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Effect of Cronobactersakazakii Experimental Infection on Layingand Hatchary Parameters of Breeder Chickens

Amer, M.M.[1*]; Manl A. Ali[1]; Hamouda, A.S[1] and Elmarakby, E.S.I[2]

[1]*Dept. of Poult. Dis, Facul. Vet. Med, Cairo University, P.O. 12211, Giza, Egypt*

[2]*Vet. Service Division, Ministry of Defense*

***Corresponding Authors:** Amer, M.M, Dept. of Poult. Dis., Facul. Vet. Med ., Cairo University, Giza, Egypt. Email: profdramer@yahoo.com*

ABSTRACT

Studies on the cycle of Cronobactersakazakii (C. sakazakii) in infected hens; the organism could recovered from outer shell of laid fresh eggs from thoracic air sac and intra croup 18.7% and 12% in 1st 15 days post infection. Incubated eggs showed hatchability was 72% and 73.3% as well as mortality in obtained chicks was 5.6% and 7.3%; respectively. C.sakazakii was isolated from liver, intestine, kidney, spleen, ovary and oviduct of 50% of air sac infected and 37.5% in intra-crop. C. sakazakii was reported to be reisolated from outer shell of fertile eggs of layers; fertility and hatchability were affected.

In conclusion; C. sakazakii was reported to be of value and affect breeder reproductively parameters. Our finding proved that C. sakazakii infection must be seriously taken in consideration especially in breeder flocks and needs more investigation.

Keywords: *C. sakazakii, breeder chickens, fertility - hatchability. Experimental infection.*

INTRODUCTION

Cronobacter spp. belongs to the family Enterobacteriaceae, a motile (peritricha) Gram-negative non-spore forming bacterium; at present, *E. sakazakii* is reported as a Cronobacter spp. species with 16 bio groups (Iversen et al., 2008, Masood et al., 2013).Recently, a taxonomic reclassification of this pathogen to consist of 5 species within a new genus "*Cronobacter*" was proposed (Baumgartner et al., 2009).

E.sakazakii was reported to contaminate fertilized eggs and may result in weak chicks, poor chick growth and low FCR (Ramnoff, 1960), increased mortality of embryos, lower hatchability and increased early chick mortality (Milakovic-Novak and Prukner , 1990, Amer et al., 2015). The organisms was also reported on the eggshell surface, cloacal swabs, commercial eggs and fertilized eggs(Al-Bahry et al., 2010,Asma-AbdEllatif, 2013,Elmmarakeby 2014).Abd El-Galil et al. (1995) studied bacterial causes of lowering hatchability and early embryonic chicken deaths in balady hatcheries. *Enterobacter sp*. isolates from non-fertile and dead in shell embryos (7.5 and 5.5%); respectively. *Enterobacter* was isolated from eggs and eggs shell of cracked and un-cracked eggs (Edema and Atayese. 2006).

Praxedes et al. (2012) identified Enterobacteriaceae of the broiler intestinal microbiota submitted from the 15th to the 23th day of life, while Amer et al. (2015) reported the pathogenicity of *E.Sakazakii*to 1-day old SPF chicks.

This study was carried out to investigate effect of experimental infection of layer breeder native chickens with *C.sakazakii* on layer and hatchery parameters.

MATERIAL AND METHODS

E.Sakazakiis Train

C.sakazakii strain isolated and identified by El-marakby (2014) was used for Experimental infection.

Experimental Chickens

Forty eight hens and 6 cocks were obtained from commercial native breeder farm at the age of 18 weeks. Cloacal swabs were taken and examined to detect *C.sakazakii* infection which proved negative.

Culture Techniques

Reisolation of *C.sakazakii* from experimental samples was adopted as recommended by FAO/WHO (2008) enrichment of samples using enrichment broth, incubated at 37 c for 24 hr. A loopful was inoculated into violet red bile agar plates and incubated overnight at 36 ^{o}C, colonies were streaked onto TSA and incubated at 25 c for 48-72 hr. only yellow pigmented colonies were selected and confirmed as *C. sakazakii* by Oxidase test and API20E.

