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ISOLATION AND SIGNIFICANCE OF AEROMONAS HYDROPHILA GROUP IN FARMED RABBITS AT ASSIUT GOVERNORATE

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ABSTRACT :

A total of 135 faecal swabs were aseptically collected from different rabbit farms at Assiut Governorate. Diarrhea and emaciation were observed in 120 out of 135 while the rest were apparently healthy.. These samples were examined bacteriologically for determination of the occurrence and frequency of *Aeromonas hydrophila*. The obtained results revealed that total isolates of *A.hydrophila* were 35 at percentage of (25.9%) which represented 33 isolates from diarrhotic rabbits at percentage of (24.4%) while 2 isolates were obtained from apparently healthy animals at percentage of (1.5%).

The experimental infection in 6-8 week-old rabbits by oral route led to 20% mortality. The clinical observation and the post-mortem lesions of experimentally infected animals were recorded Clinical observations were similar to a great extent to those of natural infection. Reisolation of infecting organism from internal organs and intestinal tract of dead and scarified slaughtered rabbits at the end of observation period were conducted.

The in vitro susceptibility of the *A.hydrophila* isolates to a variety of antibiotics revealed that highest number of isolates were sensitive to Gentamyicin (100%), Nalidxic acid (100%), Chlormphnicol (95%) and Cephoxetin (90%), while it was resistant to Penicillin and Ampicillin.

The public health significance and the economic losses arising from infection of the rabbit with *A.hydrophila* as well as suggestions for their avoidance were discussed.

INTRODUCTION:

A.hydrophila **is a Gram-negative, rod-shaped, facultative anaerobic bacterium. It had been reported in many countries in the world and isolated from a wide range of mammals (Von and Zinterhofer, 1970), surfaces water, and sewage (Hazen *et al.*, 1978), in fish, shell fish (Rippey and Cabelli, 1979), birds (Glunder and Siegmann, 1989), and rabbit (Okewole *et al.*, 1989), Efuntoye (1995) recorded that rabbits appear to be more susceptible to infection with *A.hydrophila* followed by pigs, chicken, sheep and goats during outbreak of diarrhea and enteritis.**

Pathogenicity of *A.hydrophila* in experimental animals was observed by Ali *et al* (1992) who found that experimental infected mice died between 18-24 hours with signs of septicemia, blindness and liver necrosis. So far, the mechanisms by which these organisms causing diarrhea have been only partially elucidated but it is known that they produced enterotoxins and

certain enzymes are able to adhere to cell membranes and invade them (Kirov *et al*.,1993). The pathogenicity of *A.hydrophila* is associated with the liberation of virulence factors and cell associated endotoxin. Virulence factors include the production of exotoxins (cytotoxin or enterotoxin) and α- B-hemolysins and ability to bind and to invade epithelial cells (Krovacek *et al* (1994).

***A.hydrophila* was isolated in pure form from liver, lungs, heart and spleen of rabbit with severe outbreak of hemorrhagic septicemia with highly mortality rate (Paniagua *et al*., 1998), while Kutkat *et al*. (2001) revealed that the inoculated of *A.hydrophila* in rabbits with single or double does showed a sever drop of hair, slight respiratory manifestations, profuse watery diarrhea, emaciation and mortality rate of 20%.**

The aim of the study was carried out to throw light on occurrence and pathogenicity of the *A.hydrophila* in rabbit farms, and the in vitro sensitivity test of strains isolates against different antibiotics.

MATERIAL AND METHODS:

1-Collection of samples:

Hundred thirty five faecal swabs were collected aseptically from rabbit farms in Assiut Governorate for investigation of occurrence and pathogenicity of the *A.hydrophila* in rabbit. Of these, 120 samples from diarrhotic and emaciated rabbits and 15 from apparently healthy.

2- Bacteriological examination:

It is interesting to study the relation between *A.hydrophila* and diarrhea in rabbit.

Isolation and identification: The technique recommended by Shotts and Rimler (1973), Shotts and Bullock (1975), Glunder and Siegman (1989) and Bisgaard *et al*. (1995).

