Asian Journal of Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Poultry Science

ISSN 1819-3609
DOI: 10.3923/ajpsaj.2016.78.85

Research Article
A Trial for Prevention of *Campylobacter jejuni* Infection in Broiler Chickens Using Autogenus Bacterin

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Abstract
Two strains of *Campylobacter jejuni* (*C. jejuni*) representing biotype I and II were used for preparation of bacterin. Pure, sterile and safe watery aluminium hydroxide and incomplete Freund's oil adjuvant bivalent bacterins were prepared. Both types of bacterins were evaluated in broiler chickens through subcutaneous (S/C) inoculation at one week old and boostered at three weeks of age. Results of immunoassay [mean Enzyme Linked Immuno-Sorbent Assay (ELISA) titres] and bioassay (clinical signs, mean lesion score, shedding and re-isolation rates as well as histopathological examination) proved that both types of bacterins were effective. However, oil type bacterin gave more protective effect than water type one.

Key words: *Campylobacter jejuni*, bacterin, chickens, protection, ELISA

Received: November 24, 2015 **Accepted:** February 01, 2016 **Published:** March 15, 2016

Citation: Wafaa A. Abd El-Ghany, M.H. Awaad and S.R. Nagwa, 2016. A trial for prevention of *Campylobacter jejuni* infection in broiler chickens using autogenus bacterin. Asian J. Poult. Sci., 10: 78-85.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Campylobacter jejuni (*C. jejuni*) infection in chickens has been implicated as contagious disease characterized by chronic course, high morbidity, low mortality and reduction in egg production (Achen *et al*., 1998; Meade *et al*., 2009). The organism *C. jejuni* is the primary cause of human gastrointestinal infection among *Campylobacter* species. Consumption of chickens and retail poultry products is incriminated as the main vehicle for transmission of infection to human (EFSA., 2010).

Hence, *Campylobacter* enteritis constitutes a zoonosis of major concern in public health (Ailes *et al*., 2008) and it has been shown to be greater problem than salmonellosis in several countries so, it is important to address intervention strategies by which these bacteria can be reduced or removed from the food animals. Such strategies could include vaccines. Various types of *Cammpylobacter* spp. bacterins were tested in laboratory animals like mice and rabbits with successful results (Burr *et al*., 1988; Pavlovskis *et al*., 1991; Rollwagen *et al*., 1993; De Zoete *et al*., 2007; Lin, 2009; Saxena *et al*., 2013). In chickens, different types of living and killed vaccines against *C. jejuni* have been used with variable results (Stern *et al*., 1990; Cawthraw *et al*., 1994; Rice *et al*., 1997; Lee *et al*., 1999; Ziprin *et al*., 2002).

So, the aim of this study was preparation of two types of *C. jejuni* bacterins (aluminium hydroxide and incomplete Freund's oil adjuvant) from the local field strains and evaluation of their protective potential in broiler chickens through immuno-assay (ELISA) titres and bio-assay (clinical signs, mean lesion score, shedding and re-isolation rates as well as histopathological examination).

MATERIALS AND METHODS

Strains used: Local strains of *C. jejuni* representing biotype I and II were used in bacterins preparation and as challenging bacteria.

Preparation of whole cell bivalent *C. jejuni* bacterins: Whole cell bivalent *C. jejuni* (biotype I and II) bacterins of aluminium hydroxide (AHAB) and incomplete Freund's oil adjuvant bivalent bacterins (IFAB) (10^9 colony forming unit CFU mL^{-1} *C. jejuni*) was prepared as bdescribed by Williams *et al.* (1976) and Bryner *et al.* (1978, 1988).

Quality control tests of the prepared bacterins: Purity test, completion of *C. jejuni* inactivation, sterility test and safety test were done as Pharmaceutical Society of Great Britain (1970).

Experimental design: Two hundred day-old chicks were obtained from a commercial hatchery. Twenty birds were randomly selected, necropsized and the liver and intestine were cultured for the presence of *C. jejuni* which proved to be negative. Remaining birds were kept in separate pens and fed on a commercial balanced ration *ad libitum*. Ration contained coccidiostate semduramycin at concentration of 25 ppm. No antibiotics were added to the ration or water. The chicks were vaccinated by eye drop against Newcastle disease using Hitchner B1 vaccine at 7 days-old and La Sota vaccine at 21 days-old, against infectious bursal disease using D78 at 12 days-old and against infectious bronchitis using H 120 at 17 days-old. Avian Influenza (AI) inactivated H5N2 vaccine was given subcutaneously (S/C) at 7 days-old.

