

PATHOGENICITY OF AEROMONAS HYDROPHILA IN CHICKEN EMBRYOS

H. M. Z. YOUSEIF and WAFAA M. M. HASSAN.

Dept. of Research and Diagnosis of Poultry Diseases, Animal Health Research Institute , Dokki .

Received: 19.12.2002

Accepted: 18.2.2003

SUMMARY

The pathogenicity of *Aeromonas hydrophila* to embryonated chicken eggs (ECE) at different ages of incubation was studied . Infected 5 and 17-day-old ECE revealed mortality and hatchability rates ranged between (36.7 % and 30 %) and (63.3 % and 70 %) respectively. Acidic and alkaline disinfectants (Aldekol GDA and Virkon-S) were used to control of *Aeromonas hydrophila* infection of these ECE that proved improvement in the rate of hatchability reaching (80 % and 86.6 %) and (76.6 % and 83.3 %) respectively . Post-mortem lesions as well as bacterial reisolation from dead embryos as well as hatched chicks were discussed in details .

and epidemiological importance in poultry have not been sufficiently carried out. A scanty literature on *Aeromonas* species infection in birds are available . *Aeromonas hydrophila* is considered as facultative pathogens and can cause enteritis in some birds (Aguirre et al., 1992) . *Aeromonas* species were isolated in pure cultures as the primary cause of acute death and acute nephrosis, acute haemorrhagic septicaemia and conjunctivitis lesions in : canaries and toucan , a captive ground –hornbill , pet parrot , as well as captive bustards (Panigrahy et al.,1981; Ocholi and Kalejaiye, 1990; Garcia et al., 1992; Silvanose et al., 2001). In domestic poultry, *Aeromonas hydrophila* was isolated from septicaemic condition of 3-16 week-old commercial turkeys, poultry faeces, haemorrhagic septicaemia in ducks , from faeces and carcasses of broiler chickens as well as from outbreaks in duck flocks suffered from sudden death with clinical signs of anorexia and dyspnoea (Gerlach and Bitzer1971; Stern et al., 1987; Jindal et al., 1993; FanDe et al., 1997; Akan et

INTRODUCTION

Although the genus *Aeromonas* is a Gram-negative rods motile by single polar flagella producing exotoxins, its economical significance

al., 1998 and KeMin et al. 1998). *Aeromonas* species are considered as food born pathogens and of public health importance (Gracey et al., 1982; Agger et al., 1985; Hardy et al., 1986; KiRov et al., 1990 and Sarinehmetoglu and Kuplu, 2001).

Burke et al. (1984) and KiRov et al. (1990) discussed the epidemiology of *Aeromonas hydrophila* through food contamination, infected water, food contaminated with animal faeces and infected handlers. *A. hydrophila* can contaminate treated water (chlorinated) and chill water used in poultry industries. Khurana and Kumar (1997) could isolate 125 isolates of *Aeromonas* species from poultry eggs, (liver and heart), and meat in the ratio of 0.9 %, 32.6 % and 28 % where *A. hydrophila* was the predominant isolate (77 isolates out of 125 isolates).

The present investigation was designed to study experimentally the pathogenicity of *A. hydrophila* in embryonated chicken eggs at different ages as well as the usefulness of certain disinfectants in controlling this infection.

MATERIAL AND METHODS

1- Embryonated Chicken Eggs (ECE): -

A total of 360 Hubbard ECE obtained from a Commercial Poultry Hatchery were used. They were consisting of 180 ; of 5-day-old And 180 ; 17-day-old ECE.

2 - Bacterial strain and isolation: -

A strain of *Aeromonas hydrophila* had been originally isolated from poultry meat meal was used in this study. These meals were submitted to Animal Health Research Institute from abroad for routine examination of *Salmonellae* species. Isolation of *A. hydrophila* had been carried out in trypticase soy broth containing 10 mg / ml ampicillin which was inoculated by 1 gm of sample and incubated at 28-30° C for 24 hrs. A loopful from the inoculated incubated broth was streaked onto trypticase soy ampicillin agar (TSA) and MacConkey agar plates and incubated at 28 -30° C for 48 hrs. Suspected colonies were picked up for further identification. The isolated organism was identified biochemically according to Propof and Veron (1976) including Gram stain, motility test, Voges-Proskauer, indole production, gelatin liquefaction, sugar fermentations, oxidation test and aesculin broth hydrolysis.

