Pathogenicity of Aeromonas on Embryonated Chicken Eggs

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Abstract: Pathogenicity of Aeromonas on embryonated chicken eggs was studied in three species of Aeromonas : (*Aeromonas hydrophila, Aeromonas caviae and Aeromonas sobria*) in embryonated chicken eggs either by inoculation via yolk sac route or dipping of egg incubated for till hatching . The criteria of judgment was mortality , hatchability, reisolation from dead embryo, histopathological changes in liver, yolk sac of dead embryo on hatched chicks Yolk sac inoculation of three species of Aeromonas in a dose 1.5x 10^7/ml gave 100% embryonic mortalities after 3 days and the reisolation from liver 83%, 75%, 50%, respectively and from yolk sac 91.6%,75%,66.6% respectively. In dipped eggs in media contains three Aeromonas species in adose 1.5x10^7/ml gave embryonic mortalities 25%, 33.4%, 17% respectively reisolation rate of Aeromonas species from liver 33.3%, 25%, 0% respectively and from yolk sac 100%, 50%, 100% respectively while hatchability was 75%, 66.6% and 83% respectively. Hatched chicks showed pathological changes in both liver and intestine. Finally the results indicated that, Aeromonas strains *(A.hydrophila, A.caviae, A.sobria*) were highly pathogenic for chicken and causing embryo mortalities and decrease of the hatchability.

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1. Introduction:

Aeromonas is a member of family vibrionaceae ,placed in a genus Aeromonas which include four phenotypical separated species named Aeromonas *hydrophila* (*A. hydrophila), Aeromonas Caviae,(A. Caviae), Aeromonas Sobria(A. Sobria) and Aeromonas Salmonicida (A. Salmonicida)* (Altwegg, 1988).

Aeromonas normally inhabits brackish, Fresh, estuarine, marine, chlorinated and unchorinated water supplies (slotnick, 1970; Kaper et al.,1980; Atkinson, 1986; Van Derkooj, 1988).

Aeromonas produces cytotoxins so it has a public health significance as a potential cause of food – borne illness . (Barnhart and Puncorbo 1992).

Aeromonas hydrophila, either alone or in combination with other organisms, can cause localized and systemic infection in avian species including poultry (Glunder and siegmann 1989, and Shane and Gifford. 1985). Aeromonas was recorded in chickens and turkeys suffering from enteritis and watery feces (Efuntoye, 1995) as well as in ducks suffering from salpingitis , Septicemia and/ or air saculitis (Bisgaard 1995;Li. et al., 1998 and watts, et al., 1993).

No available literature dealing with the transmission of Aeromonas through the ovary (ovo transmission of Aeromonas) while ,Aeromones has been recovered from deed – in – shell embryos and weak chicks (lin – et al., 1996).The contamination of chicken carcasses with motile Aeromonas speices was occurred in the slaughtering process from the intestinal content to carcasses via processing water (Akan et al., 1998; and Sarimeh metoglu and Kupulu, 2001).

This study was aimed to study the pathogenicity effect of Aeromonas on the embryonated chicken eggs (ECE).

2. Material and Methods

Aeromonas Strains

The *A.hydrophila, A.Caviae, A. Sobria* were kindly supplied by (first author)Dr. Zeinab Girh, Poultry Diseases Department National Research Center. These strains were maintained on nutrient agar slant by routine subculture of regular intervals. The strains were stored at 4°C and periodically tested for purity.

Media

Aeromonas ager medium (oxoid) was used for cultivation and propagation of Aeromonas species. The culture was incubated aerobically at 30C^o for 20-24 hours. The typical colonial appearance of Aeromanas species were selected and identified according to Krieg and Holt. (1984) and Havelaar et al. (1992).

Embryonated Chicken Eggs

Nighty six specific pathogen free (SPF)

embryonated chicken eggs (ECE) obtained from kom-Oshim, El – Fayom. Eggs were incubated in egg incubator at 37c° humidity 50 %. Fertile chicken eggs were used for study the pathogenic effect of A. hydrophila, A. caviae, A. sobria on the viability of the chicken embryo and its effect on hatched chicks.

Experiment Design

Aeromonas Strains,A.hydrophila, A. caviae and A. sobria were aerobically grow on Aeromanos agar medium (oxoide) at 30c° for 20-24hr. and resuspended in a concentration of (1.5×10^7) CFU/ML. SPF embryonated chicken eggs were devided into 2 equal groups 48 eggs of each,(groups A and B).

