قسم : أمـــراض الـد واجـــــن .
كليـة : الطب البيطرى ـ جامعة أسيوط .
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الدور الذى تلعبه الاصابه بالميكروبـــــات في نسبة تفريخ بيض البـــط

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- سجل تد هور في نسبة التفريخ (٣٢٪) في بيض البط في الفترة من سبتمبر الى د يسمبر ١٩٨١م في مزارع الواد ى الجد يد .
- الفحص الميكروبيولوجي أد ى الى عزل الميكروب القولوني (٣٦) ، كلبســيلا (٣٢) وبروتيوس (٢٩) سود موناس (٢٧) الميكروب السبحي (١٥) الميكروب العنقود ى (٩) الا سبرجلس (١٤) والكند يد ا البكانس (٨) .
- الكلبسيلا والبروتيوس والـميكروب السبحي والسود ومناس والميكـــــــروب العنقود ى والا سبرجلس والكند يد ا البكانس تم عزلها من المفقس وارضيات العنابـر .
- العد وى الصناعية فيأجنة بط عمر ٧ أيام باستخد ام أكثر الميكروبـــــات انتشارا أد ى الى انخفاض كبير في نسـب الفقـس.
- تمت التوصية باتباع بعض الا رشاد ات الصحية لتحسين الفقس.

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THE ROLE PLAYED BY MICROBIAL INFECTIONS ON HATCHABILITY RATE OF DUCK - EMBRYOS

(With 5 Tables)

By
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SUMMARY

Low hatchability rate (32%) was recorded among the duck-eggs during the period from September to December (1981), at El-Wadi El-Gadid. Duck Farms.

Microbiological examinations of unhatched eggs revealed the recovery of E.coli (36), Klebsiella species (32), Proteus spp. (29), Pseudomonas spp. (27), streptococci (15), Staphylococci (9), Aspergillus spp. (14) and Candida albicans (8). Klebsiella, proteus, Strept. faecalis, Pseudomonas, Staph. aureus, Aspergillus species and Candida albicans were isolated from Hatcheries and Litters.

Experimental infections of 7-days old duck-embryos with the most common isolates showed drastic drop of hatchability rate. Sanitary measures were recomended to improve the hatchability percentage.

INTRODUCTION

Bacterial entery into the egg-shell has been a subject of considerable interst for a number of years. Diseases control measures start in the farm to minimize the risk of contamintion from infected eggs.

Drastic drop in hatchability rate (32%) was recorded during the period from September to December (1981) among the breeding duck flocks at, El-Wadi El-Gadid Duck Farms, in comparison with a considerably good rate (68%) in the corresponding period of the previous season. The flocks had history of being tested against paratyphoid infections.

JUNGHER (1935) reported that hatchability losses was attributed to non-specific bacterial infections. DHAWEDKAR and DHANESAR (1960) isolated Streptococci, Staphylococci, Microcecci, Aerobacter, Proteus, Salmonella, Pasteurella, Corynebacterium and Aspergillus niger from dead in shell embryos. REID, et al. (1961) demonstrated that shell penetration by a field isolated strain of E.coli can markedly reduce hatchability. REFAI (19717 recovered A.flavus, A.niger, Mucor, Fusarium and Stemphylium from dead embryos. Pathogencity tests in 10-day-old embryonated eggs revealed that A.flavus and Fusarium were most pathogenic.

Bacterial examination of unhatched chicken eggs at Assiut Province was studied by NASHED (1981) who detected the following organisms : Enterobacteriaceae (38.2%), Micrococci (33.3%), Streptococci (12.2%), Pseudomenas (11.1%) and Anthrcoid (5.2%), out of 500 examined eggs.

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The present work was planned to fullfill the following points :-

- Detection of the microbial agents which may be responsible for hatchery losses from unhatched eggs. hatcheries and litters.
- Pathogenicity tests of the most common isolates to susceptible fertile eggs.
- Recomendations for improvment of hatchability rate.