The remaining 180 birds were divided into three groups; group 1 and 2 consisted of 70 birds each while group (3) consisted of 40 birds. Chickens of group (1) were immunized S/C with 0.2 mL $bird^{-1}$ with bivalent *C. jejuni* (biotype I and II) water type bacterin (AHAB) at 1 week-old and with another booster dose of the same bacterin (0.5 mL $bird^{-1}$) at 3 weeks-old. Group (2) was immunized by bivalent *C. jejuni* (biotype I and II) oil type bacterin (IFAB) similarly as group (1). Chickens of group (3) were kept without immunization as control group.

Measured parameters

Immuno-assay: Serum samples were collected from thirty immunized birds from each of group (1) and (2) at 0 h (just before immunization) and at weekly intervals for 4 weeks of post immunization dose. These serum samples were subjected to ELISA (Cawthraw *et al*., 1994) to detect humoral IgG antibodies.

Bio-assay: Each group (1, 2 and 3) were equally divided into two equal subgroups (a and b). Birds of subgroup (a) and subgroup (b) were challenged with *C. jejuni* biotype (I) and (II), respectively. Subgroups were (1a, 1b, 2a, 2b, 3a and 3b). Challenge was done at 4 weeks-old using 0.5 mL $bird^{-1}$ containing 5×10^8 CFU mL^{-1} of the respective *C. jejuni* biotype suspended in thioglycolate broth.

All challenged birds were kept under close observation for further 3 weeks for clinical signs and mortalities. Cloacal swabs were collected from challenged birds at 3, 7, 11, 15, 18 and 21 days post challenge to determine the frequency of *C. jejuni* shedding.

At the end of observation period survived birds were sacrificed and subjected to post mortem examination for lesion scoring (Nagwa *et al*., 1998).

Specimens were collected from liver and intestine for re-isolation and identification using motility test (Smibert, 1974), morphological (Holt *et al*., 1994) and biochemical identifications (Koneman *et al*., 1995) as well as histopathological examination (Carlton, 1967).

Statistical analysis: It was done as, Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Microscopical and biochemical identification of the grown seed culture onto Skirrow's media revealed presence of pure culture of *C. jejuni* cells. Inactivated *C. jejuni* cells showed no growth on Skirrow's agar plates after formalin inactivation. The formalin killed *C. jejuni* cells gave no bacterial growth after cultivation onto Pleuro-Pneumonia Like Organism (PPLO) agar and broth and also no fungal growth was obtained after cultivation onto Sabouraud dextrose agar plates. The prepared bivalent *C. jejuni* bacterins were found to be safe for day-old-chicks, producing neither clinical signs nor local reactions and deaths during seven successive days observation period.

The results of immuno-assay revealed that primary immunization of broiler chickens with either water type (AHAB) or oil type (IFAB) *C. jejuni* bacterins at one week of age and boostering at 3 weeks old induced seroconversion, indicating that both types were effectively antigenic (Fig. 1). However, oil type *C. jejuni* bacterin gave better mean antibody titers with steady pattern of immune response as compared with water type. This may be attributed to the oily nature of the adjuvant that gave relatively higher and steady immune response than aqueous one. This observation tend to coincide with that of Tizard (2000) who reported that when a mixture of antigen with oily emulsion is injected into the body, it stimulates local and inflammatory immune response and results in production of macrophages rich granuloma or depot around the inoculation site. The antigen within this focus slowly leeks into the body and provides a prolonged antigenic stimulus for several weeks. The depot attracts eosinophils and activates the complement which can result in increase in the antigen localization of follicular and dendritic cells and hence improve B cells memory and T cells activity (humoral immune response).

Results of immuno-assay are nearly similar to those observed by Glunder and Spiering (1992) who found that S/C vaccination of broiler chickens with Freund's adjuvant inactivated *C. jejuni* cells at different ages stimulated good humoral immune response, in addition to booster immunization induced a significant increase in the antibody titers.