3 - Disinfectants: -

a) Aldekol -GDA : -

Aldekol GDA an alkaline sanitizer, clear, yellowish solution contain an activated glutaraldehyde (243.0 g/L) in combination with second generation quaternaries (Didecyldimethyl ammonium-chloride 22.5 g/L) and inactive ingredients inerts (distilled water ad 1 liter) was used. The product is produced by EWABO ChemiGmbH Chempharmazeutische Produkte KolpingstraBe 4 , D- 49835 Wietmarschen Germany.

b) Verkon S:

An acid sanitizer powder easily soluble in water contain 1.5 % sodium chloride, 49.8 % potassium monopersulphate, potassium hydrogen sulphate, potassium trippsalt, 5% sulphoric acid, 10 % malic acid, 18.5 % sodium mexasmeta phosphate, 15 % sodium dodecylbenzene sulphate and 0.2 % lemon-peelperfume w/w was used for sanitization of hatching eggs at a ratio of 1 % in water as recommended dose (Antec International LTD, U.K.)

4 – Experimental design:

Three hundreds and eighty ECE, (190 ECE 5-day-old and 190 ECE 17-day-old) were used. Ten ECE of each age were subjected to bacteriological examination, which proved to be free from bacterial contamination. The remaining 360 ECE were divided into 12 equal groups (groups 1-6 ECE 5-day-old) and (groups 7-12 ECE 17-day-old) consisting of 30 each. ECE of groups No. 1, 2, 3, 7, 8 and 9 were dipped in a chilled 18 hours *A. hydrophila* broth culture containing 4×10^8 CFU / ml for 5 minutes while groups No. 4, 5, 6, 10, 11 and 12 kept without infection and put in a separated incubator. All groups No. (1-12) were incubated at 37.8° C. After 6 hours; the groups No.(2, 4, 8 and 10) and No.(3, 5, 9 and 11) were treated with 1 % chilled solution of Aldekol GDA and Verkon-S by spraying for 3 minutes respectively while groups No.6 and 12 kept without treatment as control blank groups. Embryos of all groups were incubated at 37.8° C with daily can-

dling for embryonic mortality. Bacterial reisolation was carried out on dead embryos as well as sacrificed chicks that hatched.

RESULTS

Obtained results are shown in tables 1 – 4.

Dead embryos (from 5 and 17-day-old ECE): -

The dead ECE were suffering from septicemia and congestion in all internal organs

Dead chick (from 17-day-old ECE hatched) :-

There was one-hatched chick dead of 7-day-old (represent 4.7 %) suffering from dullness, inappetance and diarrhoea. Postmortem lesions showed moderate congestion in heart, kidneys and intestine with slight congestion in liver, yolk sac and lungs. There was severe enteritis with participation of urate in ureters .

Postmortem lesions of sacrificed chicks :-

1-A.hydrophila infected group: (17-day-old ECE): -

No observed lesions could be detected in the survived sacrificed chicks after 1st or 2nd weeks of hatching except slight congestion in kidneys, liver and lungs in some chicks.

2- Other groups: -

No observed lesions could be detected in the survived sacrificed chicks after 1st or 2nd weeks of hatching.

Table (1): Results of *A. hydrophila* infected 5-day-old chicken embryos:

Group No.	Bacterial infection	Disinfectant		No. of ECE	Rate of embryonic mortalities				Chick hatching	
		Aldekol GDA	Verkon - S		Early	Late	Total No.	%	No.	%
1	<i>A. hydrophila</i>	-	-	30	8	3	11	36.7	19	63.3
2	<i>A. hydrophila</i>	+	-	30	4	2	6	20	24	80.0
3	<i>A. hydrophila</i>	-	+	30	4	3	7	23.4	23	76.6
4	Control	+	-	30	2	1	3	10	20	90.0
5	Control	-	+	30	1	3	4	13.4	26	86.6
6	Control	-	-	30	1	-	1	3.4	29	96.6

