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STUDIES ON YOLK SAC INFECTION IN OSTRICH AND RELEVANT CONTROL TRIALS

(With 4 Tables and 2 Figures)

By

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دراسات على عدوى كيس المح في النعام وطرق السيطرة المناسبة

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أجريت هذه الدراسة الحقلية لمعرفة الأسباب البكتيرية لعدوى كيس المح في صغار النعام والإجراءات الوقائية لتقليل معدل حدوثها وذلك بتطهير بيضة النعام المعد للتحضين بواسطة استخدام ثلاث طرق للتطهير (محلول اليود المخفف بنسبة ٧% - التيك تيرول المطهر - التبخير باستخدام غاز الفورمالدهيد). وقد تم تجميع عدد ٢٥ جنين نعام نافق داخل البيض و ١٥ من صغار النعام النافق حديثاً بسبب عدم امتصاص كيس المح بالإضافة إلى عدد ٢٥ مسحة بكتيريولوجية من المستقيم لأمهات النعام البياض من ثلاث مزارع للنعام تشكو من ارتفاع معدل الإصابة بعدوى كيس المح. وقد تم إجراء الفحص الظاهري والتشريحي للأجنة وصغار النعام وكذلك الفحص البكتيريولوجي. وقد أظهرت لنتائج عزل ميكروب السالمونيلا والميكروب القولوني والسيدوموناس ايروجينوزا والكابسيلا وكذلك الميكروب العنقودى والبروتيس والانتيروكوكاس بنسب متفاوتة. وقد وجد من التصنيف السيرولوجى لعترات الميكروب القولوني المعزول إنها تنتمى سيرولوجيا إلى 01، 02، 055، 078 وأوضحت الدراسة انخفاض معدل الإصابة بعدوى كيس المح في مجموعات البيض المعالجة بمطهرات عن المجموعة الضابطة وإن غسيل البيض باستخدام محلول اليود بنسبة ٧% قبل تحضينه كان له تأثير ايجابي ملحوظ في انخفاض معدل الإصابة بعدوى كيس المح عن استخدام التيك تيرول أو بخار الفورمالدهيد.

SUMMARY

This study was intended to study the possible bacterial causes of yolk sac infection in ostrich chick under field conditions and the effect of egg treatments with different disinfectants (Iodine 7% - TH4+ -[Tek-trol] and formaldehyde fumigation) on the incidence of yolk sac infection in hatched chicks. A total of 25 dead in-shell, 15 freshly died 2 weeks old ostrich chicks and 25 cloacal swabs from breeder ostrich hens were

obtained from 3 ostrich farms suffered from increased incidence of yolk sac infection. The clinical signs, postmortem and bacteriological investigation, were carried out. The results of bacteriological examination revealed the isolation of *Salmonella spp.*, *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella spp.*, *Staphylococcus aureus*, *proteus spp.* and *enterococci* with variable percentage, isolated *E.coli* strains belonged to serotypes 01, 02, 055, 078 and untyped. The incidence of yolk sac infection was reduced in hatched chicks from treated groups than control one and washing the eggs with Iodine 7% pre-incubation could prevent incidence of yolk sac infection than Tek-trol (2/25) and formaldehyde fumigation (4/24).

Key words: *Yolk SAC, Ostrich.*

INTRODUCTION

It has been said that ostrich production and world-wide ostrich industry entering their third era. The first era revolved around feather and its demand on world market at the beginning of 20th century, the second revolved around skin and its demand world wide because of its durability and attractiveness, the third era seems to revolved around the demand for meat. Ostrich meat is low in cholesterol as well as tasty, for this reason there is a high demand (Hallam, 1992).

As ostrich production become commercialized and moved toward marketing livestock producer pay attention the management program and control of infectious diseases in this species. Yolk sac constitute about 20% of the initial egg mass and some time 30-40% of the body mass and the time of absorption ranged from 7-10 days post hatching when most chicks had used all of the yolk with absence of infection (Deeming, 1995a).

Yolk sac infection, omphilitis and retention of yolk sac were all different aspects of the same entity and was a common cause of mortality in ostrich chicks, with most birds dying within 14-21 days of hatching (Deeming *et al.*, 1996), (Huchzermeyer, 1998).