Experimental Infection

The 24 h broth culture was adjusted to 0.5 MacFarland standards. Each chicken and cock was infected with 1 ml of 24 hours *C.sakazakii* broth culture contains 1.5×10^{8}cfu/ml. Birds of group 2 were infected via with 1 ml of 24 hr. *C.sakazakii* broth culture contains 1.5×10^{8}cfu/ml intra-crop or left posterior thoracic air sac.

Experimental Design

The chickens were divided equally into 3 groups; 16 hens and 2cock each. Each was kept in a separate pen till 70% egg production at 32 weeks old. Birds of group 1 were inoculated intra-crop, group 2 was injected in left posterior thoracic air sac and those of group 3 were kept as non infected control.

The laid eggs during 4 weeks after infection were collected separately in sterile container. Half of the obtained eggs/group was daily subjected to bacteriological examination and the other half was marked and grouped and incubated to detect fertility, hatchability and 1 day old chicks. Hatched chicks were kept under observation for 10 days for signs and mortalities. Unfertile eggs, dead embryos and dead chicks were subjected to bacteriological examination. At the end of the 4thweek post infection all hens were sacrificed and exposed to post-mortem examination and samples from liver, spleen, intestine, oviduct and ovary were collected for bacteriological examination. The results obtained are shown in tables (1-4).

RESULTS

In thoracic air sac infected group (1st-15 days post infection) *C.sakazakii* was reisolated from outer shell of 14 out of the 75 examined fresh eggs in rate of 18.7% (table 1), while In intra-croup infected group *C.sakazakii* was reisolated from 11 out of the 75 eggs in rate of 14.7% .Egg yolk of both groups was negative.

Table1. *Reisolation rate of C.sakazakii from examined fresh eggs of laying in air sac and intra-crop infected chickens (n=5 eggs/ day).*

+ve Days PI	Air sac infected chickens			Days	Intra-crop infected chickens		
	No of +ve	No of -ve	% of +ve reisolation		No of +ve	No of -ve	% of +ve reisolation
2	1	4	20	2	2	3	40
3	2	3	40	3	1	4	20
4	2	3	40	4- 5	-	5	0
5	2	3	40	6	1	4	20
8	2	3	40	7	1	4	20
9	1	4	20	8	2	3	40
10	1	4	20	9-12	-	5	0
11	-	5	0	13	1	4	20
12	3	2	60	14	1	4	20
13-15	-	5	0	15	2	3	40
	14	61	18.7		11	65	14.7

The obtained chicks from the incubated eggs 75 eggs are 54 chicks (72% hatchability), 55chicks (73.3% hatchability) and 60 chicks (90% hatchability) in air sac infected, intra-crop infected and control negative; respectively (Table 2). Reisolation of *E.sakazakii* from non fertile eggs and dead in shell embryos were 12.5% and 14.28% of air sac infected; respectively as well as 7.69% and 28.6% ; respectively of intra-crop infected (Table 2).

Table2. *Rate of C.sakazakiire isolation from non and dead in shell eggs as well as hatchery parameter of eggs of infected and non infected incubated of laying (n= 75 eggs/group).*

Group No.	Infection	Non fertile		Fertility	Dead in shell		No. of chicks	Hatcha-bility
		No	% of + ve reisolation		No	% of +ve reisolation		
1	Air sac	8	12.5	89.33	13	14.28	54	79.1
2	Intra-crop	7	28.6	90.66	13	7.69	55	80.8
3	Control	5	-	93.33	7	-	63	90

Table3. *Distribution and rate of C.sakazakiire isolation from dead hatched chicks.*

Group N.	Route of Infection	No of chicks	Age/days				Total	Rate of mortali ty	No of +vereis olation
			1	2	3	4-10			
1	Air sac	54	-	1	2	-	3	5.6	1
2	Intra-crop	55	1	2	1	-	4	7.3	1
3	Control -ve	63	-	-	-	-	-	-	-