Group A was subdivided to 4 subgroups 12 eggs of each (A1-A4), inoculated via yolk sac at 5 days -old with 0.2ml of the bacterial culture of *A. Hydrophila, A. Caviae and A. Sobria* respectively and sub group A_4 was kept as negative control.

Group B was also Subdivided into 4 subgroup (B1-B4) each dipped in bacterial Culture containing 1.5×10^7/ml of *A. hydrophila, A. Caviae and A. Sobria* while B4 were kept as negative control. ECE were incubated and candled every 24 for recording mortality till hatch. Livers and yolks of the dead embryos from each group were subjected to reisolation of Aeromonas species. The hatched chicks from each group were killed. Livers and intestines were collected for reisolation of Aeromonas spp. and histopathological examination.

Histopathological examination:

Histopathological examination was carried out according to the method of (Shane and Gifford, 1985) Representative samples from livers and intestines of dead embryos were immersed and fixed in 10% formalin saline. These samples were dehydrated, cleared, embedded and cut to 7um then they were transferred to glass slides and stained with hematoxylin and eosin. Then they were examined by ordinary microscope.

3. Results:

Table (1): Mortalities of embryoneted chicken after inoculation with 1.5 x *10^7/ml of A. hydrophila,A. caviae,A.sobria,* via yolk sac route

Group Code	Aeromonas SP	No. of Inoculated Eggs	Days Post inoculation of Aeromonas													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A1	A.hydrophila	12	3D*	6D*	3 D	-	-	-	-	-	-	-	-	-	-	-	-
A2	A.caviae	12	7D	5D		-	-	-	-	-	-	-	-	-	-	-	-
A3	A.sobria	12	6D	6D		-	-	-	-	-	-	-	-	-	-	-	-
A4	Control	12	1D	1D		-	-	-	-	-	-	-	-	-	-	-	-

*D; Died embryo

Table (2): Mortality of embryoneted chicken eggs after dipping in media containing 1.5 x 10^7/ml of *Aeromonas (A.hydrophila,A.caviae and A.sobria)*

Group Code	Aeromonas Species	No of Eggs	Days Post Dipping in Aeromonas														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B1	A.hydrophila	12	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
B2	A.caviae	12	0	0	0	0	2	0	1	1	0	0	0	0	0	0	0
B3	A.sobria	12	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
B4	Not inoculated	12	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0

Table (3): Reisolation of *Aeromonas* species from liver and Yolk of dead embryos

Group Code	Aeromenas spp.	Total No of Eggs	No of Dead embryo	Reisolation of Aeromonas species from internal organ			
				Liver	%	Yolk	%
1A	A. hydrophila	12	12	10	83	11	91.6
2A	A. caviae	12	12	9	75	9	75
3A	A. sobria	12	12	6	50	8	66.6
4A	Control	12	0	0	0	0	0
1B	A. hydrophila	12	3	1	33.3	3	100
2B	A. caviae	12	4	1	25	2	50
3B	A. sobria	12	2	-	0	2	100
4B	Control	12	3	0	-	0	-

Table (4): Reisolation of *Aeromanos species* from liver and Yolk of hatched chicks hatchability Percentage

Group Code	Aeromenas Species	Total No ,of Eggs	No, of Hatched embryo	Hatchability %	Site of reisolation			
					Liver	%	Yolk	%
B1	*A.hydrophila*	12	9	75	4	44.4	6	66.6
B2	*A.caviae*	12	8	66.6	4	50	5	62.6
B3	*A.sobria*	12	10	83	1	10	3	30
B4	Not treated	12	9	75	0	-	0	-

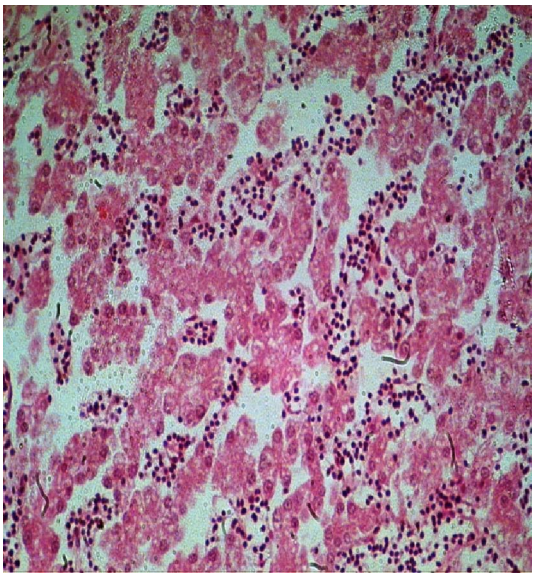

Fig (1): liver showing sever congestion in the hepatic blood vessels and sinosoids they were distended and encorged with blood.

