Vet. Med. J. Giza 38, No. 2, 245-257 (1990)

THE EFFICACY OF ASCORBIC ACID DURING EXPERIMENTAL CAMPYLOBACTER INFECTION IN CHICKENS

By

A.M. KHALID*, A.S. EL-GED*, MAYSA H. MOHAMED**
AMAL EL-BAGOURY**, A.BASSIOUNY* and M.S.SABER***

*: Fac. Vet. Med., Zagazig University
Benha Branch, Moshtohor
**: Animal Health Research Institute; Dokki-Giza
***: Fac. Vet. Med., Cairo University

(Received: 3.4.1990)

INTRODUCTION

Organisms of the genus Campylobacter, mainly *Campylobacter jejuni* and to a lesser extent *Campylobacter fetus* subsp., *fetus* have been associated with enteritis in man (Bokkenheuser et al., 1979 and Blaser et al., 1980). Recently, campylobacter infections have been incriminated in several intensive poultry farms to form a serious attractive problem (Smibert, 1969 and Peckham, 1972). The organism was isolated from ailing and butchered poultry(Skirrow, 1977). Moreover, Grant et al. (1980) were able to isolate *C.jejuni* from the faeces of ailing birds at poultry market. *C.jejuni* was recognized as the aetiological agent of campylobacteriosis causing vibrionic hepatitis in poultry (Delaplane et al., 1955; Hofstad et al., 1958 and Peckham, 1972).

Although ascorbic acid can be synthesized by poultry the addition of considerable amounts of this vitamin to the poultry diet proved to improve the growth response of chicks (Baldissera-Nordio, 1957). Also,Hill and Garren (1955) showed that ascorbic acid (AA) increased the resistance of chicks to fowl typhoid when fed with other vitamins. Moreover, Thaxton (1984)

observed that AA supplementation provided a significant level of protection from the lethal effects of infectious bursal disease. Edrise et al. (1986) noticed that dietary supplementation of AA to experimental chicken has potentiated their immune response against Newcastle disease. In addition, Gross and Cherry (1987) noted a substantial improvement in the livability associated with the addition of AA to chicks diet following the inoculation with *E.coli*.

This study was undertaken to extend knowledge on therapeutic efficacy of ascorbic acid, to determine whether repeated ingestion of ascorbic acid would hasten elimination of campylobacter from infected chickens and to assess the carryover of the organism.

MATERIAL AND METHODS

Experimental Birds:

Fifty 4-week old broiler Hubbard chickens were obtained from the General Poultry Company. All birds were kept on basal diet, for at least one week, free from ascorbic acid. Intially, all birds negative for camplobacter infection were used in the subsequent investigations.

Organisms:

The Campylobacter strains used were supplied from the Animal Reproduction Institute, Al-Haram. They were originally isolated from chickens. Before the present study began, cultures of campylobacter were grown separately in semisolid bacto-thiol medium at 37°C for 3 days. Prior to inoculation, the organisms were diluted with sterile saline solution to produce a campylobacter inoculum containing approximately 1.26×10^{-10} viable organism per m. according to the method adopted by Truscott and Stockdle (1965).

Experiments:

Five groups of ten were used in this experiment.

Group I : Inoculated by *C.jejuni* (1ml per os)
Group II : Inoculated by *C.jejuni* (0.5ml I/P)
Group III : Inoculated by *C.fetus* subsp.*fetus* (1ml per os)
Group IV : Inoculated by *C.fetus* subsp.*fetus* (0.5ml I/P)
Group V : Uninoculated were kept as control.

Each group was divided into two subgroups, subgroup (A) were supplemented by vitamin C (AA) 500 mg/kg diet and subgroup (B) were fed only on the basal diet. Each subgroup was kept separately in a clean cage throughout the course of the experiment.

The clinical signs of all birds was observed twice daily to record their general health conditions. Three weeks post-infection, the surviving chicks were sacrificed and examined for the pathological appearance of the internal organs and attempts were done for re-isolation of the organisms from heart blood, liver, lungs kidneys, spleen and duodenum. Specimens for histopathological studies were obtained. Samples were fixed in 10 % neutral buffered formaline, embedded in paraffin. Appropriate sections were cut and routinelly stained by Harris haematoxyline and eosin (Harris, 1898). Moreover, the weight of each bird was registered during the course of the experiment.

Blood samples were successively withdrawn from the ailing birds at 0, 5, 10 and 15 days intervals from the onset of the experiment. Serum was separated and used for measuring ascorbic acid (AA) level according to the standard modified method recorded by Jagota and Dani (1982).

