

Studies on *Pseudomonas Aeruginosa*, *Proteus Vulgaris* and *S. Typhi-Murium* Infection in Ducklings

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ONE DAY to 2 weeks old ailing duckling and duck eggs were examined bacteriologically for the isolation of pseudomonas, proteus and salmonella species.

Pseudomonas aeruginosa, *Proteus vulgaris* and *Salmonella* species were isolated in the ratio of 15 %, 10% and 9% from 400 ailing ducklings and of 11 %, 8.5 % and 12 % from 200 duck eggs respectively.

Experimentally, *Ps. aeruginosa*, *Pr. vulgaris* and *S. typhi-murium* were infected orally and subcutaneously in one day old ducklings. *Ps. aeruginosa* and *S. typhi-murium* were pathogenic, the ratio of mortality was 60 % and 65 % (oral) and 75 % and 80 % (subcutaneous) infection respectively. *Pr. vulgaris* do not show any sign of illness and no death occur.

The importance of ducks and duck eggs can not be ignored as one of the important sources of animal protein for humanbeings.

The significance of Pseudomanasiasia in poultry has been already mentioned by many investigators (Goldsby and Eveleth, 1950; Rossi, 1955; Fontaneli, 1958; Valadao, 1961; Williams and Neukrky, 1966 Awaad *et al.*, 1980 and Saad *et al.*, 1981). Contrary to that, few literatures have dealt with proteus infection in birds.

Accordingly this work was designed to investigate :

1. Incidence and types of microorganisms in duck eggs.
2. Incidence and types of infection in ducklings.

3. Pathological role played by *Ps aeruginosa* and *Pr. vulgaris* isolated from ducklings in the infection process as compared with *S. typhi-murium*.

Material and Methods

Specimens

Four hundred (one day-old to 2 weeks-old) ailing ducklings, and 200 infertile duck eggs were collected from the General Poultry Company as well as private farms during the years 1980-1982 were used.

Bacteriological examination

Egg-shells were scrubbed with a stiff brush and warm water then rinsed well and drained . The eggs were then immersed in ethyl alcohol 70% for 5 min, after which they were allowed to drain before being flamed quickly.

Samples from heart blood, liver, spleen, gail-bladder, intensine and yolk sacs as well as heavy inoculum from the whole mixed egg contents were examined bacteriologically by inoculating of selenite F broth and incubated at 43° for 18 hr, then subcultured on blood agar, nutrient agar, S-S agar and MacConkey agar media, and incubated at the same temperature for further 24 hr. Suspected colonies were microscopically examined then isolated in a pure culture for biochemical and serological identifications after Edwards and Ewing (1972).

Experimental Work

One hundred and fifty, one day-old Pekin ducklings were divided into 7 groups consisting of 20 each (groups 1-7).

Before infection, random samples of 10 ducklings were subjected to post-mortem and bacteriological examination which proved to be negative for salmonella infection.

The groups 1 and 2;3 and 4;5 and 6 were orally and subcutaneously inoculated with *Ps. aeruginosa*, *Pr. vulgaris*, and *S. typhi-murium* respectively. The used infecting doses were 10^6 , 2.5×10^7 and 5×10^7 viable cells/ml for *Ps. aeruginosa*, *Pr. vulgaris* and *S. typhi-murium* respectively using McFarland tube No. 1 for standardization. Ducklings of group 7 were left without inoculation as control group.

Birds of all groups were kept separately for 21 days observation period with record of the clinical signs and mortalities. At the end of the experiment survived ducklings were sacrificed. Dead, as well as sacrificed ducklings were subjected to post-mortem, as well as bacteriological examinations for reisolation of the inoculated microorganisms.

Results

Examination of four hundred ailing ducklings and 200 duck eggs resulted in the isolation of *Ps. aeruginosa* *Pr. vulgaris* and *Salmonella* species in a rate of 15%, 10% and 9% respectively for ducklings and 11%, 8.5% and 12% respectively for duck eggs (Table 1). Further of *Salmonella* isolates revealed different serotypes shown in Table 2. *S. typhimurium* was isolated from duck eggs and ducklings at rate of 3.5 % and 3.25%.

TABLE 1. The types and incidence of isolation of different microorganisms from duck eggs and ducklings.

