

AUCES

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

A.M. Abdel-Gwad*; A.A. Abdel-Rahman**

*Animal Health Research Institute Assiut Lab.
**Animal Health Research Institute EL-Minia Lab.

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 Gentamicin (100%), Nalidixic 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 μg) of Ampicillin and incubated at 28°C for 24 hour. The primary isolation of the organism was obtained by culturing the broth on Rimler-Shotts medium and incubated at 28°C for 18-24 hours. Suspected colonies were picked up and streaked onto the surface of Starch Ampicillin Agar (SAA) at 28°C 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°C 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- **Pathogeneity 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 weekly 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), Chloromphenicol (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 diarrhoeic and apparent healthy rabbits from different rabbit 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°C. 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 convex 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), Hsu *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, Aesclun, 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 Efuntolye (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 diarrhea 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 pathogeneity 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.

Pathogeneity 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 Efuntolye (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 (Hazan 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 pathogeneity and is important in determining the occurrence of infection.
Table (1): The frequency percentage of Aeromonas hydrophila isolated from 135 rabbits samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. samples</th>
<th>+ve samples</th>
<th>Frequency %</th>
<th>All over frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhotic</td>
<td>120</td>
<td>33</td>
<td>27.5%</td>
<td>24.4%</td>
</tr>
<tr>
<td>Apparently healthy</td>
<td>15</td>
<td>2</td>
<td>13.3%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td>35</td>
<td>25.9%</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group No</th>
<th>No of infected rabbit</th>
<th>Route of infection</th>
<th>Does of inoculums A.hydrophila</th>
<th>Daily deaths post infection</th>
<th>Total No. of death</th>
<th>No. of survivors</th>
<th>Mortality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>Orally</td>
<td>9x10^3 C.F.U</td>
<td>1-4</td>
<td>0</td>
<td>4</td>
<td>20%</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Control</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

3- Antibiotic susceptibility:

Susceptibility of Patterns of A. hydrophila to antimicrobial agents have varied, but isolates were usually susceptible to Chloramphenicol, Tetracycline and Trimethoprin-Sulfamethoxazol 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 diarrhoea, emaciation and deaths resulting in 20% mortality.

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

<table>
<thead>
<tr>
<th>Antibacterial agent</th>
<th>Aeromonas hydrophila isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Gentamicin (10/µg)</td>
<td>20</td>
</tr>
<tr>
<td>Chormphnicol (30/ µg)</td>
<td>19</td>
</tr>
<tr>
<td>Tetracycline (30/µg)</td>
<td>17</td>
</tr>
<tr>
<td>Ampicillin (10 /µg),</td>
<td>0</td>
</tr>
<tr>
<td>Penicillin (10/ µg),</td>
<td>0</td>
</tr>
<tr>
<td>Cephoxetin (30/ µg)</td>
<td>18</td>
</tr>
<tr>
<td>Kanamicin (30/ µg)</td>
<td>19</td>
</tr>
<tr>
<td>Nalidixic acid (30/ µg)</td>
<td>20</td>
</tr>
<tr>
<td>Streptomycin (10/µg)</td>
<td>14</td>
</tr>
<tr>
<td>---------------------</td>
<td>----</td>
</tr>
<tr>
<td>R (-ve) = Resistant</td>
<td>1 (+ve, ++ve) = Intermediate</td>
</tr>
</tbody>
</table>

REFERENCES:


Zentralblatt fur hygiene und Unweltmed., 201, 423-436.


عزل وأهمية مجموعة الأوبروناس هيدروفيلا في أراض المزرعة

فی محافظة أسيوط

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