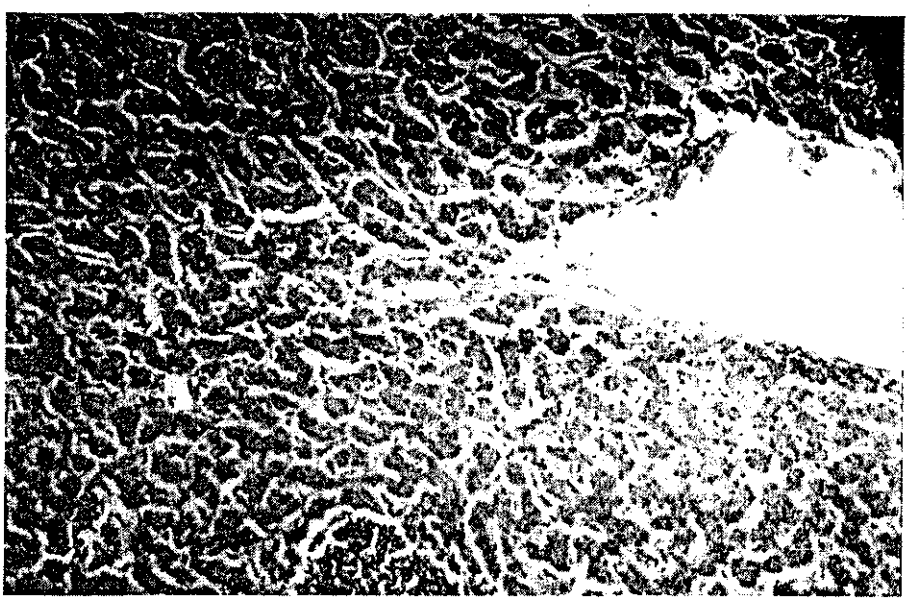

Fig. (3): Liver showing focal aggregation of round cells and Kupffer cells activation. H & E stain, X 125.

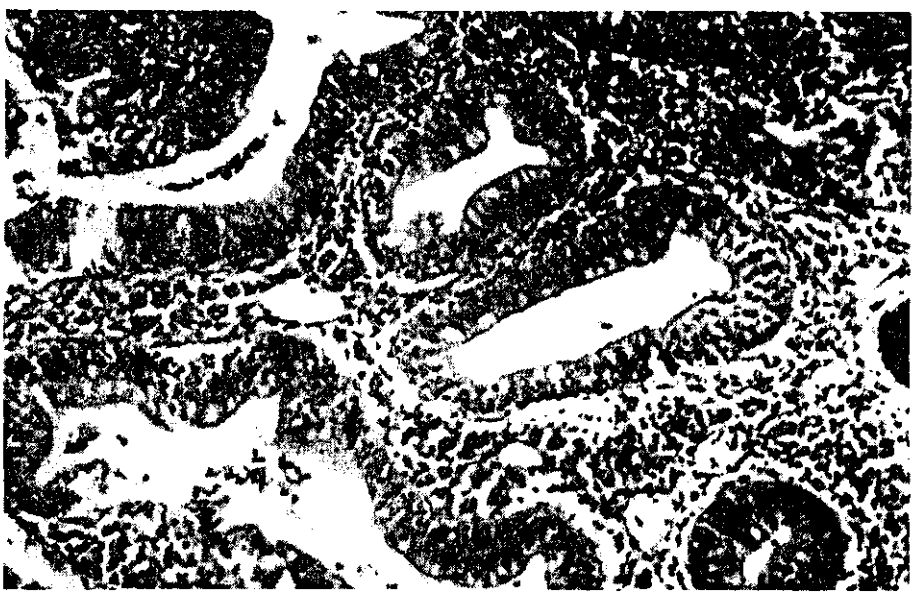

Fig. (4): Intestine showing infiltration around intestinal glands with round cells. H & E stain, X 250.

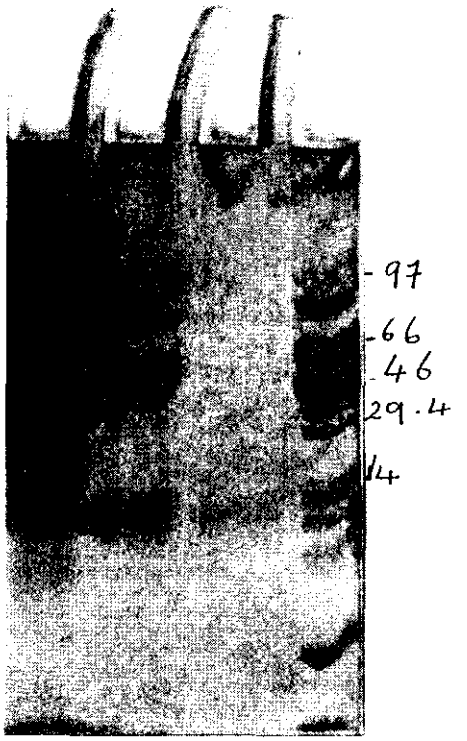

Fig. (5): Polyacrylamide gel electrophoresis patterns of OMP *C. jejuni* separated from samples.