Basic dilution of faecal swabs were made (ten-fold serially diluted with sterile saline up to 10^{-10}) for bacteriological examination. From this basic dilution ten ml of initial dilution was inoculated into 10 ml of Tripticase Soy broth (TSB) added with (20/ ug) of Ampicillin and incubated at 28^0C for 24 hour. The primary isolation of the organism was obtained by culturing the broth on Rimler-Shotts medium and incubated at 28^0C for 18-24 hours. Suspected colonies were picked up and streaked onto the surface of Starch Ampicillin Agar (SAA) at 28^0C and for 24 hour. Suspected colonies were transferred onto 5% sheep blood agar, nutrient agar, and Tripticase Soy Agar (TPA) plates and Trible Sugar Iron (TSI) slant and incubated at 28^0C for 24 hour.

The isolated bacterial was identified by culture morphology, Gram-stain and biochemically according to (Bullock *et al*., 1971, Popoff, 1984, Palumbo et. al. 1985, Glunder and Siegman, 1989 and Bisgaard *et al*., 1995). The colonies that showed typical reaction in TSI and positive for cytochrom oxidase test, oxidation and fermentation reaction in of glucose and catalase test were confirmed as *A.hydrophila*.

3- Pathogencity test:

Twenty seven, 6-8 week-old balady rabbits obtained from private farms at Assiut Governorate were used in this study. The animals were kept in cages and observed for a period of a week. A random sample of 3 rabbits was slaughtered and exposed to post-mortem, parasitological examination for coccidia and other parasites and bacteriological examinations for *Staph. aureus* and other pathogenic bacteria, which proved their health status and free from diseases Faecal swabs were examined for three

successive days to be sure that rabbits were free from *A.hydrophila*

Experimental test: Twenty four, rabbits were classified into 2 groups:

Group 1 (20) rabbits were inoculated orally with 0.5 ml of 24h. broth culture ($9x10^8$ viable organism/rabbit).

Group 2 (4) rabbits were kept without inoculation as control.

All rabbits were kept for 30 days (period of observation with daily examination for clinical signs and mortality rate. Faecal swabs were taken weekling for bacteriological examination. At the end of observation period all rabbits were recorded as well as trials for reisolation of infecting organism from liver, kidney, lungs, and intestinal were recorded.

4- Antibiotic sensitivity test :

a-Culture Media:

Mueller-Hinton agar : This medium was used for the disk diffusion test. It produces large and clear zone of inhibition when sensitive organisms are in contact with susceptible antibiotic.

b-Antibiotic sensitivity disks:

A total of 9 chemotherapeutic agents (Oxoid), were used (Gentamicin (10/ug), Chlormphnicol (30/ug), Tetracycline (30/mg), Penicillin (10/µg), Ampicillin (10/µg) Cephoxetin (30/µg), Kanamycin (30/µg), Nalidixic acid (30/ µg) and Streptomycin (10/ µg),

c-Methods:

Disk diffusion test: The disk diffusion technique was applied according to FineGold and Martin (1982). The degree of sensitivity was determined and interpretation of their sensitivity were done according to Oxoid Manual (1982) and Koneman *et al.* (1983).

RESULTS AND DISCUSSIONS:

1-Isolation and Identification of *A.hydrophila* in rabbits:

In contrast to the large number of publications on the role of A. hydrophila causing diarrhea in large animals, humans, birds and fish, there are few papers handily the effect of *A.hydrophila* in rabbit. In this study faecal swabs were collected from dirrhroeic and apparent healthy rabbits from different rabbits farms localities in Assiut Governorate for isolation and identification of *A.hydrophila*. According to morphological and biochemical characters, 20 isolates (26.7%) were identified to be *A.hydrophila* that grew on RS media after 24 hr. incubation at 28^0C. These colonies were rounded, 2-3 mm in diameter, and yellow to orange in color. This agrees with the findings of Shotts and Rimler (1973) who reported that a characteristic type of colony was obtained when *A. hydrophila* was inoculated on to RS Media and these type of colonies indicating maltose fermentation, also our results agree with that reported by Hazen *et al* (1978) who stated that RS Media was 94% efficient for isolation of *Aeromonas hydrophila* and Hsu *et al* (1981) who noted that all 127 strains of *A. hydrophila* tested produced yellow colonies on RS Media, while were. White to pale pink, round and covex colonies appear on nutrient agar. The isolates proved to be a Gram-negative, rod-shaped, facultative anaerobic and motile.

