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EPIDEMIOLOGICAL STUDIES OF PSEUDOMONAS AERUGINOSA IN CHICKENS, FISH AND HUMAN

(With 5 Tables)

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(Received at 8/6/1989)

دراسة وبائية لميكروب السودموناس ايرجينوزا في الدجاج ، السمك ، الإنسان

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تم فى هذا البحث فحص عدد ٢٥٠ عينة من أجنة بيض الدجاج الكابس من المفرخات الحكومية والخاصة بمحافظة أسيوط وقد أمكن عزل ٢٨ عترة من ميكروب السودموناس بنسبة٢ر١١٪ كما تم عزل ٢٠ عترة أخرى عند فحص ٤٠٦ دجاجة نافقة ذات أعمار ومصادر مختلفة بنفس المحافظة بنسبة ٩ر٤٪ وكذلك ٢٣ عترة من فحص عدد ١٢٢ حالة سمك و ٣٠ عترة من بول الإنسان كما تم تصنيف العترات المعزولة من السودموناس بيوكيميائياً وإتضح أنها تنتمى إلى سودموناس ايرجينوزا · وبإجراء العدوى الصناعية بالعترات المعزولة من المصادر المختلفة (دجاج-سمك - إنسان) فى أجنة الدجاج عمر ٧ أيام بالحقن فى كيس المح ثبت أن الميكروب ضارى جداً حيث بلغت نسبة الوفيات في المجموعات المختلفة ١٠٠٪ ولكن بأزمنة متباينة وكذلك ثبت ضراوة تلك العترات عند حقن كتاكيت عمر ٣ أيام تحت الجلد بنسبة وفيات ١٠٠٪ وعند حقن الفئران تحت الجلد والأرانب فى التجويف البريتوني بالعترات سابقة الذكر فقد إتضح إختلافها في الضراوة بالنسبة لهذه الحيوانات ·

SUMMARY

28 (11.2%) isolates of pseudomonas aeruginosa were detected from 250 dead chicken embryos in addition 20(4.9%) isolates of the same organism were recovered from 406 examined dead chickens collected from different sources at Assiut Governorate, also 23 & 30 isolates of ps.aeruginosa were isolated from internal organs of fish as well as human urine respectively.

Pathogenicity tests in 7 day old chicken embryos by yolk sac inoculation as well as in 3 day old chicks by s/c route proved that the organism isolated from poultry, fish and human was highly pathogenic with high mortality rate, on the other hand the experimental infection in mice

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and rabbit illustrated that human strain is the more pathogenic in mice and the poultry strain is the most pathogenic in rabbit. Reisolation of the organism in experimentally infected chicken embryos, baby chicks, mice and rabbits were conducted.

INTRODUCTION

Many microbial agents are responsible for losses in different chicken farms, Ps. aeruginosa is not only causing embryonic mortality but also for deaths of chickens of different ages (VALADAE, 1961; SRINIVASAN, 1977; NASHED, 1981; SAAD, et al. 1981; ANDREEV, et al. 1982 and BAPAT, et al. 1985.

The biochemical reaction of Ps.aeruginosa strains isolated from dead in shell embryos and chickens were reported by SADASIVAN, et al. 1979 and KIM, et al. 1982.

Ps.aeruginosa strains isolated from dead in shell embryos and chickens were pathogenic to both chicken embryos and young chicks with mortality rate up to 100% as recorded by ALI, 1980; AWAAD, et al. 1981 and SAIF-EDIN, 1983.

BROWNING & MACKIE (1949), stated that s/c injection of small dose of Ps.aeruginosa in rabbits may produce a local suppuration, but if the dose be large, spreading hoemorrhagic oedema results which attended by septicaemia. Intravenous injection may produce according to the dose, rapid septicaemia with nephritis.

CRUICKSHANK, et al. (1972) noticed that s/c injection of the organism in rabbits, guinea pigs & mice produces Fever & local abscess formation. Death is associated with the i/v injection of large doses in these animals.

The aim of the present study was designed to give an idea about the following:

1- The prevalence of Ps.aeruginosa in dead chicken embryos, chickens, fish and human.
2- Experimental infections using Ps.aeruginosa strains isolated from poultry, fish and human in chicken embryos, baby chicks, mice and rabbits to detect pathogenicity and if there is host specificity or not.