Specimens	No. of examined specimens	No. of positive sample	No. of positive sample	Bacterial species					
				<i>Ps. aeruginosa</i>		<i>Pr. vulgaris</i>		<i>S. typhimurium</i>	
				No.	%	No.	%	No.	%
Duck eggs . .	200	63	31.5	22	11	17	8.2	24	12
Ducklings . .	400	136	34.0	60	15	40	10.0	36	9

S. gallinarum-pullarum, *S. manhattan*, *S. newlands*, and *S. tshingwe* were isolated only from ducklings at a rate of 2.5%, 1.25%, 1% and 1% respectively, *S. newport*, *S. eastbourne* and *S. haidelberg* were isolated from duck eggs at a rate 3%, 3%, and 2.5% respectively.

Experimental infection of the isolated 3 species, *Ps. aeruginosa*, *Pr. vulgaris* and *S. typhi-murium* to one day old ducklings using oral and subcutaneous routes of inoculation were shown in Table 3. Where there were a considerable variation.

Oral infection with *Ps. aeruginosa* (group 1) resulted in the death of 12 out of 20 infected ducklings within the first 7 days from infection.

Duckling infected subcutaneous with *Ps. aeruginosa* (group 2) showed clinical signs of loss of appetite, dullness, depression ruffled feathers and pasty vent 24 hr after infection. Fifteen out of 20 infected duckling died within the first 5 days after subcutaneous infection while the remained 5 birds showed stunted growth as compared with non infected control.

TABLE 2. Serotyping of *Salmonella* isolated from duck eggs and ducklings.

Salmonella serotypes	Duck eggs (200)		Ducklings (400)	
	No. of Salmonella species	%	No. of Salmonella species	%
<i>S. typhi-murium</i>	7	3.5	15	3.25
<i>S. gallinarumpallorum</i>	—	—	10	2.50
<i>S. newport</i>	6	3.0	—	—
<i>S. eastbourne</i>	6	3.0	—	—
<i>S. heidelberg</i>	5	2.5	—	—
<i>S. manhattan</i>	—	—	5	1.25
<i>S. newlands</i>	—	—	4	1.00
<i>S. ishtionwe</i>	—	—	4	1.00

() Number of examined samples. % was calculated according to number of samples.

TABLE 3. Results of pathogenicity of the isolated microorganisms to one-day old ducklings.

Group No.	No. of infected duckling	Inoculated micro-organism	Route of inoculation	Dose bacteria /ml	No. of survivors	No. of dead birds	Rate of mortality
1	20	<i>Pseudomonas aeruginosa</i>	oral	10^8	8	12	60%
2	20		S/C		5	15	75%
3	20	<i>Proteus vulgaris</i>	oral	2.5×10^7	20	—	0%
4	20		S/C		20	—	0%
5	20	<i>S. typhi-murium</i>	oral	3.0×10^7	7	13	65%
6	20		S/C		4	16	80%
7	20	Control	—	—	20	—	0%

The recorded post-mortem lesions in *Ps. aeruginosa* infected ducklings which died 24 hr after infection were congested subcutaneous blood vessels, greenish yellow gelatinous subcutaneous oedema, distended gall-bladder, petechial haemorrhages in heart, liver, spleen and kidneys. Similar post-mortem lesions were also reported in those died 72 hr after infection but the subcutaneous oedema was more obvious, while the liver developed mottling with greenish appearance and coagulated subcutaneous exudates with adhesion to the underlying tissue.

Ducklings of groups 3 and 4 which were inoculated orally and S/C respectively with *Pr. vulgaris* did not develop any obvious clinical signs or mortalities during the observation period till the end of the experiment. The post-mortem examination of those slaughtered ducklings did not reveal any noticeable lesions.

Experimentally infected ducklings with *S. typhimurium* (groups 5 and 6) showed variations in the incubation periods from 1-7 days for orally infected ducklings and 1-2 days after subcutaneous injection.

The noticed clinical symptoms were loss of appetite, increased thirst, dullness, discharge from the eye, followed by diarrhoea and nervous manifestation observed, as staggering gait, trembling incoordination of movement, laying on back kicking and keeling over.

Death occurred within 24 hr from the onset of symptom.

Thirteen out of 20 and sixteen out of 20 were died in oral and subcutaneously infection respectively. The obtained post-mortem findings within the first week after infection were typical picture of septicemia including congestion of the internal organs with petechial haemorrhages on heart fat, liver and spleen, while those died later showed necrotic foci in the liver and heart with the enlargement of gallbladder and brain congestion.