Table (2): Results of *A. hydrophila* infected 17-day-old chicken embryos

Group No.	Bacterial infection	Disinfectant		No. of ECE	Rate of embryonic mortalities				Chick hatching	
		Aldekol GDA	Verkon - S		Early	Late	Total No.	%	No.	%
7	<i>A. hydrophila</i>	-	-	30	-	9	9	30	21	70.0
8	<i>A. hydrophila</i>	+	-	30	-	4	4	13.4	26	86.6
9	<i>A. hydrophila</i>	-	+	30	-	5	5	16.7	25	83.3
10	Control	+	-	30	-	2	2	6.7	28	93.3
11	Control	-	+	30	-	3	3	10	27	90.0
12	Control	-	-	30	-	-	-	-	30	100.0

* One chick died at 7-day-old (4.7 %) from total hatched chicks

Table (3): Results of reisolation of *A. hydrophila* from infected 5-day-old dead chicken embryos and sacrificed survive chicks

Group No.	Chick age/ week	Bacterial infection and treated	Embryonic death reisolation			Chick organs positive for reisolation						
			Total No.	+ve No.	%	Total No.	Heart	Liver.	Yolk .sac	Lungs	Inestine	%
1	1st	<i>A. hydrophila</i>	11	9	81.8	10	5/10	7/10	4/10	3/10	6/12	70.0
	2nd					9	1/9	3/9	-	1/9	5/9	55.5
2	1st	<i>A. hydrophila</i> + Aldekol GDA	6	3	50.0	12	2/12	8/12	5/12	3/12	8/12	66.6
	2nd					12	-/12	4/12	-	1/12	5/12	41.6
3	1st	<i>A. hydrophila</i> + Virkon-S	7	4	57.1	12	1/12	6/12	4/12	2/12	8/12	66.6
	2nd					11	-/11	3/11	-	-	5/11	45.4

Table (4): Results of reisolation of *A. hydrophila* from infected 17-day-old dead chicken embryos and sacrificed survive chicks

Group No.	Chick age/ week	Bacterial infection and treated	Embryonic death reisolation			Chick organs positive for reisolation						
			Total No.	+ve No.	%	Total No.	Heart	Liver.	Yolk .sac	Lungs	Inestine	%
7	1st	A.hydrophila	9	9	100	10	2/10	7/10	5/10	4/10	8/10	80.0
	2nd					11	-	3/11	-	1/10	7/11	63.8
8	1st	A.hydrophila + Aldekol GDA	4	2	50	13	1/13	7/13	6/13	3/13	8/13	61.8
	2nd					13	-	2/13	-	1/13	6/13	46.1
9	1st	A.hydrophila + Virkon-S	5	2	40	12	2/12	8/12	5/12	4/12	9/12	75.0
	2nd					13	-	3/13	-	-	7/13	66.6

DISCUSSION

Aeromonas hydrophila received an increasing attention as a food-born diarrhoeal disease in human beings which isolated from poultry eggs (Varnam and Evans, 1991 and Khurana and Kumar, 1997). Reviewing the available literature; as far as the authors knows this investigation seems to be the first time in Egypt that deals with the pathogenicity of *A. hydrophila* in embryonated chicken eggs.

The present results revealed that embryonic mortalities reaching 36.7% and 30% for 5-day-old and 17-day-old ECE as compared with 3.4% and zero% in control blank groups respectively (Tables 1 and 2). Dead ECE were suffering from septicaemia and congestion in all the internal organs with the rate of reisolation reaching 81.8% and 100% from 5-day-old and 17-day-old respectively (Tables 3 and 4).

As regards to the results illustrated in Table (2) *A. hydrophila* infection resulted in one dead hatched chick at 7-day-old (4.7%) suffering from dullness, inappitance and diarrhoea. Post-mortem lesions were moderate congestion in heart, kidneys and intestine with slight congestion in liver, yolk sac and lungs. There was severe enteritis with participation of urate in ureters. These results are incomplete accordance with that reported by Ocholi and Kalejaiye (1990) who isolated *A. hydrophila* from liver, lungs and intestine of ground Hornbill suffering from haemorrhagic septicaemia with haemorrhage in the internal organs. Gerlach and Bitzer (1971) described a septicaemic condition in commercial turkeys aged 3-16 weeks that was attributed to *A. hydrophila* infection with 10-30% morbidity and 1-5% mortality. Meanwhile, Saif and Busch (1974) studied the synergistic relationship of *Salmonella infantis* and *A. hydrophila* in newly hatched poults and found that both organisms

together produced 30 % mortality but neither organism produced mortality when inoculated individually.