Concerning the biochemical characterization of the isolates the uniformly positive and uniformly negative results were confirmatory of those reported by others authors including Popoff and Vern (1976), Hus *et al* (1981) and Toranzo *et al* (1986). The biochemical reactions of the isolates showed that typical reaction in TSI, and positive for each of

cytochrom oxidase test, oxidation and fermentation reaction in O/F glucose, catalase,indol production, Aesculin, starch hydrolysis, gelatin liquefaction and B-haemolysis on 5% sheep blood agar except two isolates produced α- haemolysis were confirmed as *A.hydrophila*. The results recorded in Table (1) revealed that, 35 (25.4%) out of 135 faecal swabs of rabbit samples were positive for *A.hydrophila* The positive isolates were represented of 33 (27.5%) out of 120 diarrhoetic rabbits samples and 2 (13.3%) out of 15 apparent healthy. The all over frequency of positive isolates of diarrhoetic rabbits were (24.4%) while were (1.5%) in apparent healthy. These results agreement with reported that by Efuntoye (1995) who recorded that the lower level of *A.hydrophila* in apparent healthy rabbits while were higher rate in diarrhoetic rabbits, suggested that *A.hydrophila* is closely associated with outbreaks of diarrhea in rabbits.

2- Experimental infection:

The clinical signs noticed were: loss of appetite, ruffed fure, depression, disinclination to move, inclination to separate in the corner of the cage followed by profuse watery diarrhoea after the second week post infection, slight respiratory manifestation with coughing, sneezing, catarrhal nasal discharge and a sever drop of hair occurs after seven days post inoculation. In the last stage sick animals showed progressive emaciation followed by death.

The P.M. lesions of dead and scarified rabbits include general congestion of all carcasses in severely emaciated cases, congestion with petechial haemorrhages in liver, kidney, spleen, lungs are pale in some cases. Intestine showed sever enteritis, filled with watery fluid and distended with gases.

No abnormal symptoms were observed in control group. The results of pathogencity test are given in Table (2).

Reisolation of the inoculated organism from internal organs especially liver, kidney, lungs and intestine from dead and scarified rabbits at the end of the experimental were positive.

Pathogencity test of *A.hydrophila* conducted on 6-8 weeks old healthy rabbits by oral route proved the pathogenic nature of the tested isolate with 20% mortality. Exactly the same results reported by Kutakat *et al* (2001) who found that inoculation of *A.hydrophila* led to 20% mortality in four weeks-old Newzealand rabbits when infected with the same does and route of inoculation, while more higher observations was recorded by Efuntoye (1995) who found that *A.hydrophila* causes (42.8%) mortality in rabbits

For the clinical findings and P.M. pictures in the present study was a nearly similar reported by Kutkat *et al* (2001). Regarding to respiratory manifestations and dropping of hair which occurs in some infected groups of rabbits, there is no available literature dealing with those cases in rabbits but several authors recorded sporadic cases of pneumonia, skin ulcer caused by *A.hydrophila* in goat, fish, human as Stoskopf (1993), Neves *et al* (1994) and Alonso *et al* (1996). Other reports suggested injuries caused by other parasite and mechanical means will expose the epithelial to this bacterium (Hazen *et al* (1978) and Elliot and Shotts (1980). *A.hydrophila* which adhere to epithelial cells are believed to colonize, produce lesions, therefore the interaction with the epithelial cells is the first step towards pathogenicity and is important in determining the occurrence of infection.

Table (1): The frequency percentage of Aeromonas hydrophila isolated from 135 rabbits samples

Samples	No. samples	+ve samples	Frequency %	All over frequency
Diarrhotic	120	33	27.5%	24.4%
Apparently healthy	15	2	13.3%	1.5%
Total	135	35		25.9%

Table (2) Showing of results of pathogenicity of A.hydrophila in rabbits

Group No	No of infected rabbit	Rout of infection	Does of inoculums A.hydrophila	Daily deaths post infection						Total No of death	No. of survivors	Mortality rate
				1-4	14	15	16	17	18-30			
1	20	Orally	$9x10^8$ C.F.U	0	2	0	0	2	0-0	4	16	20%
2	4	Control	0	0	0	0	0	0	0-0	0	4	0

3- Antibiotic susceptibility:

Susceptibility of Patterns of *A.hydrophila* to antimicrobial agents have varied, but isolates were usually susceptible to Chloramphenical, Tetracycline and Trimethoprin-Sulfamethoxzol and relatively resistant to Penicillin, Polymixin and Cephalasproins Fass and Barnishan (1981) and Davis *et al.* (1978). In vitro susceptibility of the *A.hydrophila* isolates to a variety of antibiotics shown in (Table 3). These data revealed to 100%of the *A.hydrophila* isolates sensitive to Gentamyicin and Nalidixic acid, 95% to Kanamyicin, 90% to Cephoxetin, 85% to Tetracycline and 70% to Streptomycin, while all isolates of *A.hydrophila* were resistant to Penicillin and Ampicillin property due to beta-lactamase production, These results agree with obtained by Soliman (1988) who showed that most of the *A.hydrophila* isolates to be sensitive to Chloramphenicol, Nalidixic acid, Streptomycin, Kanamycin and Colistin while all of them were resistant to Ampicillin and Novobiocin and were similar to those recorded by MacCracken and Barkley (1972), Mascher *et al* (1988), Molero *et al* (1989) and Sohair and Eman (2002).those reported that a great number of strains seemed to be more sensitive to Gentamyicin, Kanamyicin, Chloramphenicol and Tetracycline while resistance to Ampicillin and Penicillin. Finally, the present study result conclude that *A.hydrophila* is considered a highly pathogenicity to rabbits since it causes sever dirrhoea, emaciation and deaths resulting in 20% mortality.

Table (3): Antibiotic sensitivity test for Aeromonas hydrophila isolates

Antibacterial agent	Aeromonas hydrophila isolates					
	Sensitive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Gentamicin (10/µg)	20	100%	0	0	0	0
Chormphnicol (30/ µg)	19	95%	1	5%	0	0
Tetracycline (30/µg)	17	85%	3	15%	0	0
Ampicillin (10 /µg)	0	0	2	10%	18	90%
Penicillin (10/ µg),	0	0	0	0	20	100%
Cephoxetin (30/ µg)	18	90%	1	5%	1	5%
Kanamicin (30/ µg)	19	95%	1	5%	0	0
Nalidixic acid (30/ µg)	20	100%	0	0	0	0
Streptomycin (10/ µg)	14	70%	2	10%	4	20%

R (-ve) = Resistant I (+ve, ++ve) = Intermediate S (+++ve) = Sensitive

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عزل وأهمية مجموعة الأيروموناس هيدروفيلا فى أرانب المزرعة فى محافظة أسيوط

عبد التواب محمد عبد الجواد، عبد الرحمن عبد المجيد عبد الرحمن

تم عمل استبيان لدراسة مدى انتشار عدوى ميكروب الأيروموناس هيدروفيلا فى مزارع الأرانب فى محافظة أسيوط أجريت الدراسة على١٣٥ عينة براز من مزارع الأرانب شملت على ١٢٠ حالة تعانى من الإسهال والضعف العام و١٥ حالة سليمة ظاهريا وتم فحص العينات بكتريولوجيا لاستبيان مدى تواجد ميكروب الأيروموناس هيدروفيلا فى أرانب المزارع وعلاقتها بالإسهال وكانت نتائج الفحص البكتريولوجى تشير إلى عزل ٣٥ عترة من الميكروب من جميع الحالات بنسبة ٢٥,٤% منها ٢٤,٤% من حالات الأسهال و١,٥% من الحالات السليمة ظاهريا و بإجراء العدوى الصناعية بهذا الميكروب فى الأرانب عمر ٦-٨ أسابيع بواسطة الحقن عن طريق الفم وجدت أنها ضارية للأرانب حيث بلغت نسبة النفوق إلى ٢٠%، وقد سجلت الأعراض الإكلينيكية والآفات التشريحية ووجد آن الأعراض الأكليكينية تشبه إلى حد كبير تلك التى لوحظت فى العدوى الطبيعية٠ هذا وقد تم عزل الميكروب مرة أخرى من الأعضاء الداخلية والأمعاء من الأرانب النافقة والمصابة٠ كما تم عمل اختبار الحساسية للميكروب المعزول وكان شديد الحساسية لكل من الجنتاميسين والنلادكس اسيد والكولورمنيفينكول والكاناميسين بينما كان مقاوم لكل من البنسلين والامبسلين٠ وتم مناقشة الأهمية الصحية من تواجد هذا الميكروب وعلاقته بحالات الإسهال فى الأرانب