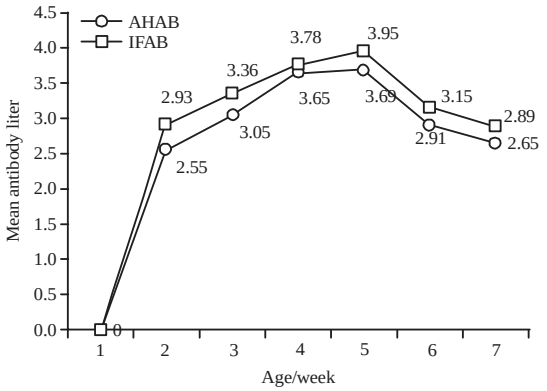

Fig. 1: Comparison between the results of ELISA in immunized and un immunized broiler chickens vaccinated with AHAB and IFAB, AHAB: Aluminium hydroxide adjuvant bacterin, IFAB: Incomplete Freund's adjuvant bacterin

Although generally accepted that *C. jejuni* colonizes its avian host as a commensal, *C. jejuni* inefficiently adheres to and invades cells of the chicken gut epithelium (Larson *et al*., 2008). This is initially followed by an inefficient innate immune response by the chick resulting eventually in the production of specific antibodies (Shoaf-Sweeney *et al*., 2008). Although such a response is not able to clear *C. jejuni* from the gut, reduced bacterial counts have been observed (Shoaf-Sweeney *et al*., 2008).

On the other hand, the results of bioassay after challenge of subgroups with both *C. jejuni* biotypes (I) and (II) indicated that there was difference between immunized and un-immunized control birds relative to clinical signs. Signs of depression, sleepy appearance and mucoid greenish diarrhoea were observed in un-immunized challenged control subgroups (3a and 3b). Deaths were neither recorded in un-immunized or immunized subgroups. This observation suggests the chronicity of *C. jejuni* infection in poultry as reported previously (Peckham, 1984).

Moreover, There was significant difference ($p<0.05$) between immunized and un immunized control subgroups in the mean gross lesion scoring of sacrificed birds 3 weeks post challenge, where the lesions are more evident in un immunized than immunized birds. Nevertheless, *C. jejuni* bivalent oily bacterin (IFAB) superseded water type (AHAB) in its protective effect when lesion scoring was taken in consideration as a criterion (mean score 0.6 in subgroup 2a immunized with IFAB and challenged with biotype I and 1.2 in subgroup 2b challenged with biotype II, vs. 1.8 and 2.2 in

subgroups 1a and 1b, respectively in birds immunized with AHAB and challenged with biotype I and II. Control (un immunized and challenged) birds showed total lesion scores 2.4 and 2.9, respectively for groups 3a and 3b.

Concerning the rate of *C. jejuni* shedding during 3 weeks post challenge observation, the results revealed a decreasing tendency in both immunized groups with (AHAB) and (IFAB) as compared with the un immunized chickens. The differences between immunized and un immunized subgroups in the rate of *C. jejuni* shedding during 3 weeks observation period were significant ($p<0.05$). In subgroups 1a and 1b that immunized with AHAB vaccine and challenged with biotype I and II, respectively where it was 100% at the 3rd day post challenge ad reached to 20% at 21st days post challenge. However, groups 2a and 2b that immunized by IFAB and challenged by biotype I and II, respectively, showed the least shedding rate as it declined from 100-10% at the previous intervals. This finding disagree with Glunder and Spiering (1992) who concluded that parental vaccination with killed *Campylobacters* has no or little influence on the excretion rate of the organism and that the excretion period of the organism may be dependent on strain characteristics.

The results in Table 1 reveals that the re-isolation rate of *C. jejuni* from both liver and intestine of immunized and un immunized groups at the end of 3 weeks observation period were 90 and 75% for un immunized 3a and 3b subgroups; respectively and was significantly ($p<0.05$) lower in immunized subgroups (10% in 1a, 20% in 1b, 0% in 2a and 10% in 2b). The results confirmed the efficacy of immunization with both prepared bacterins and the superiority of the oily type (IFAB) over watery type (AHAB) in reduction of re-isolation rate.

The liver of immunized and challenged birds showed congestion of central veins, sinusoids and portal blood vessels. The wall of some blood vessels was destructed with the presence of mononuclear cells in their lumen (Fig. 2). Multifocal necrosis of hepatic parenchyma was predominant in which the cellular details of hepatocytes were lost while the cellular architecture was present (Fig. 3).