MATERIALS and METHODS

Bacteriological examinations of unhatched eggs :-

560 dead duck embryos of different ages, were collected cleaned and sterilized externally to get rid of faecal contamination. About one ml. of yolk was taken by sterile pipette, inoculated in 10 ml. Selenite F. broth, incubated for 16 hours, followed by subculturing on S.S. agar media, incubated at 37 °C for 24 - 48 hours.

Loopfuls from yolk were directly streaked on MacConkey agar, Cetrimid-agar, Blood-azide-agar and Mannitol-salt-agar plates which were incubated at 37 °C for 24 - 48 hours and suspected colonies were picked up for purification and further identification according to EDWARDS and EWING (1972).

Mycological examinations of unhatched eggs :-

Loopfuls from he internal surfaces of the egg-shell were streaked on Sabauroud-maltose-agar medium " containing 0.5 mg. of Chloramphenicol/ml. ". The plates were incubated at 25 °C for 5 days before being examined and suspected colonies were subjected for further identifications according to AJELLO, et al. (1963) for isolated moulds and ROSE and HARRISON (1971) for isolated yeasts.

Microbiological examinations of hatchery fluffs and dust :-

Hatchery fluffs and dust were collected into sterile plastic bags using sterile wooden tongue blade. One table spoonful was inoculated into 100 ml. of both nutrient and enriched broth incubated for 24 hours at 37 °C followed by subculturing on the previously mentioned solid media. incubated for 24 - 48 hours at 37 °C. Further identifications were conducted as described before.

For mycological examinations the samples were inoculated into Sabauroud-dextrose-broth, incubated at 25 °C for 48 hours followed by subculturing on Sabauroud-agar.

Microbiological examinations of litters :-

Samples of 2 - 3 gm. fine, dry floor litter from the upper 1 - 2 inches of litter were collected on a sterile plastic bags. The same procedures of microbiological examinations of hatchery fluffs and dust were carried out with litter samples.

Pathogenicity tests :-

300 embryonated duck-aggs of 5 - 7 days-old supplied from Assiut Agriculture College Farm were used in this study. The embryos were devided into six equal groups each of (50), the first five groups were dipped in 24 hours broth cultures of different bacterial isolates (table V) containing 14 x 10 viable cells/ml. for 4 minutes, while the last group of embryos were dipped in sterile broth " REID, et al. (1961) ".

Daily candling of the incubated embryos was carried out, deaths were recorded and reisolations of the inoculated organisms were conducted.

RESULTS

The results of microbial isolations from examined eggs, hatcheries and litters were illustrated in Tables I - V.

DISCUSSION

Hatchability is the most essential measure for the reproductive efficiency of domestic birds depending on many factors. Microbial contamination appeared to be one of them having tremendous effect on the survival of embryos and final hatchability rate.

The bacteriological examinations of 560 unhatched and dead in-shell egges collected from duck farms suffering from low hatchability rate (32%) revealed the detection of the following organisms : E.coli (36), Klebsiella (32), Proteus (29), Pseudomonas (27), Streptococcus (15) and Staphylococcus (9), while 412 eggs were negative for such isolates. Almost the same organisms were previously isolated by DHAWEDKAR and DHANESAR (1960), VENUGOPALAN, et al. (1974), KARIM and ALI (1976), FALADE (1977), ROKIA (1980), NASHED (1981) and Kamel (1982).

Salmonellae isolated from duck-eggs which were considered to be responsible for high hatchery lesses by CHOWDHURY, et al. (1976), EL-SAWY (1976), and ROKIA (1980) were not recoered by the authors in the present study. This may be attributed to the strict measures conducted to eradicate paratyphoid-carrier birds by annual testing of the flocks and for the use of a prophylactic therapeutic programe to the newly hatched ducklings.

Concerning the mycological examinations of unhatched eggs, 14 isolates were identified to belong to different Aspergillus species and 8 isolates to Candida albicans. Our results agreed with those of REFAI (1971) and SAMBYAL, et al. (1981).