RESULTS AND DISCUSSION

The clinical signs noticed among chicks (subgroups-B) infected by *C.jejuni* or *C.fetus* subspecies *fetus* and

Table (1): Healthy (non-infected) chicks:

Days of experiment	Group supplemented by AA			Group not supplemented by AA		
	mW	A	I	mW	A	I
0	300	4.90 ± 0.02	-	300	4.90 ± 0.02	-
5	315	4.99 ± 0.03	-	310	4.90 ± 0.02	-
10	335	5.11 ± 0.03	-	320	4.90 ± 0.02	-
15	350	5.11 ± 0.03	-	330	4.90 ± 0.02	-

Table (2): Chicks infected with Campylobacter jejuni

Days post infection	Inoculation per Os						Inoculation Intraperitoneally					
	a			b			a			b		
	mW	A	I*	mW	A	I	mW	A	I	mW	A	I
0	300	4.99 ± 0.03	-	300	4.80 ± 0.01	-	300	4.90 ± 0.02	-	300	4.50 ± 0.001	-
5	305	4.90 ± 0.02	70	305	4.60 ± 0.002	80	305	4.86 ± 0.01	80	300	4.14 ± 0.02	90
10	315	4.90 ± 0.02	50	310	4.43 ± 0.01	60	310	4.65 ± 0.004	70	310	4.10 ± 0.01	80
15	330	4.80 ± 0.01	50	320	4.20 ± 0.003	60	320	4.60 ± 0.02	60	315	3.80 ± 0.02	80

* Calculated according to the number of examined cases.

fed on basal diet that did not contain ascorbic acid were: loss of body gain, ruffled feathers, dropping of the wings, dullness, depression and scaly shrunken combs (Tables 1-3). Similar clinical symptoms were described by Grant et al. (1980).

In the present work diarrhoea and pasty vent were detected only in one chick from each of subgroup II (B), III (B); IV (B) after 10-days post infection. Nearly similar clinical manifestations have been described by Ruize-Palacios et al. (1981) who showed that chickens experimentally infected with *C. jejuni* were free from clinical signs, gross and macroscopic changes but only few cases revealed diarrhoea. Similar findings have also been reported by Altmeger et al. (1986); Kohler et al. (1987) Shanker et al. (1988).

Post-mortem examination after 15 days post infection revealed that 95% of infected birds deprived of AA had gross liver lesions. The lesions included enlarged liver with greesy consistancy, small necrotic foci in the parenchyma, haemorrhagic areas and haemorrhagic foci under the capsule, friable liver with large blood clots adhering to the surface. The lungs showed congestion nearly in all examined cases and haemorrhagic patches were also seen on the periphery of the lung lobes. Kidneys were pale and enlarged. The heart in some cases was flabby, in group II, three cases revealed gelatineous fat around the coronary grooves. The spleen was enlarged and dark red in colour in most cases. The duodenum showed petechial haemorrhages scattered on the mucosal layer. These pathological changes and their explanation are similar to a great extent to what had been stated by Peckham (1958) and Hofstad et al. (1958). On the other hand, birds supplemented by AA showed less gross lesions, there were no pathological alterations in the liver, lungs and intestines.

The histopathological findings in liver in all groups

Table (3): Chicks infected with *Campylobacter fetus* subsp. *fetus*.

Days post infection	Inoculation per Os			Inoculation Intraperitoneally								
	a						b					
	mW	A	I[x]	mW	A	I	mW	A	I	mW	A	I
0	300	4.90 ± 0.03	-	300	4.80 ± 0.01	-	300	4.90 ± 0.02	-	300	4.95 ± 0.001	-
5	310	4.85 ± 0.02	60	310	4.70 ± 0.11	70	305	4.87 ± 0.01	70	298	4.80 ± 0.02	80
10	330	4.65 ± 0.01	50	315	4.50 ± 0.21	60	310	4.72 ± 0.13	70	305	4.28 ± 0.013	80
15	335	4.60 ± 0.05	40	320	3.90 ± 0.001	60	320	4.15 ± 0.013	60	315	4.56 ± 0.014	60

a : Group supplemented with AA.

b : Group not supplemented with AA.

mW : Mean weight per grams.

A : Ascorbic acid level in serum.

I : Re-isolation rate of Campylobacter organisms.

x : Calculated according to the number of examined cases.