Many factors involved in this condition including poor hygiene during egg handling and incubation which allow bacterial pathogens to penetrate through the egg shell to vittelo-intestinal duct, into yolk sac, then the duct inflamed, closed up and preventing the extrusion of the yolk into the intestine (Deeming, 1997).

The aim of this work is to study the possible bacterial causes of yolk sac infection in ostrich under field conditions and the hygienic

measures that should be taken to prevent yolk sac infection in ostrich chicks.

MATERIAL and METHODS

[1] Birds and sampling:

Twenty-five fertile unhatched ostrich eggs (dead in shell) and fifteen freshly dead ostrich chicks of 2 weeks of age suffered from yolk sac infection. Besides twenty-five cloacal swabs from breeder ostrich hens were obtained from 3 ostrich farms in Ismailia governorate. The postmortem findings were carried out for both ostrich's embryo and chicks as well as clinical science in the later bacteriological isolation was done from yolk sac contents and liver in case of dead in-shell embryos, while from liver, heart, spleen, intestine and yolk sac from freshly dead ostrich chick and from cloacal swabs of breeder hens.

[2] Bacteriological examination:

Samples were cultured on Nutrient, Rapport and Selenite F broth (oxoid), incubated at 37°C for 24hrs followed by sub-culturing on MacConkey agar, S.S agar blood agar, EMB agar and nutrient agar (oxoid). Suspected colonies were subjected to morphological and biochemical identification according to Quinn *et al.* (1994 and 2002) isolated *E.coli* strains were serologically serotyped according to Edward and Ewing (1972).

[3] Field treatment:

This field trial was carried out to determine the effect of egg treatment with different disinfectants in order to minimize incidence of yolk sac infection in hatched ostrich chicks.

A) Birds:

One flock of apparently healthy ostrich was used in this experiment, reared under extensive system of housing (28 birds/4Feddan) with sex ratio of 1 male / 2 females.

The flock fed upon balanced ration contained 16% protein, the eggs were collected once daily, then stored for one week in temperature of 18°C and humidity ranged 60-70% with broad end up.

B) Hatchery used (Prohatch):

South African ostrich incubation system was followed. This incubator is specialized for ostrich. The temperature was 37.7°C and humidity was 23-28% in setter while in hatchery the temperature was 35.7-36.3°C and humidity was 28-32%. Turning of eggs was carried out

each hour and candling of eggs was carried out in 14, 21, 28 and 35 days of incubation. The incubator supplied with ultraviolet lamp.

C) Disinfection of hatching eggs:

This was done according to Button (1996). A total of 200 ostrich eggs were divided into five groups and used in this experiment as the following:

Group 1: Hatched eggs were washed with TH4+ (sogeval laboratories – laval –france).

Group 2: Hatched eggs were washed with iodine 7% (Betadine).

Group 3: Hatched eggs were subjected to formaldehyde fumigation before setting in incubator by chemical reaction of 40 ml formaline 40% and 20 g pot. permanganate per 2.83 m^3.

Group 4: Hatched eggs were sprayed with Tek-trol 10% (Bio-tek industry- USA).

Group 5: Control untreated Hatched eggs. All hatched chicks were kept under observation until reach one month old, clinical signs and mortality rate were recorded to indicate yolk sac infected chicks.

RESULTS

The clinical signs of ostrich chicks with yolk sac infection were observed 10-14 days after hatching. Chicks showed anorexia, loss of weight again, depression, and abdominal enlargement, isolated from other chicks, hold their head down or against their body. The abdominal palpation of such chicks revealed presence of abdominal mass, while the embryos failed to hatch and found dead in their shell (sticky chick).

The P.M examination of ostrich chick was hepatomegaly, splenomegaly and generalized venous congestion with unabsorped yolk sac of viscous content, watery yellow brown with caseous materials. The navel inflamed with presence of pea size abscess as shown in Fig. (1). The P.M examination of dead in shell showed distended abdomen and adherence of embryos to inner eggshell surface (sticky chick), the yolk sac content was viscous, turbid and of unpleasant smell. The navel area inflamed with general congestion of the carcasses and yolk sac as shown Fig. (2).

The result of beateriological examination and field treatment were recorded in Tables 1, 2, 3 and 4.