DISCUSSION

This study was done to evaluate the humoral immune response of chickens immunized with outer membrane protein of *C. jejuni* using ELISA. Table (1) demonstrated that sera collected from the immunized chickens showed higher antibody titre than the control group that gradually increased 4 weeks immunization reached to the highest at 3rd and 4th week post immunization. This result indicates that the protection could be manifested by antibodies of outer membrane protein. These results agree with Noor et al. (1995), who found that high titres in antibodies of immunized chicken and *C. jejuni* is a common environmental pathogen in chicken. It was not surprising that low titres of antibody were also detected in unimmunized chicks. Widders et al. (1996), found that there is a significant increase in specific antibody titres in serum only when primary and secondary immunization with whole cells was administered intraperitoneally.

Reduction in reisolation percent in this study from vaccinated challenged chickens (16.66%) as compared with non vaccinated challenged (85%), table (2) indicates that the OMP oil adjuvant mixture of C. jejuni is capable of inducing protective

immunity against *C. jejuni*. On the other side, preliminary reports suggest that by 8 - 9 weeks post-infection, *C. jejuni* colonization is significantly reduced and even eradicated in some cases, suggesting an effectiveness of the immune response (Cawthraw et al., 1994).

C. jejuni in our study could be reisolated from cases of liver and intestinal lesions. This result was confirmed with histopathological lesions which shows centrolobular necrosis and hepatic tissues were infiltrated with macrophages and there is inflammation with variable degrees in the liver. There was periglandular oedema of intestinal gland associated with necrosis of some. This agree with Boukraa et al. (1991), who could isolate *C. jejuni* from focal necrotic hepatitis in broiler chickens. Histopathological lesion of other cases which gave negative reisolation for *C. jejuni* show focal aggregation of round cells and Kupffer cells activation and infiltration of intestinal glands with round cells that may be due to post-vaccinal reaction. Given the apparently commensal association of Campylobacter in poultry, it remains to be seen whether complete eradication of this human pathogen from commercial poultry will be an achievable goal. Even if eradication is not achieved, reduction in the levels of colonization may still be an important means of reducing the bioload of enteropathogens to which the consumer is exposed on retail products. Hence vaccination may provide an important means of this end (Rice et al., 1997).

The high titres of antibodies in immunized birds than unimmunized group associated with reduction in reisolation rate of *C. jejuni* from immunized challenged birds than challenged control group indicated that there was an immune response to antigen preparation (OMP) which reduce infection of *C. jejuni* and such antigenic preparation may contain immunogenic proteins.

The clearance of *C. jejuni* from internal organs of bird vaccinated with the OMP indicating that, OMP from gram negative bacteria can induce protective immunity. So the vaccines can trigger the cell mediated component of the immune system may be able to afford greater protection against this organism. Because lipophilic substances have an important role in stimulation of cell mediated responses and conjugation of lipid to protein molecule will create an antigen with enhanced effect on the cell mediated component of the immune system (Frank, 1974). The main proteins in this antigenic preparation was shown by poly acrylamide gel electrophoresis pattern as given in the present results.

Previous studies indicated that 45 KDa protein is believed to be a major outer membrane protein and has immunogenic effect (Demelo and Pechere, 1990). Lam (1992) found high immune response to 45 KDa protein when injected S/C in chicks. Blaser et al. (1983), reported that 45 KDa represents more than 50% of the total bacterial

protein. Dubreuil et al. (1990), found that the immunodominant protein antigen of *C. jejuni* is subunit molecular weight of 59.000 to 61.000 (59 - 61 KDa). Blaser et al. (1984) and Nechamkin and Hart (1985) reported that the 61.45 and 31 KDa proteins are immunogenic. Blaser et al. (1984), observed that major (OMP) molecular weight between 41.45 KDa and minor bands at 29 KDa and 50 KDa, they all were immunogenic. Mills and Bradbury (1984) found the major outer membrane protein was 43 KDa and there were 8 to 10 minor bands ranging from 92 KDa to 14 KDa.

Our results of this study agree with all these previous findings where the antigen preparation (OMP) protein of 44.316, 50.345, 55.10, 60.304 and 80.012 KDa are suggested to be immunogenic proteins.

Our results indicate that immunization with Campylobacter outer membrane protein can reduce the level of infection with *C. jejuni* but did not give complete protection. Encouraging future development of successful vaccine for the control of campylobacteriosis in poultry .

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