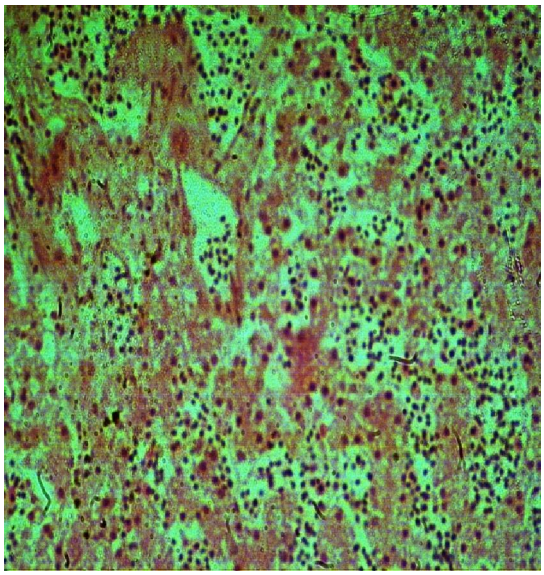

Fig. (2): liver showing focal areas of coagulative necrosis with disturbance in the arrangement of hepatocytes.

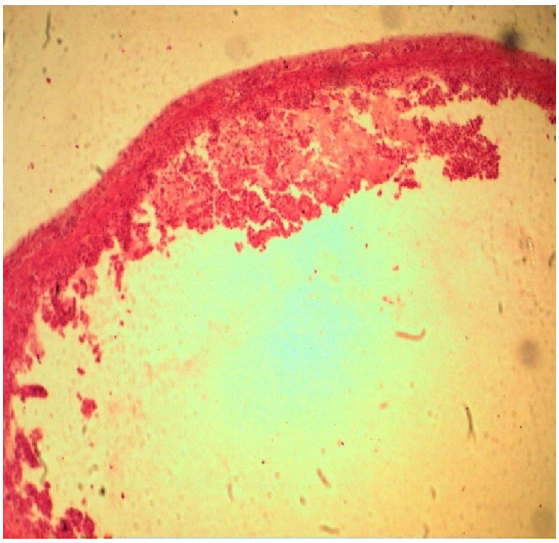

Fig (3): Intestine showing excessive Mucous secreation in intestine .

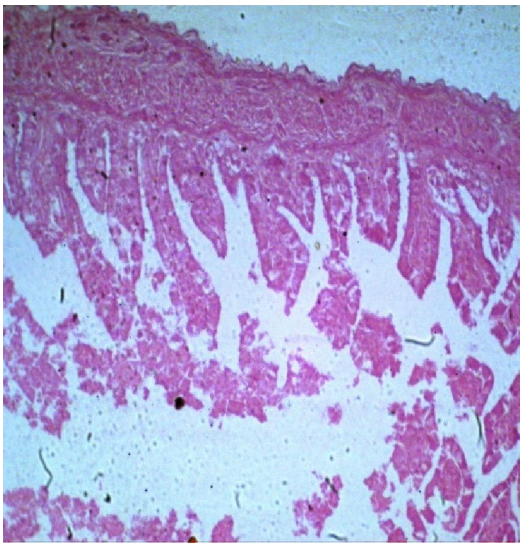

Fig (4): Intestine showing hyperplasia in intestinal epithelium with slight congestion of blood vessels

The embryonated chicken eggs treated with *Aeromonas species (A.hydrophila, A.Caviae, and A.Sobria)* either by inoculation via yolk sac route or dipping were greatly affected. In group A which inoculated via yolk sac route the embryo mortality was 100% (3 days post inoculation) while in group B which dipped in Aeromonas species the mortality rate ranged from 25%, 33.4% and 17% (11 days post dipping) for *Aeromonas A.hydrophila, A.caviae and A.sobria*, respectively.

Reisolation of Aeromonas from liver of dead embryos of group A inoculated by yolk sac route were 83%, 75% and 50% while from yolk results were 91.6%, 75%, 66.6% for *A. hydrophila, A.caviae and A.sobria,* respectively as shown in Table (3).

Group B which had dipped in media containing Aermonas spp. showed 33.3%, 25% and 0% for isolation of *A. hydrophila, A. caviae, A.sobria* from liver while isolation from yolk were 100%, 50, 100% for *A. hydrophila, A.caviae and A.sobria* respectively.