The number of hepatocytes was completely lost and replaced by large numbers of mononuclear cells aggregation. Focal mononuclear cells aggregation and heterophils were also found in the portal areas, central veins and in between hepatocytes. Dissociation and disorganization of hepatic plates were observed in addition to activation of Kupffer cells. The hepatocytes appeared swollen with granulation or vacuolation of their cytoplasm (Fig. 4). Hyperplasia of the

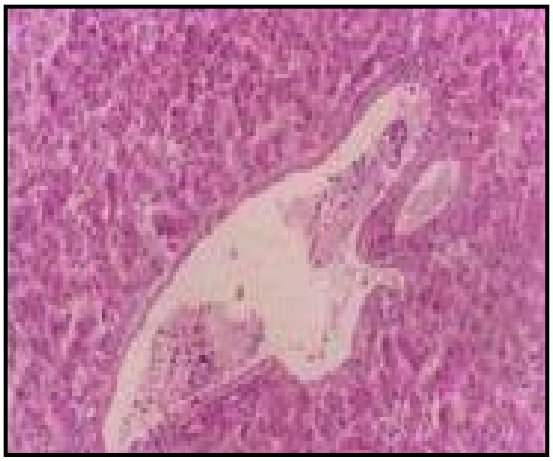

Fig. 2: Liver of broilers immunized with IFAB and infected with *C. jejuni* showing few numbers of inflammatory cells in blood vessels (H and E×33)

Table 1: Re-isolation of *C. jejuni* in immunized and unimmunized broiler chickens sacrificed at the end of 21 days observation period post-challenge

	Results of *C. jejuni* re-isolation											
	1a		1b		2a		2b		3a		3b	
Case No.	L	I	L	I	L	I	L	I	L	I	L	I
1	-	-	-	-	-	-	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-	+	+	+	-
3	+	-	-	-	-	-	-	-	+	+	+	+
4	-	-	+	-	-	-	-	-	+	+	+	+
5	-	-	-	-	-	-	-	-	+	-	+	-
6	-	-	+	-	-	-	-	-	+	+	+	-
7	-	+	-	-	-	-	-	-	+	+	+	+
8	-	-	+	+	-	-	-	-	+	+	+	+
9	-	-	-	-	-	-	-	-	+	-	+	-
10	-	-	-	-	-	-	-	-	+	+	+	-
Total Positive/total examined	2/20		4/20		0/20		2/20		18/20		15/20	
%	10*		20*		0*		10*		90		75	

L: Liver I: Intestine, +: Positive, -: Negative, Subgroup (a): Challenged with *C. jejuni* biotype I, Subgroup (b): Challenged with *C. jejuni* biotype II, *Significant decrease over their un immunized control group ($p<0.05$)

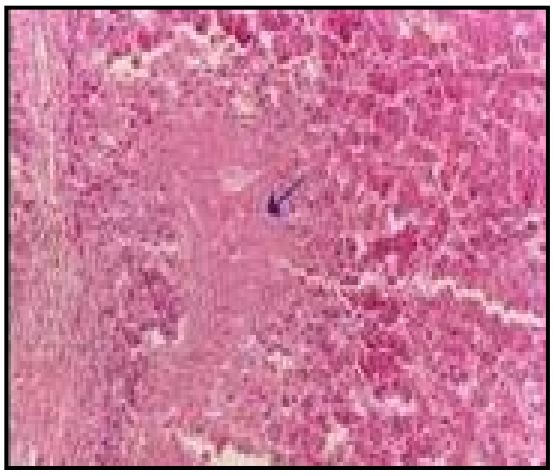

Fig. 3: Liver of broilers immunized with AHAB and infected with *C. jejuni* showing focal area of necrosis (arrow) (H and E×33)

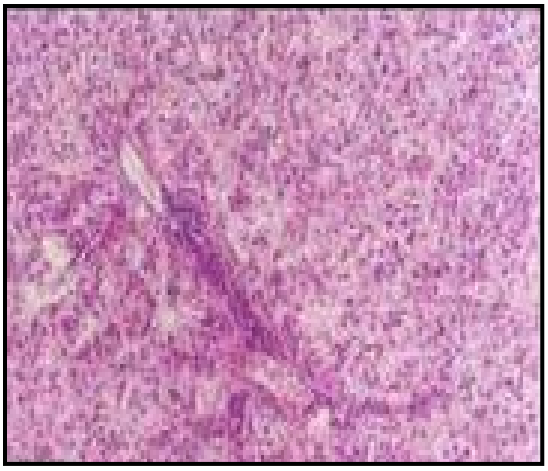

Fig. 4: Liver of broilers immunized with IFAB and infected with *C. jejuni* showing mild vacuolation of hepatocytes (H and E×66)

epithelial lining of the bile ducts was only noticed in un immunized challenged birds accompanied by newly formed bile ductules (Fig. 5).