Klebsiella, Ps.pyocyanea, Pr.vulgaris. Staph.aureus, Strept. faecalis, Candida albicans, A.niger, A.flavus and A. fumigatus were isolated from hatcheries and litter samples. Our results were closely resembling those of PLESSER, et al. (1975) who recovered Klebsiella from hatcheries in which the eggs failed to hatch. BRUCE and JOHNSON (1978) who isolated members of Enterobacteriaceae, Micrococci, Streptococci, Staphylococci and Pseudomonas from hatcheries contained unhatched-eggs and SAMBYAL, et al. (1981) who found that A.funigatus isolated from both dead eggs and hatcheries was responsible for hatchery losses in two farms.

Experimental infections of embryonated eggs 5 - 7 days-old using the most common bacterial isolates showed that all the tested organisms were pathogenic and had a drastic effect on hatchability of infected embryos ranged from 44% to 12% in comparison wih the control group (76%). Some-what similar results were recorded by KAMEL (1982) who founed that the hatchability rates of Proteus and E.coli infected eggs were 14 & 16% repectively.

The present study indicated that the low hatchability rate of duck embryos was mainly attributed to microbial contaminations. Detection of microorganisms from both hatcheries and litters draw the attention towards these sites which may be the source of infections.

Periodical egg collection, pre-incubation fumigation, early and late-funigation of incubated eggs in addition to cleaning and disinfections of incubators, hatcheries and equipments may be of value in solving such problem.

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TABLE (I): Showing different species of bacterial isolates.

Sr. No.	Bacterile species	Frequency	
		No.	%
1	E.coli	36	24.3
2	Klebsiella spp.	32	21.6
3	Proteus sop.	29	19.5
4	Pseudomonas spp.	27	18.2
5	Strept. faecalis	15	10.1
6	Staph. aureus	9	6.8
		148	

TABLE (II): Showing different bacterial serotypes isolated from unhatched eggs.

Sr. No.	Isolated serotypes	Frequency
1	E.coli 0128 : K67 (B12)	28
2	E.coli 0126 : K71 (B16)	8
3	Proteus vulgaris	18
4	Proteus mirablis	8
5	Proteus rettgeri	6
6	Pseudomonas pyocyanea	15
7	Pseudomonas flurescens	12
8	Streptococcus faecalis	15
9	Staphylococcus aureus	9

TABLE (III): Showing different fungal species isolated form unhatched eggs.

Sr. No.	Fungal species	Frequency No.	Frequency %
1	Candida albicans	8	36.4
2	Aspergillus niger	6	27.2
3	Aspergillus fumigatus	5	22.7
4	Aspergillus flavus	3	13.6

TABLE (IV): Illustrated microbial isolations from hatcheries and litters.

Examined samples	I solated organisms
Hatchery-dust and fluffs	Candida albicans, A.niger, A.fumigatus, A.flavus, Staph.aureus, Klebsiella, Ps. pyocyanea and Pr.vulgaris.
Litters	Strept.faecalis, Klebsiella, Ps.pyocyanea, Pr.vulgaris, A.niger, and A.flavus.

MICROBIAL INFECTIONS & HATCHABILITY

TABLE (V)

Showing results of microbial experimental infections

Inoculated organisms	No.of embryos	Daily deaths of embryos (post-infection)												Piped dead in shell	Total deaths No.	Total deaths %	Frequency of hatchability No.	Frequency of hatchability %
		2	5	6	8	9	11	12	13	15	16	18	19					
E.coli 0128:K67(B12)	50		1		2		6		1	1	4			29	44	88	6	12
Pr.vulgaris	50			1			4			2		3		32	42	84	8	16
Klebsiella spp.	50	1			2	3				2	3		1	28	40	80	10	20
Ps.pyocyanea	50		3	1	5	3	2	1		1		3		20	39	78	11	22
Ps.fluorescens	50			4		2	3	1				2		16	28	56	22	44
Sterile-broth	50	1		1		1		2				1		6	12	24	38	76