Table4. *Reisolation of C.sakazakii from infected hen's organs.*

Group No.	Infection route	Case No.	Organs					
			Liver	Spleen	Intestine	Kidney	Ovary	Oviduct
1	Air sac	1	+	+	+	-	-	+
		2	+	-	+	-	+	+
		3	+	-	+	+	+	+
		4	+	+	+	-	-	+
		5-8	-					
2	intra-crop	1	+	-	+	-	+	+
		2	+	+	+	-	-	+
		3	+		+	-	-	-
		4-8	-					
3	non	1-8	-					

+ = positive *- = negative*

Reared hatched chicks from air sac and intra-crop infected hens showed death of 3 chicks (mortality 5.6%) and 4 chicks (mortality 7.3%); respectively; in the 1st 3 days of life (observation period) and *C. sakazakii* was isolated from 1 chick out of each (Table 3).

*C.sakazakii*was isolated from liver, intestine, ovary and oviduct of laying hens where 4 /8 (50%) are positive in air sac inoculation while in intra-crop infection 3 hens from 8 (37.5%) are positive (Table 4).

DISCUSSION

Study the cycle of *C..sakazakii* infection in chickens. 5 months old chickens were experimentally infected with *C.sakazakii* broth culture through the air sac or intra-crop and reared for egg production. Samples were taken for the reisolation of *C.sakazakii* from freshly laid eggs and from dead in shell embryos of eggs during 21 days incubation. The outer shell and egg yolk of freshly laid eggs were examined.

In thoracic air sac group (1st 15 days of laying) *C.sakazakii* was reisolated from 14 out of the 75 outer shell samples examined (18.7%), no organism could be found in the egg yolks of this group. Out of 75 eggs incubated for hatching (2nd 15 days of laying) 54 chickens were obtained(72%) from which 3 chicks died during the period of observation(5.6%) and *C.sakazakii* could be isolated from only 1 of them. Out of 21 dead in shell embryos 3 revealed *C.sakazakii* on bacteriological examination.

In intra-crop group (1st 15 days of laying) *C.sakazakii* was reisolated from 12 out of the 75 outer shell examined samples (12%) (Edema and Atayese, 2006; Musgrove et al. 2008, Amin and Abdel-Hameed, 2009).

No organism could be found in the egg yolks of this group. Out of 75 eggs incubated for hatching 55chickens were obtained (73.3%)

from which 4 chicks died during the period of observation (7.3%) and C.*sakazakii* could be isolated from only 1 of them. Out of 20 dead in shell embryos 3 revealed *C.sakazakii* on bacteriological examination. This result agrees with those of Ali (1993) who reported that *Enterobacter* are involved in the bacterial agents of yolk sac infection. Also, Ardrey et al. (1968***)*** reported that oral or into the air sac inoculation of pathogenic *E.coli* developmented the carrier hens. Timoney et al. (1989) showed that oral inoculation of laying hens with *S.entretidis* could produce infection of the reproductive. ***Cox et al. (2000)*** stated that oral challenge of 10^6 salmonella cells caused the ovary and oviduct to become infected.

C.sakazakii was reported to be re-isolated from outer shell of fertile eggs obtained from infected layers. Isolation of *E.sakazakii* from egg shell was reported (Edema and Atayese, 2006, Musgrove et al. 2008). Furthermore both fertility and hatchability were affected. AbdEl-Galil et al. (1995) studied bacterial causes of lowering hatchability and early embryonic chicken deaths and reported that *Enterobacter sp*. isolates from non-fertile and dead in shell embryos (7.5 and 5.5%); respectively.

Results obtained from this experiment indicate that laying chickens exposed to C.sakazakii infection, the infection passes to some of their eggs and affect hatchability. This result is suggestive the cycle of such infection.

It can be concluded that our finding about *C.sakazakii* needs more investigation especially under our field conditions.

Conflict of Interest

The authors have no conflict of interests to declare regarding the publication of this paper. Also, the authors declare that the work was self-funded.

Authors' Contributions

M.M.A. and M.A.A designed and planned this study. A.S.H and E.S.M.E performed experimental work, collects samples and all laboratory tests. All authors shared samples collection, performing the tests, manuscript writing, drafted, revised the manuscript and approved the final manuscript.

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