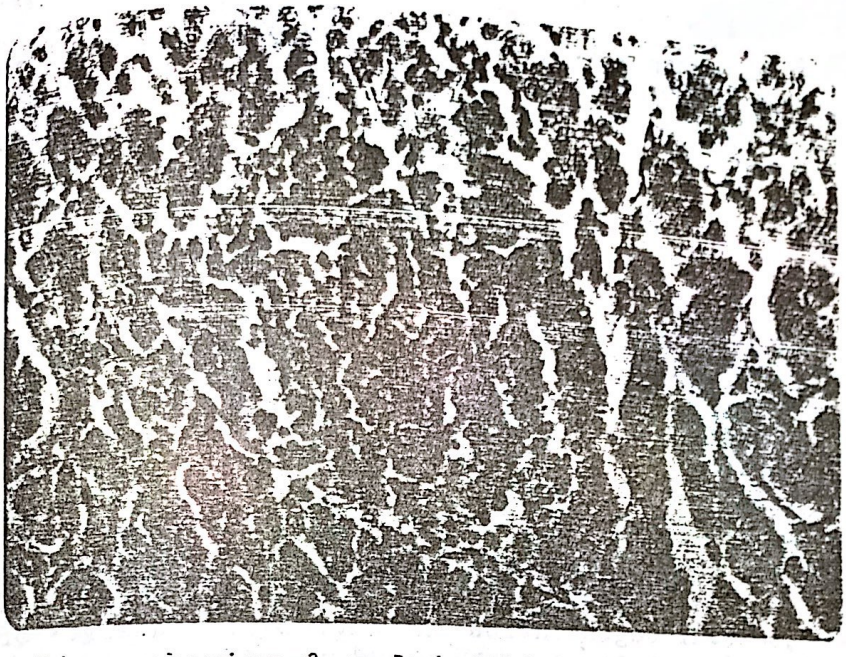

Fig. (1): Liver showing foccal infiltration with mononuclear cells.
(H & E stain, X 40).

Fig. (2): Liver showing proliferation of bile ducts.
(H & E, X 40).

varied in intensity between the treated and non-treated groups. The common lesions were minute petechial haemorrhages and focal infiltration by mononuclear cells (Fig. 1). Focal areas of coagulative necrosis were seen. Activation of an Kupffer cells was pronounced in all cases, indicating phagocytic function of these cells. The portal areas showed slight or severe fibrous tissue formation and hyperplastic proliferation of bile duct (Fig. 2). These lesions might have developed as a result of the toxic effect of the microorganism. Similar lesions were shown by Osburn and Hoskins (1970). Lungs showed alveolar pneumonia and peribronchial lymphocytic infiltration (Fig. 3). Intestinal mucosa suffered from slaughing of lamina epithelialis, mononuclear cellular infiltration in lamina propria were shown. Necrosis of glandular epithelium was also pronounced (Fig. 4). Kidneys showed granular degeneration and necrobiotic changes of some epithelial linning the renal tubules.

Moreover, the results revealed that the re-isolation rate of campylobacter organisms from infected birds showed higher incidence in birds deprived from AA and those supplemented with the vitamin showed markedly low frequency of reisolation.

These results indicate that dietary supplementation of vitamin C to the experimental chicks had potentiated their immune response against campylobacter infection. Similar findings were reported regarding other infectious agents (Hill and Garren, 1958; Thaxton 1984; Edrise et al., 1986 and Gross and Cherry,1987).

The results presented in tables (1-3) also showed that AA levels were lower in birds infected with campylobacter species than those of the non-infected medicated groups. These results nearly agree with those described by Chatterijee (1987) who stated that the synthesis of AA in poultry was markedly reduced