The rate of hatchability improved after using aldekol-GDA and virkon-S disinfectants reaching (80 % and 86.6 %) and (76.6 % and 83.3 %) for 5 and 17-day-old ECE respectively as shown in tables (1 & 2) . While these rates in infected non-treated groups with *A. hydrophila* reached 63.3 % and 70 % for 5 and 17-day-old ECE respectively.

Control groups treated with aldekol-GDA, virkon-S and blank control No; (4 and 10), (5 and 11) and (6 and 12) ; the hatchability rate reached (90 % and 93.3 %), (86.6 % and 90 %) and (96.6 % and 100 %) respectively. The slight bad effect of Aldekol-GDA on embryonic hatchability might be due to the presence of quaternary, which causes low early embryonic loss. Scott et al. (1993) found that there was no gross toxic effect on embryo viability treated with glutaraldehyde . Virkon-S gave the mildest bad effect on hatchability. These results are incomplete accordance with that obtained by, Youseif et al. (2000 and 2001) who concluded that virkon-S and Aldekol -GDA were safe for sanitization of ECE infected with bacteria.

Results of bacterial reisolation from the dead embryos which infected at 5-day-old , and treated with Aldekol GDA and Virkon-S were 50 % and 57.1 % respectively as compared with 81.8 % in

their control . While the rare of reisolation reached 41.6 – 66.6 % and 45.4 – 66.6 % in sacrificed hatched chicks respectively as compared with 55.5 – 70.0 % in their control (Tables 3) .

Results of bacterial reisolation from the dead embryos which infected at 17-day-old , and treated with Aldekol GDA and Virkon-S were 50.0 % and 40.0 % respectively as compared with 100.0 % in their control . While the rare of reisolation reached 46.1 – 61.8 % and 66.6 – 75.0 % in sacrificed hatched chicks respectively as compared with 63.6 – 80.0 % in their control (Tables 4) .

In conclusion , *Aeromonas hydrophila* infection in ECE is a potent pathogen that should be considered on putting strategies for control measures and biosecurity in hatcheries as well as a public health hazard. The roles possibly played by faecal and water contamination for this pathogen must be taken in consideration for controlling this pathogen.

REFERENCES

- Agger, W.A.; McCormick, J.D. and Gurwith, M.J. (1985): Clinical and microbiological features of *Aeromonas hydrophila*-associated diarrhea. *J. of Clin. Microbiol.*, 21: 909 – 913.
- Aguirre A.A. ; Quan, T. J. ; Cook, R.S. and McLean, R.G. (1992): Cloacal flora isolated from wild black-bellied whistling ducks (*Dendrocygna autumnalis*) in Laguna La Nacha, Mexico . *Avian Dis.*, 36: 459 – 462.