MATERIAL and METHODS

Specimens and bacteriological work:

250 dead in shell embryos as well as 406 growing and adult dead chickens and 122 samples of fish were examined for isolation of Ps.aeruginosa on pseudomonas cN supplement sR 102 which are consisted of pseudomona s agar base (oxoid, CM 559) and cetrimide Naldixic acid (C.N) supplement sR 102. The isolated strains were subjected to identification according to BUCHANEN and GIBBONS, 1975. One strain of Ps.aeruginosa from each source were chosen for Experimental infection, the isolated strains were kept in 0.4% semisolid agar and when need for inoculation were subcultured in nutrient broth for 24 hours at 37°C. Serial dilutions were carried out in 0.9% sterile saline solutions and number of the organism per ml was determined by plate count method (CRUICKSHANK, 1965).

Pathogenicity tests:

Embryonated chicken eggs, baby chicks, mice and rabbits proved to be free from pseudomonas infection as well as other pathogenic organisms by bacteriological examination.

Experimental infection to chicken embryos:

10 groups (1-10) each containing 5 chicken embryos of 7 day old. Group No. 1, 4, 7 were inoculated via yolk sac route by 0.1 ml of 24 hours broth cultured contained 10^4, 10^6 and 10^8 viable cells of Ps.aeruginosa/ml of poultry origin respectively, the same was repeated with organism of human origin in Group 2, 5, 8 and with organism of fish origin in Group 3, 6, 9.

Group No. 10 was left uninoculated as control, all groups reincubated and candled daily till end of incubation period, dead embryos during this period were subjected to bacteriological examination for reisolation of the organism.

Experimental infection in babychicks:

10 groups (1-10) each containing 5 birds of 3 day old chicks Groups 1, 4, 7 were inoculated s/c with 10^4, 10^6, 10^8 viable cells of Ps. aeruginosa of poultry origin respectively and the same was repeated with strain of human origin in group 2, 5, 8 and with strain of fish origin in group 3, 6, 9. Group 10 was left uninoculated as control.

All groups were kept under observation for one week clinical signs, mortality rate as well as p.M. lesions were recorded. Reisolation of the inoculated organism was conducted from experimentaly infected chicks.

Experimental infection in mice:

10 groups (1-10) each containing 5 mice, the same groups, route and dose as described under experimental infection of baby chicks. All groups were kept under observation for 14 days; dead mice were subjected to bacteriological examination for reisolation of the organism.

Experimental infection in rabbits:

7 groups (1-7) each containing 5 rabbits, Group No. 1, 4 were inoculated 1/p with 10^4 and 10^8 viable cells of ps. aeruginosa of poultry origin and the same was repeated in group 2, 5 with strain of human origin and in group 3, 6 with strain of fish origin respectively.

Group No. 7 was left uninoculated as control, all groups of rabbits were kept under observation for one week, dead rabbits were subjected to bacteriological examination for reisolation of the organism.

RESULTS

Table (1): Shows the frequency and percentage of ps. aeruginosa.
Table (2): Shows the results of pathogenicity in chicken embryos.
Table (3): Shows the results of pathogenicity in baby chicks.
Table (4): Shows the results of pathogenicity in mice.
Table (5): Shows the results of pathogenicity in rabbits.

DISCUSSION

In the last years pseudomonas infection drew our attention due to economic losses in embryos and chickens of different ages to poultry industry, in this present study, out of 250 dead embryos, 28 isolates were identified to be ps. aeruginosa, in similar studies the same organism have been isolated by other investigators, ZAGOEVSKI (1956); ALI (1980) and BECIREVIC and POPOVIC (1986). The percent of the isolated ps. aeruginosa by the authors (11.2%) was nearly the same percentage mentioned by RANES and SZALY (1974) and NASHED (1981) from dead embryos and unhatched eggs.

Bacteriological examination of dead grower and adult chickens revealed that the organism was isolated in a low percent (4.4 and 5.3%) respectively this is due to the uncontrolled administration of drugs, our results agreed to some extent with MAZAETTI (1972); MARKARYAN (1975) and AWAAD, et al. (1981).

Experimental infection of 7 day old chicken embryos via yolk sac with strains of ps. aeruginosa isolated from different sources proved that the strains were highly virulent with 100% mortality, the period at which mortality occur varies from 24 to 60 hours post infection according to the dose used in infection. The dead embryos showed greenish discolouration and turbidity of the embryonic fluid. Reisolation of ps. aeruginosa were carried out from infected embryos but not from control. The pathogenic effect of ps. aeruginosa in chicken embryos was studied by SATO, et al. (1961), SRINIVASAN (1977), SAAD, et al. (1981), NASHED (1981) and SAIF-EDIN (1983) who concluded that the organism of a high percent of embryonic mortality.

