Vet.Med.J.,Giza. Vol.51, No.2. (2003) :205 - 212.

PATHOGENICITY OF AEROMONAS HYDROPHILA IN CHICKEN EMBRYOS

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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 renged 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 hatchablity reaching (80 % and 86.6 %) and (76.6 % and 83.3 %) respectively . Postmortem lesions as well as bacterial reisolation from dead embryos as well as hatched chicks were discussed in details .

INTRODUCTION

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

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; Ki-Rov et al., 1990 and Sarinehmetoglu and Kuplu-lu, 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 meals 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* has 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 inculated 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 Propoff and Veron (1976) including Gram stain, motility test, Voges-Proskauer, indole production, gelatin liquefacation, sugar fermentations, oxidation test and aesculin broth hydrolysis.

3 - Disinfectants: -

a) Aldekol –GDA : -

Aldekol GDA an alkaline sanitizer, clear, yellowish solution contain an activated glutaraldehyd (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 tripplsalt, 5% sulphoric acid, 10 % malic acid, 18.5 % sodium mexameta 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 x 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 candling 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	A.hydrophila	-	-	30	8	3	11	36.7	19	63.3
2	A.hydrophila	+	-	30	4	2	6	20	24	80.0
3	A.hydrophila	-	+	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	A.hydrophila	-	-	30	-	9	9	30	21	70.0
8	A.hydrophila	+	-	30	-	4	4	13.4	26	86.6
9	A.hydrophila	-	+	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	A.hydrophila	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	A.hydrophila + 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	A.hydrophila + 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. hydrophif* 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 humanbeings which isolated from poultry eggs (Varnam and Evans,1991 and Khurana and Kumar, 1997) . Reviewing the available literature; as far as the authers 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 resultted 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 haemorragic 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 hatchablity improved after using aldekol-GDA and verkon-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 hatchablity 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 glutradehyde . 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.

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