The hatchability of the inoculated ECE was 0%in group A while it ranged from 66.6 – 83% in group B *A. hydrophila* was reisolated from 44.4%, 66.6% from liver and yolk of hatched chicks respectively, *A. caviae* was reisolated from 50 and 62.6 from liver and yolk of hatched chicks. And *A. sobria* was reisolated from 10% and 30% of liver and yolk of hatched chick.

Histopathological examination of liver, intestine of hatched chicks revealed that changes in liver in form of sever congestion in the hepatic blood vessels and sinosoids they were distended and encorged with blood. There was focal areas of coagulative necrosis with disturbance in the arrangement of hepatocytes. The intestine, there was excessive Mucous secreation in intestine as a result of hyperactivity of mucous gland, and sever hyperplasia in the intestinal epithelium with slight congestion of blood vessels in the mucosa. Fig. (1-4).

Discussion

Aeromonas species has public heath significance . It's one of most important organisms which cause a food borne illness (Barnhart and Puncorbo 1992). *Aeromonas hydrophila* cause localized and systemic infections in avian species (Glunder and Siegmann 1989, and Shane and Gifford 1985).

This study aimed to study the effect of *Aeromanas* species: *(A.hydrophila, A.caviae, A.sobria)* on the viability and hatchability of embryonated chicken eggs as well as its pathological effect on hatched chicks.

Results showed that, *A. hydrophila, A.caviae and A.Sobria,* kill the chicken embryos. There was a correlation between the route of infection and mortality rate. inoculation via Yolk sac revealed mortality 100% of the embryos chicken eggs within 3 days post inoculation in group A. . The dipping of ECE in media containing(*A.hydrophila, A.caviae and A.sobria)* revealed mortalities 25%, 33.4% and 17% respectively after 11days post dipping In group B(Tables 1 and 2).

Aeromonas species were reisolated from liver and yolk of dead embryos in both groups A were 83%, 75% and 50% and from yolk were 91.6, 75, 66.6 for *A.hydrophila, A.caviae and A.sobria* respectively.

In group B which dipped in Aeromonas suspension the isolation percentage from liver were 33.3, 25 and 0 while from yolk were 100%, 50% and

100% for *A.hydrophila, A.Caviae and A.sobria*, receptivity.

The differences in mortality percentage and reisolation percentage from liver and yolk of dead embryos could be attributed to the method of infection with Aeromenas . It was found that the yolk sac inoculation route was more effective than the dipping method. Also, the reisolation the of Aeromonas from liver and yolk of group A which inoculated with yolk sac route were higher than group B which dipped in the Aeromonas species. These results aggreeded with the finding abtaned by (Kutkat et al., 2001) as they found that *A. hydrophila* can cause mortality in embryos ranged from 10-20% (after 6 days post inoculation) there was a correlation between the level of *A. hydraphila* and the mortality.

As the inoculation rate results in insert of high level of Aeromonas Inside the ECE & result in 100% mortality while dipping of ECE in the *Aeromonas* suspension results of eggs. From the previous results we can expect the effect of Aeromonas on ECE from infected females may be due to contamination of egg shell by dropping will be less than infection from the ovary.

Aeromonas speices effected on the percentage of hatchability of ECE as shown on Table (1 , 2) the inoculation of *Aeromanas* in the ECE cause mortality of 100% of the so the hatchability percentage was 0% while in the group B which dipped in Aeromonas suspension should hatchability percentages 75, 66 and 83 in *A. hydrophila A.caviae and A.sobria* respectively and these were no significant difference in between different group in hatchability.

The reduction in the hatchability mainly due to mortality of the weak embryos these results agreed with that abtained by (Yadav and Verma, 1998).

Reisolation of the Aeromonas from liver and yolk of the hatched chicks was 44.4% and 66.6% for A. hydrophila 50% and 62% in *A. caviae* and 10 and 30% in *A. sobria*. There was no difference in reisolation percentage from internal organs between A. hydrophila and A.caviae but there were high difference in reisolation percentage from liver and yolk in group dipped in *A. sobria*, in the other hand; the reisolation rate was lower then the other two groups these may be explained by the lower invasion of the of Aeromonas species to the embryo tissues.

Aeromonas strains may be produce Toxins which cause pathological changes in the liver and intestine of the hatched chicks, the liver change were in form of focal areas of coagulative necrosis with disturbance in the arrangement of hepatocytes. In intestine there was excessive mucous secretion and sloughing of the epithelium tissues of intestine with destructed villi.

Results of the present study indicated that, Aeromonas strains A.*hydrophila, A.caviae, A.sobria* were highly pathogenic for chicken and causing embryo mortalities and decrease of the hatchability.

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