Experimental infections of 3 days old baby chicks s/c with different strains of ps. aeruginosa proved that the organism is highly virulent to baby chicks, infection with various doses of poultry and fish strains of the organism lead to 100% mortality with 24 hours post infection. Pathogenicity of human strain to baby chicks depend upon the dose used in infection i.e. infection with 10^8 and 10^6 /ml lead to 100% mortality with 24 and 48 hours post infection respectively while infection with 10^4 /ml resulted in 80% mortality at 48 hours post infection. The clinical signs of morbid chicks was characterized by depression, ruffled feathers, drooping of wings and diarrhea, the post-mortem lesions showed congestion of lungs, intestinal B.Vs. liver, spleen,

heart B.Vs. musculature of thigh muscle, out side and inner side of the keel bone. The systemic reaction may be related to the multiplication and toxin production of the organism, reisolation of the organism from dead baby chicks was conducted and this result agree with the finding of SAIF EDIN (1983) and disagree with result obtained by EL-NASSAN, et al. (1973) who failed to isolate organism from experimentally infected chicks. Nearly similar observation were noticed by VALADAE (1961), WILLIAMS and NEWKREK (1966), RAY and BANERJI (1969), AWAAD, et al. (1981), TRENCHI, et al. (1981), ANDREEV, et al. (1982) and SAIF-EDIN (1983). With respect to the results obtained by AWAAD, et al. (1981) and SAIF-EDIN (1983) that virulence of the organism was parralel to the inoculated dose, in the present study only human strain gave similar finding.

Experimental infection of mice s/c injected with poultry, human and fish strain revealed that the virulence of the organism varies according to the source of the strain as well as the dose used in infection. Higher dose (10^8/ml) of human strain was more virulent to mice resulted in 80% mortality, while the same dose of poultry strain produced 60% mortality, the lower doses (10^4/ml and 10^6/ml) of human and poultry strains produced 20 and 40% mortality respectively. With respect to fish strain, only dose 10^8/ml is pathogenic to mice with 40% mortality. Reisolation of the organism from dead mice was carried out. Nearly similar observations were obtained by CRUICKSHANK, (1972) who reported that large doses lead to death of mice, MARKARYAN (1975) proved that poultry strain is pathogenic to mice experimentally, while SEDIK, et al. (1987) reported that the exoproduct of ps. aeruginosa exhibited lethal effect when injected into mice intraperitonealy.

Rabbit I/p infected with ps. aeruginosa revealed that pathogenicity varied according to source of strain as well as the dose used in infection. Higher dose (10^6/ml) of poultry strain was more virulent to rabbit and produced 100% mortality while the lower dose (10^4/ml) resulted in 80% mortality, on the other hand, only high dose of human strain was pathogenic with 40% mortality, while groups of rabbits infected with lower dose were survived during the experimental period. Fish strain was virulent to rabbit and the virulence did not depend upon the dose used in infection, higher and lower doses produced equal mortality (80%) reisolation of the organism was carried out from dead infected rabbit. Macroscopic lesions showed signs of septicemia. BROWNING and MACKIE (1949) reported that s/c injection of small dose of ps. aeruginosa in rabbit produce local lesions and large dose may be attended by septicemia.

The present study proved that ps. aeruginosa is highly significant for poultry industry in our country, moreover there is no host specificity for this organism which are isolated from different sources i.e. infected persons working in poultry farms and also fish used in feeds of poultry can be considered as a source of infection and vice versa, moreover the result of experimental infection in mice and rabbit can be

used to differentiate between ps. aeruginosa strains isolated from different sources, yet this point needed more details study.

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Table (1): Frequency and percentage of ps. aeruginosa.

Examined specimens	Source	No.of the examined samples	No. of isolates	%
Dead embryos	balady and Governmental incubators	250	28	11.2
grower chickens	chicken farms	180	8	4.4
adult chickens	chicken farms	226	12	5.3
human urine	laboratories *	-	30	-
fish	Markets	122	23	18.8

* ps. aeruginosa were collected from laboratories.