The enterocytes lining the villi in both immunized and un immunized challenged birds wee necrosed in some areas leaving vill denuded from their epithelial linings. Others showed hyperplasia of enterocytes accompanied by goblet cells transformation (Fig. 6 and 7). The epithelial lining crypts of Lieberkuhn showed hyperplasia and increased numbers of mitotic figures (Fig. 8). Mononuclear cells aggregation in lamina propria was prominent.

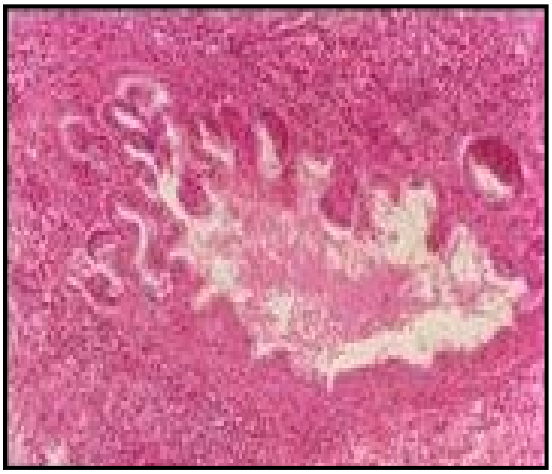

Fig. 5: Liver of control (unimmunized and infected) broilers showing severe hyperplasia of the epithelial lining the bile duct (H and E×66)

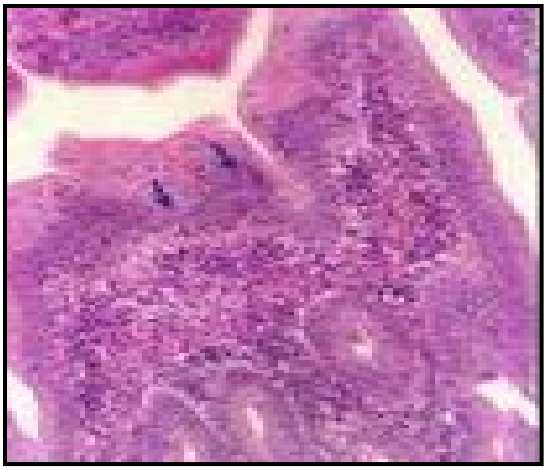

Fig. 6: Intestine of control (unimmunized and infected) broilers showing severe hyperplasia of enterocytes (arrow). Notice severe inflammatory cells aggregation in lamina propria (H and E×66)

The main histopathological lesions in liver as well as intestine and degree of severity in chicken broilers immunized with two types of *C. jejuni* bacterins are shown in Table 2 and 3, respectively. The lesions in the liver of un immunized challenged control subgroups 3a and 3b were characterized by varying degrees of hepatocytes degeneration and inflammatory cells infiltration, Kupffer cells activation, hyperplasia of the bile ducts with formation of new ductules and mild engorgement of the hepatic blood vessels. These changes can be attributed to the direct effect of

Table 2: Main histopathological lesions in liver and degree of severity in chicken broilers immunized with two types of *C. jejuni* bacterins

Lesions	Control	Broilers immunized with (AHAB)	Broilers immunized with (IFAB)
Congested blood vessels	++	Absent	Absent
Inflammatory cells in blood vessels	++	+	+
Multifocal necrosis	+++	++ specially biotype I	++
Cellular infiltration	++++	+++	++
Bile duct hyperplasia and newly formed bile ductules	+++	Absent	Absent
Hepatocytes vacuolation	++++	++ specially biotype II	Absent
Hepatocytes granulation	Absent	++ specially biotype II	Absent
Activation of Kupffer cells	+++	Absent	Absent

++++: Severe, +++: Moderate, ++ and +: Mild

Table 3: Main histopathological lesions in intestine and degree of severity in chicken broilers immunized with both bacterins