- Akan, M.; Eysegul, A. and Diker, K.S. (1998): Motile Aeromonads in the feces and carcasses of broiler chickens in Turkey. *J. Food Prot.*, 61 (1): 113 – 115.
- Burke, V. ; Robinson, J. ; Gracey, M. ; Peterson, D. ; Meyer, N. and Haley, V. (1984): Isolation of *Aeromonas* spp. from an unchlorinated domestic water supply . *Appl. Environ. Microbiol.*, 48: 367 – 370.
- FanDe , K. ; Yinyao , H. ; WenZhong , Wu. ; Qiong , C. ; Kong-FD ; Huang-YY ; Wu-WZ and Chen-Q. (1997): Isolation and identification of two strains of *Aeromonas*. *Chinese J. Vet. Sci. Technol.*, 27 (2): 23 – 24.
- Garcia, ME. ; Domenech , A. ; Dominguez , L. ; Ramiro , F. ; and Fernandez , G.JF . (1992): *Aeromonas hydrophila* conjunctivitis in a pet parrot (*Amazona versicolor*) . *Avian Dis.*, 36 (4): 1110 – 1111.
- Gerlach, H. and Bitzer, K. (1971): Infection with *Aeromonas hydrophila* in young turkeys. *Dtsch. Tieraerztl , Wochenschr* , 78 : 593 – 608 .
- Gracey , M. ; Burke , V. and Robinson , J. (1982): *Aeromonas* – associated gastroenteritis. *Lancet*, 2 : 1304 – 1306
- Hardy , J.C. ; Todd , L.S. and Stringer , M.F. (1986): Toxin production by *Aeromonas hydrophila* in bacteriological media and foods. *Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene I. Abteilung Suppl.* 15: 175 – 176.
- Jindal ,N.S.R. and Kumar , A. (1993): Comparison of *Aeromonas* spp. isolated from human, livestock and poultry feces . *J. Vet. Med.* 48: 80 – 83.
- KeMin, Li.; WenXian , H. ; JinHe , Y. ; WenRu , Yu. ; KM, Li.; WX, H.; JH, Y. and WR, Y.U. (1998): Pathogen identification and immunization experiments of *Aeromonas hydrophila* disease in duck. *Chinese J. Vet. Med.* 24 (12) : 13 – 14.
- Khurana, R. and Kumar, A. (1997): Prevalence of motile Aeromonads in foods of animal origin. *J. Sci. Tech. (Mysore)* , 34 (3) : 228 – 229 .
- KiRov S.M., Anderson M.J. and Mc Meekin T.A. (1990): A note on *Aeromonas* spp from chickens as possible food-borne pathogens. *J. Appl. Bacteriol.*, 68 : 327 – 334.
- Ocholi, RA. And Kalejaiye, J.O. (1990): *Aeromonas hydrophila* as cause of septicaemia in a ground-hornbill (*Bucorvus abyssinicus*) . *Avian Dis.*, 34 (2): 495 – 496 .
- Panigrahy, B.; Mathewson , JJ. ; Hall, CF. And Grumbles LC. (1981): Unusual disease conditions in pet and aviary birds. *J. Am. Vet. Med. Assoc.*, Feb 15; 178 (4) : 394 – 395.
- Propoff, M. and Veron, M. (1976): A taxonomic study of *Aeromonas hydrophila* and *Aeromonas punctata* group. *J. Gen. Microbiol.* , 94 : 11 – 22.
- Saif, Y.M. and Busch, W.F. (1974): *Aeromonas* and *Salmonella* infections in turkey poults. Report Ohio Agr. Res. And Dev. Center. PP. 119 – 120 .
- Sarnehmetoglu, B. and Kuplulu, O. (2001): Isolation and identification of motile *Aeromonas* species from chicken. *Dtsch Tirarztl Wochenschr* , Nov. ; 108 (11) : 465 – 467 .
- Scott, T.A. ; Swetnam , C and Kinsman , R. (1993): Screening of sanitizing agents and methods of application for hatching eggs. III- Effect of concentration and exposure time on embryo viability. *J. App. Poult. Res.*, 2 (1): 12 – 18
- Silvanose, C.D. ; Bailey, TA. ; Naldo , JL. and Howlett , JC. (2001): Bacterial flora of the conjunctiva and nasal cavity in normal and diseased captive bustards *Avian Dis.*, 45 (2): 447 – 451.

Stern, N.J.; Drazek, E.S. and Joseph, S.W. (1987): Low incidence of *Aeromonas* spp. in livestock feces. *J. Food Prot.*, 50: 66 – 69.

Varnam, A.H. and Evans, M.G. (1991): *Aeromonas* in food-borne pathogens: An illustrated text. Walf. Publishing Ltd, London, England, pp. 185 – 198.

Youseif, H.M.Z.; Ali, M.M. and Hassanein, Z.A.W. (2000): The efficiency of some disinfectants against chicken

embryo bacterial infections. *J. Egypt Vet. Med. Assoc.*, 60 (1): 89 – 96.

Youseif, H.M.Z.; Hassanein, Z.A.W. and Jihan, M. B. (2001) : The sanitizing effect of Glutraldehyde-Quaternary Ammonium combination (Aldekol GDA) on bacterial infection in hatching eggs (Laboratory and Field trials) . *J. Vet. Med.(Giza)*, 49 (4) : 531 – 541.