Table 1: The bacterial pathogens isolated from both ostrich chicks and dead in-shell embryos with infected yolk sac.

Type of isolated bacteria	Ostrich chick		Ostrich embryo	
	Freq.	%	Freq.	%
Salmonella spp.	6/15*	40	5/25	20
E. coli	13/15	86.67	25/25	100
Pseudomonas aeruginosa	6/15	40	19/25	76
Klebsiella spp.	9/15	60	18/25	72
Staph. aureus	3/15	20	7/25	28
Proteus spp.	12/15	80	22/25	88

(*) Number per total

Table 2: The member of bacterial pathogens isolated from cloacal swabs of breeder ostrich hens.

Type of isolated bacteria	Frequency of isolation	%
Salmonella spp.	8/25	32
E. coli	20/25	80
Klebsiella spp.	9/25	36
Proteus spp.	14/25	56
Enterococci	15/25	60

Table 3: Serotyping of *E.coli* isolated from ostrich chicks, embryos and cloacal swabs of ostrich hens.

E. coli	Ostrich chicks		Ostrich embryos		Cloacal swabs	
Serotypes	Freq.	%	Freq.	%	Freq.	%
01	2/13	15.38	3/25	12	1/20	5
02	2/13	15.38	7/25	28	3/20	15
055	2/13	15.38	4/25	16	2/20	10
078	4/13	30.77	9/25	36	10/20	50
Untyped	3/13	23	2/25	8	4/20	20

Table 4: The effect of egg treatment on the incidence of yolk sac infection in ostrich chicks.

Groups	Disinfectant used	No. of incubate eggs	No. of Fertile eggs	No. of hatched chicks	No. of live chicks up to 1 month post hatching	No. of affected chicks with yolk sac (dead)*
1	**Iodine 7%**	40	29	25	25	--
2	**TH4+**	40	28	24	21	3/24
3	**Tek-trol spray 10%**	40	28	25	23	2/25
4	**Formaldehyde fumigation**	40	27	24	20	4/24
5	**Control (untreated)**	40	27	24	19	5/24

(*) Affected chicks with yolk sac died 7-12 day, post-hatching.

DISCUSSION

The clinical signs of affected ostrich chick with yolk sac infection were anorexia, depression, loss of weight gain and abdominal enlargement while the ostrich embryo failed to hatch and found dead in-shell. The P.M examination of ostrich chicks revealed the presence of hepato-splenomegaly, and generalized congestion. The yolk sac contents were viscous, watery yellow brown with caseous material, navel inflamed and sometimes presence of pea size abscess. While P.M examination of ostrich embryo revealed the adherence of the embryo to the inner shell membrane (sticky chick), yolk sac inflamed, viscous content, unpleasant smell and generalized congestion of the carcass, these observations and P.M findings agree with that reported by (Blue-Mclendon and Homco, 1995; Deeming, 1995a; Deeming *et al.*, 1996 and Huchzermeyer, 1998).

Deeming, (1995c) mentioned that few days before hatching the whole yolk sac was drawn into the abdominal cavity and the navel began to close over the yolk sac if the infection not recorded.

Yolk sac of the embryo is the focus of infection and many embryos die before hatching particularly late in incubation and some dies shortly after hatching with losses up to 3 weeks (Tully and Shane,

1998). Concerning the bacteriological examination, revealed *Salmonella spp.* (40%, 20%), *E.coli* (86.67%, 100%), *P. aeruginosa* (40%, 76%), *Klebsiella spp.* (60%, 72%), *Staph. aureus* (20%, 28%) and *Proteus spp.* (80%, 88%) were isolated from the affected ostrich chicks and embryos respectively. Similar findings were reported by Shivaprasad, (1993); Deeming, (1995a) and Welsh *et al.* (1997). They reported that a great variety of organisms such as *Salmonella spp. E. coli, Pseudomonas spp., Klebsiella spp., Proteus spp., Bacillus spp., Enterococci* and *Staphylococcus spp.* were frequently isolated (often a mixture cultures) from yolk sac infected chicks and embryos. Also (Grilli *et al.*, 1996) reported that out of 38 ostrich chicks which died before 20 days of age, 30 cases of yolk sac infection were recorded. The isolated organisms from these yolk sac including *Escherichia coli, Pseudomonas spp., Staphylococcus aureus, Proteus spp* and *Streptococcus.*

Ratites including ostrich, emus and rheas have increased in infection with *Salmonella spp.* over the last 10 years (Dempsey *et al.*, 1997).