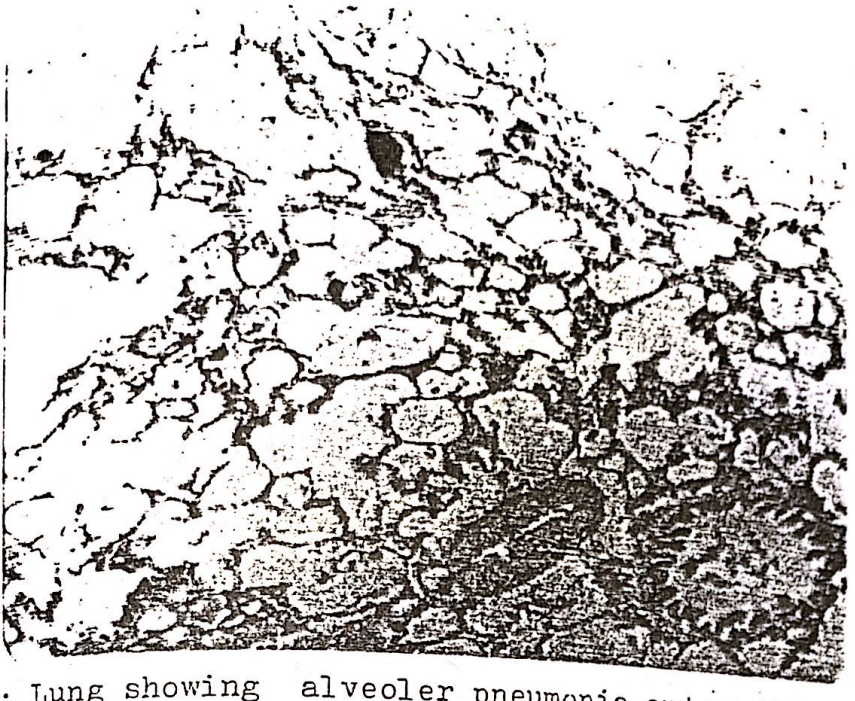

Fig. (3): Lung showing alveoler pneumonia and peribronchial lymphocytic infiltration.
(H & E, X 20)

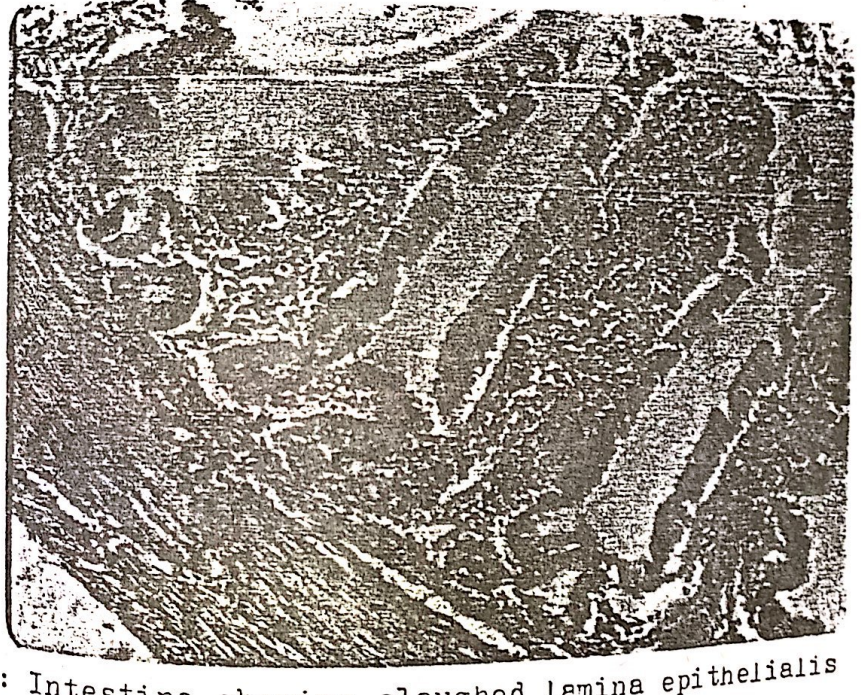

Fig. (4): Intestine showing slaughed lamina epithelialis and mononuclear infiltration.
(H & E, X 20).

under certain stress factors such as infection and trauma. Moreover, Squibb et al. (1955) recorded lower serum AA levels in birds infected with Coryza and Newcastle diseases. Meanwhile, Hill and Garren(1955 and 1958) reported a decrease in the plasma AA levels in chickens infected with fowl typhoid.

It can be concluded that supplementation of AA improved the health status of campylobacter infected chicken in the form of weight gain, better resistance to infection, potentiating the immune response leading to a lesser degree of morbidity and pathological changes.

SUMMARY

Campylobacter jejuni and *C.fetus* subsp. *fetus* were inoculated into fifty chickens by different routes. Ascorbic acid (AA) was supplemented in a dose of 500 mg/kg diet. The clinical, postmortem, bacteriological and histopathological findings were recorded. The re-isolation rate of campylobacter organisms was much lower in chickens supplemented with AA than other birds. Infected chickens kept on basal diet only showed loss of body weight. Birds infected with campylobacter organisms showed lower serum AA levels than those of the non-medicated group.

The addition of AA proved to minimized the lesions in infected cickens and seemed to improve the weight gain. AA also potentiated the healthy conditions in birds supplemented with high doses of this vitamin. The importance of AA as food additive in poultry diet to protect chickens and consequently human beings from infectious agents was discussed.

REFERENCES

1. Altmeger, M.; Krabisch, P.; Dorn,P. (1986): Prevalance and distribution of *Campylobacter jejuni* and *C coli* in broiler production. II-Characterization, drug resistance and pathogenicity. Dtsche Tieraerztl. Wschr. 93 (10): 469-472.