Discussion

In the present investigation bacteriological examination of duck eggs resulted in the isolation of *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Salmonella* species in a rate of 11%, 8.5% and 12% respectively. Moreover, these microorganisms were similarly isolated from ailing ducklings in a rate of 15%, 10% and 36% respectively. Safwat *et al.* (1984) reported that the incidence of contamination of duck eggs reached 9.7% for *pseudomonas*, 6.8% for *proteus* and 19.4% for *salmonella* species.

Serotyping of *salmonella* species revealed the isolation of *S. typhimurium*, *S. newport*, *S. eastbourne* and *S. heidelberg* for duck eggs in rates of 3.5%, 3%, 3%, 2.5% of the isolated *salmonella* species. On the other hand *S. typhimurium*, *S. gallinarum-pullorum*, *S. manhattan*, *S. newlands*,

and *S. tshiongwe*, were also isolated from ducklings in a ratio of 3.25%, 2.5%, 1.25%, 1% and 1% of the isolated salmonella species. Koppel and Polony (1958) that *S. typhi-murium* was responsible for mortality in ducks, also El-Akkad *et al.* (1967) isolated *S. typhi-murium* and *S. gallinarumpullorum* from Pekin ducks, but El-Rafaie (1967) reported the infection of ducklings with *S. typhi-murium* and *S. gallinarumpullorum*. Shouman and Mostafa (1975) could isolate *S. gallinarum-pullorum* and *S. typhi-murium* in infertile and dead in shell duck eggs as well as one day old ducklings. Safwat (1976) isolated and studied experimentally the pathogenicity of *S. newport*, *S. typhi-murium* and *S. tshiongwa* to one day old ducklings, with mortality rate 75%, 70% and 60% respectively.

The role of ducks as being an important reservoir for salmonella transmission to other animals and man as well as through their meat, egg and droppings has been reported by many investigators (Clarenburg and Vink, 1950; Blaxland and Blowers 1951; Linsert, 1958; Rasmussen, 1962; Shouman and Moustafa, 1975; Safwat, 1976 and 1979).

Pathogenicity test to one day old ducklings for *Ps aeruginosa Pr. vulgaris* and *S. typhi-murium* revealed that pseudomonas aeruginosa was pathogenic for one day old ducklings. Similar clinical signs and mortalities together with post-mortum pictures were reported in chicken by Mazetti (1971) and Markaryan (1975). Our results showed the *Proteus vulgaris* had no pathogenic effect on one day-old ducklings under the condition of our experiment. On the other hand *S. typhi-murium* was pathogenic for one day old ducklings a result which completely similar to those reported by Safwat (1976 and 1979) who proved experimentally that *S. typhi-murium* was pathogenic to one day old ducklings.

In conclusion, it is apparently clear that this investigation is the first which deals with the possible infection and the pathogenicity of *Ps. aeruginosa* to one day old ducklings as well as the ailing ducks.

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دراسات عن العدوى بميكروب بسيدومونس ابروجونوزا وبروتيس فولجارس والسالمونيلا تيفس ميوريم في كتاكيت البط

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فحص ٤٠٠ كتكوت بط يكينى تتراوح اعمارهم من سن يوم الى ٢ اسبوع
وكذلك ٢٠٠ بيضة بطه بكتولوجيا .

وجد ان نسبة الاصابة فى كتاكيت البط بميكروب بسيدومونس ١٥٪
وبروتيس فولجارس ١٠٪ وسالمونيا ٩٪ . كذلك نسبة الاصابة فى بيض البط
بتلك الميكروبات على الترتيب ١١٪ ، ٨٥٪ ، ١٢٪ .

اثبتت العدوى الصناعية بالفم والحقن تحت الجلد فى كتاكيت بط سن
يوم ان ميكروب بسيدومونس وسالمونيا تيفس ميوريم تعطى ضراوة بنسبة ٦٠٪
٦٥ عن الحقن بالفم و٧٥ و٨٥ عن الحقن تحت الجلد .

اما ميكروب بروتيس فولجارس فلم تثبت ضراوته ولم يعطى اى اعراض
او وفيات بعد الحقن بالفم او تحت الجلد .