Lesions	Control	Broilers immunized with (AHAB)	Broilers immunized with (IFAB)
Necrosis of enterocytes	++++	+++	++
Hyperplasia of enterocytes	+++	Absent	+
Goblet cells transformation	Absent	Absent	+ especially biotype II
Hyperplasia of Crypt	+++	++ especially biotype II	+ especially biotype II
Cellular infiltration in lamina propria	++++	+++	+

++++: Severe, +++: Moderate, ++ and +: Mild

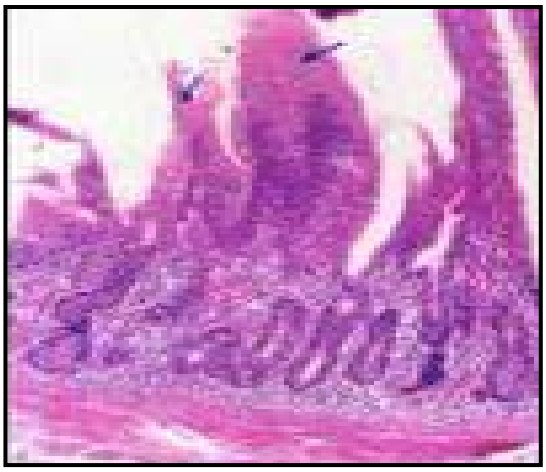

Fig. 7: Intestine of broilers immunized with IFAB and infected with *C. jejuni* showing mild hyperplasia of enterocytes, goblet cells transformation (arrow) and mild inflammatory cells aggregation (H and E×33)

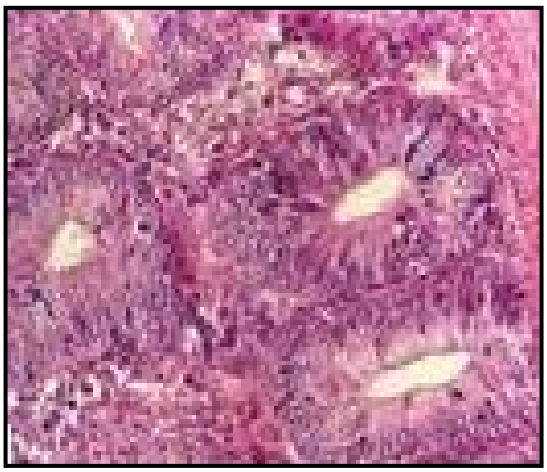

Fig. 8: Intestine of broilers immunized with AHAB and infected with *C. jejuni* showing hyperplasia of crypt of Lieberkuhn with moderate numbers of mitotic figures (arrow) (H and E×66)

C. jejuni organism and/or its endotoxins. This finding agrees with those reported by pervious investigators (Moore, 1958; Whenham *et al*., 1961; Thackrey and Johnstone, 1964; Nagwa, 1992). The liver of challenged and immunized broilers with either (AHAB) or (IFAB) revealed absence of engorged blood vessels, bile ducts hyperplasia and activated Kupffer cells. Other lesions were less severe in those birds than control ones. Moreover, the hepatocytes of IFAB immunized birds weren't completely degenerated (Absence of vacuolation or granulation) and that emphasize the efficacy of the prepared bacterins. The intestine un immunized challenged control subgroups showed severe necrosis (denudation) of the enterocytes. This could be explained by the direct effect of Campylobacter organism on the epithelial lining the intestinal mucosa (Wallis, 1994). Other enterocytes showed moderate hyperplasia and that may be due to the toxic effect of the organism on the epithelial cells which agree well with those reported by Moore (1958) and Moustafa (2002). The lamina propria was characterized by severe mononuclear cells infiltrations which could be due to enterotoxins production. These changes coincide with those recorded by Welkos (1984).

On the other hand, the severity of histopathological changes in the liver and intestine of AHAB and IFAB immunized and challenged subgroups were milder and some

were even completely absent when compared with control birds. Moreover, IFAB immunized broilers generally revealed milder microscopic lesions than AHAB immunized birds.

CONCLUSION

In conclusion, application of water-type or oil-type *C. jejuni* bacterin was effective in prevention of infection in broiler chickens but the later was more effective than the former. These bacterins gave short period of immunity so, they need to be boostered to prolong the period of immunity.

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