2. Baldissera-Nordio, C. (1957): Die Rolle des vitamins C in der Physiologie und Ernachrung des Geflugels, F. Hoffman-Laroche.

3. Blaser, M.J.; LaForce, F.M.; Wilson, n.A.; Wang, W.L. (1980): Reservoirs for human cmpylobacteriosis. J. Infect. Dis., 141 (5): 665-669.

4. Bokkenheuser, V.D.; Richardson, N.J.; Bryner, J. H.; Roux, D.J.; Schutte, A.B., Koorhof, H.J.; Freiman, I.; Hartman, E. (1979): Detection of enteric campylobacteriosis in children. J. Clin. Microbiol., 2: 227-232.

5. Chatterjee, G.C. (1967): In "The vitamins". Vol. 1, P. 407 (W.H. Sebrell, Jr and R.S. Harris Editors), New York and London Academic Press.

6. Delaplane, J.P.; Smith, H.A.; Moore, R.W. (1955): An unidentified agent causing a hepatitis in chickens. S. West. Vet., 8: 356-361.

7. Edrise, B.M.; Khair El-Din, A.W.; Soliman, R. (1986): The immunopotentiating effect of ascorbic acid against Newcastle disease in chicken. Vet. Med. J., 34 (2): 251-264.

8. Grant, I.H.; Richardson, N.J.; Bokkenheuser, V.D. (1980): Broiler chickens as potential source of campylobacter infections in humans. J. Clin. Microbiol., 11 (5): 508-510.

9. Gross, and Cherry (1987): Recent findings on vitamin C supplementation. Technical symposium, March 11, 1987, Daytona Beach, Florida, USA.

10. Harris, H.E. (1898): Cited by Carleton, M.A.; Drury, R.A.B.; Cameron, H., 1967. In "Carleton's Histology Technique. 4th Ed., Oxford Univ. Press. New York, Torento.

11. Hill, C.H. and Garren, H.W. (1955): The effect of high levels of vitamins on the resistance of chicks to fowl typhoid. Ann. New York Acad. Sci., 63: 186-194.

12. Hill, C.H. and Garren, H.W. (1958): Plasma ascorbic acid levels of chicks with fowl typhoid. Poultry Sci., 37: 336-337.

13. Hofstad, M.S., McGehee, E.H.; Bennett, P.C.(1958): Avian Infectious Hepatitis. Avian Dis., 2: 358-364.

14. Jagota, S.K. and Dani, H.M. (1982): A new colorimetric technique for the estimation of vitamin C using folin phenol reagent. Analytical Biochemistry, 127: 178-182.

15. Kohler, B.; Richter, U.; Mochmann, H.; Wendland, B. (1987): Experimental infection of chicks with *Campylobacter jejuni* and C.coli. Archiv fur Experimentelle Veterinarmedizin, 41 (1): 37-50.

16. Osburn, B.I. and Hoskins, P.K. (1970): Experimentally induced *Vibrio fetus* var intestinalis infection in pregnant cows. Am. J. Vet. Res., 31: 1733-1741.

17. Peckham, M.C. (1958): Avian vibrionic hepatitis. Avian Dis., 2: 348-398.

18. Peckham, M.C. (1972): Diseases of Poultry. 6th Ed., Ames. Iowa State Univ.

19. Ruiz-Palacios, G.M.; Escamilla, E.; Torres, N. (1981): Experimental campylobacter diarrhoea in chicken. Infection and Immunity, 34 (1): 250-255.

20. Shanker, S.; Lee, A. Serrell, T.C. (1988): Experimental colonization of broiler chicks with *Campylobacter jejuni* Epidemiology and infection, 100 (1): 27-34.

21. Skirrow, M.B. (1977): Campylobacter enteritis a new disease. Br. Med. J., 2: 9-11.

22. Smibert, R.M. (1969): *Vibrio fetus* var intestinalis isolated from the intestinal contents of birds. Am. J. Vet. Res., 30 (8): 1437-1442.

23. Squibb, R.L.; Braham, J.E.; Guzman, M.; Scrimshaw (1955): Blood serum total proteins, riboflavin, ascorbic acid, carotenoids and vitamin A of New Hampshire chickens infected with coryza, cholera or Newcastle diseases. Poultry Sci., 34: 1054-1058.

24. Thakton, J.P. (1984): Recent findings on vitamin C supplementation. Technical symposium, March 11, 1987. Daytona Beach, Florida, USA.

25. Truscott, R.B. and Stockdle, H.G. (1966): Correlation of the identity of bile and cecal vibrios from the same cases of avian vibrionic hepatitis. Avian Dis., 10: 67-73.