Cloacal swabs from breeding hens indicated the isolation of *Salmonella spp., E. coli; Klebsiella, Proteus spp.* and *Enterococci.* These results coincided with that reported by Foggin, (1992); Phalen, (1995) and Kamel *et al,* (2000), where faecal contamination of the eggs is considered to be the most important source of infections, other sources may be through the vertical transmission (Husein *et al.*, 2002).

The incubated ostrich eggs showed a higher incidence of microbial contamination with 45% of fertile and 67% of dead in-shell. This may be attributed to their infection with varieties of faecal and soil bacteria (Foggin and Honywill, 1992., Button *et al.* 1994). Also, Brown *et al.* (1996) and Deeming, (1996) reported that near-term embryonic mortality of ostrich eggs, bacterial contamination was observed in all examined eggs and the organisms isolated were typical of soil and faecal origin.

Concerning the serological serotyping of *E. coli* isolated from chicks, embryos and breeder hens, the results indicated that they belong to 01, 02, 055, and 078 and they were of a common serotypes of poultry species. This result is supported by Huchzermeyer (1998) who reported that all *E. coli* serotypes isolates of ostrich were corresponding to poultry pathogens.

The infection of yolk sac can take place via different routes, contamination through the shell (shell contamination route), umbilical infection at hatch and general infection. Hence some embryos suffering

from contamination through the shell will succumb to generalized infection and die before hatching Huchzermeyer (1998) and Deeming, (1999).

The sanitation programmes for ostrich eggs are variably based on procedures employed for poultry eggs and vary from egg washing through to simply brushing off soil Deeming (1997), also Deeming (1995b) declared that the problems associated with microbial infection of eggs can only be solved by the preventive measures.

Our results of field treatment for prevention and minimizing the yolk sac infection as shown in Table (4) indicated that there was a reduction in incidence of yolk sac infection in hatched chicks in treated groups with different disinfectants than non treated one (control). This observation was also recommended by Button *et al.* (1994) who reported that lower infection rate and higher hatchability rate were obtained in farms where the egg sanitized than on farms with no sanitation practiced. Accordingly the prevention of the infection was a better course of action Deeming, (1995a); Dunn (1995); Wade (1995) and Speer (1996). Moreover, to prevent the yolk sac infection, strictest incubator hygiene is necessary, disaffection of the eggs after collection and/or before setting and nest hygiene (Huchzermeyer, 1997).

Also the results indicated that washing of eggs, with 7% iodine solution gave the best result than other disinfectants and minimized incidence of yolk sac infection in hatched chicks. Such result agrees to some extent with that reported by Hallam, (1992), However Stewart (1996) mentioned that formaldehyde gas fumigation was extremely irritant for human and respiratory tract of the hatched chicks so, it is not recommended. Also Badley (1997), founded that acidic disinfectant will be inactivated in the shell pores by the buffering action of calcium carbonate of the shell. In contrast Tully and Shane, (1996) considered that, washing of eggs will remove cuticle and allow bacterial penetration through the shell and recommended dry cleaning of eggs by non abrasive material.

It can be concluded that yolk sac infection is a significant cause of early mortality in ostrich chicks with most bird dying within 14-21 days of hatching as a matter of fact are incriminated many factors, in such infection including poor hygiene during egg handling and incubation, but the bacterial contamination by (*Salmonella spp., E. coli, Pseudomonas aeruginosa, Klebsiella spp., Staphylococcus, proteus*....) had the upper hand in such infection either from breeder hens or through the faecal contamination during oviposition or handling (shell

contamination). Accordingly egg handling and disinfection are the key factors in prevention of yolk sac infection in young ostrich. Anyhow to minimize the infection of eggs and chicks, keep the nest clean and dry as possible. On the other hand, eggs should be collected as soon as possible after laying and cleaned, washed using warmed water with a disinfectant solution. Such eggs should be stored for short time and at hatching the navel of each chick should be disinfected.

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Fig (1): Two weeks old ostrich chick shows inflamed navel.

Fig (2): Yolk sac infection in ostrich embryo.