Table (2): Results of pathogenicity test of pseudomonas aeruginosa in chicken embryos

Group No.	Source of strain	Route of infection	Dose/ml	No.of infected chicken embryos.	Deaths of chicken embryos post infection									Mortality.		Hatchability	
					24 hrs	36 hrs	48 hrs	60 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs	No.	%	No.	%
1	poultry	yolk sac	0.1ml(10^4/ml)	5	-	-	5	-	-	-	-	-	-	5	100	-	-
2	Human	yolk sac	0.1ml(10^4/ml)	5	2	-	-	3	-	-	-	-	-	5	100	-	-
3	fish	yolk sac	0.1ml(10^4/ml)	5	1	-	-	4	-	-	-	-	-	5	100	-	-
4	poultry	yolk sac	0.1ml(10^6/ml)	5	2	-	3	-	-	-	-	-	-	5	100	-	-
5	Human	yolk sac	0.1ml(10^6/ml)	5	3	-	-	2	-	-	-	-	-	5	100	-	-
6	fish	yolk sac	0.1ml(10^6/ml)	5	4	-	-	1	-	-	-	-	-	5	100	-	-
7	poultry	yolk sac	0.1ml(10^8/ml)	5	5	-	-	-	-	-	-	-	-	5	100	-	-
8	Human	yolk sac	0.1ml(10^8/ml)	5	5	-	-	-	-	-	-	-	-	5	100	-	-
9	fish	yolk sac	0.1ml(10^8/ml)	5	5	-	-	-	-	-	-	-	-	5	100	-	-
10	control	-	-	5	-	-	-	-	-	-	-	-	-	-	-	5	100

Table (3). Results of pathogenicity test of pseudomonas aeruginosa in baby chicks.

Group No	Source of strain	Route of infection	Dose/ml	No.of infected baby chicks	Daily deaths post infection							Mortality		Survival	
					1	2	3	4	5	6	7	No	%	No.	%
1	poultry	s/c	10^4/ml	5	5	-	-	-	-	-	-	5	100	-	-
2	Human	s/c	10^4/ml	5	-	4	-	-	-	-	-	4	80	1	20
3	Fish	s/c	10^4/ml	5	5	-	-	-	-	-	-	5	100	-	-
4	Poultry	s/c	10^6/ml	5	5	-	-	-	-	-	-	5	100	-	-
5	Human	s/c	10^6/ml	5	4	1	-	-	-	-	-	5	100	-	-
6	Fish	s/c	10^6/ml	5	5	-	-	-	-	-	-	5	100	-	-
7	Poultry	s/c	10^8/ml	5	5	-	-	-	-	-	-	5	100	-	-
8	Human	s/c	10^8/ml	5	5	-	-	-	-	-	-	5	100	-	-
9	Fish	s/c	10^8/ml	5	5	-	-	-	-	-	-	5	100	-	-
10	Control	-	-	5	-	-	-	-	-	-	-	-	-	5	100

Table (4). Results of pathogenicity test of pseudomonas aeruginosa in mice.

Group No.	Source of strain	Rout of infection	Dose/ml	No.of infected mice	Daily deaths post infection														Mortality		Survival	
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	No	%	No	%
1	Poultry	s/c	10^4/ml	5	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	20	4	80
2	Human	s/c	10^4/ml	5	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	20	4	80
3	Fish	s/c	10^4/ml	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	100
4	Poultry	s/c	10^6/ml	5	-	1	-	-	1	-	-	-	-	-	-	-	-	-	2	40	3	60
5	Human	s/c	10^6/ml	5	-	1	-	-	-	-	1	-	-	-	-	-	-	-	2	40	3	60
6	Fish	s/c	10^6/ml	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	100
7	Poultry	s/c	10^8/ml	5	-	1	-	-	2	-	-	-	-	-	-	-	-	-	3	60	2	40
8	Human	s/c	10^8/ml	5	-	2	1	-	-	-	-	-	-	-	-	-	1	-	4	80	1	20
9	Fish	s/c	10^8/ml	5	-	2	-	-	-	-	-	-	-	-	-	-	-	-	2	40	3	60
10	Control			5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	100

Table (5): Results of pathogenicity test of pseudomonas aeruginosa in rabbits.

Group No.	Source of strain	Route of infection	Dose/ml	No.of infected rabbit	Daily deaths post infection							Mortality		Survival	
					1	2	3	4	5	6	7	No.	%	No.	%
1	Poultry	1/p	10^4/ml	5	4	-	-	-	-	-	-	4	80	1	20
2	Human	1/p	10^4/ml	5	-	-	-	-	-	-	-	-	-	5	100
3	Fish	1/p	10^4/ml	5	4	-	-	-	-	-	-	4	80	1	20
4	Poultry	1/p	10^8/ml	5	5	-	-	-	-	-	-	5	100	-	-
5	Human	1/p	10^8/ml	5	2	-	-	-	-	-	-	2	40	3	60
6	Fish	1/p	10^8/ml	5	4	-	-	-	-	-	-	4	80	1	20
7	Control	-	-	5	-	-	-	-	-	-	-	-	-	5	100