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## **SOME STUDIES ON *LISTERIA* *MONOCYTOGENES* IN RABBITS**

(With 3 Tables and 5 Figures)

By

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بعض الدراسات عن ميكروب الليستيريا مونوسيتوجينس في الأرانب

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لدراسة مدى تعرض مزارع الأرانب الحكوميه والخاصه بمحافظة أسيوط للأصابه بميكروب الليستيريا مونوسيتوجينس . تم فحص عدد ٥٠ أرنب بعضها مصاب والأخرى حديثه الوفاة يشتبه في أصابتها بمرض الليستيريا. أوضحت الخواص المورفولوجيه والتفاعلات البيوكيميائيه والبيولوجيه والفحص الهستوباثولوجي عزل ٩ حالات ايجابية لميكروب الليستيريا مونوسيتوجينس بنسبه ١٨% . وقد وجد ان التغيرات الباثولوجيه في الكبد والرحم والمخ تشبه تماما تلك التى وصفت في حالات الأرانب المصابه بالليستيريا مع ملاحظة شدة هذه التغيرات في رحم الأرانب الحوامل أو التى حدث لها أجهاض . كما لوحظ نزيف وقله في عدد الخلايا الليمفاويه فى الطحال . بأجراء اختبار الحساسيه فى المعمل للعترات المعزوله وجد أنها جميعا حساسه للجينتاميسين والتتراسيكلين ، والسبكتينومايسين بدرجة عاليه.

### **SUMMARY**

In studying *Listeria monocytogenes* in rabbits, a total of "50" diseased as well as freshly dead animals suspected clinically and pathologically to suffer from the disease were examined. The affected rabbits were collected from different governmental and private farms at Assiut Governorate. They were subjected to post mortem as well as bacteriological examinations. Only nine positive cases of *Listeria monocytogenes* were isolated with an incidence of 18%. Identification of the causative organism was based on morphological,

biochemical and biological characters as well as histopathological lesions. Liver, uterus and brain showed the typical lesions of listeriosis in rabbit. The gravid and aborted uteri were severely affected than the non gravid one. The spleen showed severe hemorrhage in the red pulp and exhaustion of the lymphocytic elements. In vitro antibiotics sensitivity tests showed that the examined isolates were highly sensitive to gentamycin, tetracycline and spictinomycin.

*Key words: Rabbits - Listeria monocytogenes*

## INTRODUCTION

*Listeria monocytogenes* is widely distributed in nature and has been isolated from a variety of species including mammals and birds (Wardrop and Macleod, 1983; Barlow and McGroome, 1985; Cooper, 1988; Seman et al., 1990 & Jubb and Huxtable, 1993.)

In rabbits, this infection is considered as one of the major disease problem facing rabbit health and breeding in the form of infertility, abortion and high mortality, resulting in economic losses in the rabbitaries, as reported by Gray and Killinger (1966), Devois (1983), Abd El waneas (1985) and Abd EL-Motelib et al. (1990). It is becoming recognised that intercurrent disease, environmental stresses appear to be very potent factors in activating clinical signs of listeriosis. This in addition to the possible transmission of listeriosis to other animals, birds and humans. So the work reported in this paper was undertaken to give an idea about the following:

- The incidence of *Listeria monocytogenes* in naturally affected rabbits in Assuit Governorate.
- The main pathological lesions of listeriosis in rabbits as a tool of differential diagnosis.
- The antibiogramme of isolated *Listeria monocytogenes* strains from rabbits.

## MATERIALS and METHODS

### Materials:-

#### 1- Samples:

A total of 50, diseased and freshly dead rabbits of various ages, sex and breeds, were collected from governmental and private farms at Assiut Governorate. These rabbits were suffering from conjunctivitis, diarrhoea, retention of urine, nervous signs including locomotor disturbances and



paralysis as well as abortion. Tissue samples from brain, liver, spleen, kidneys, urinary bladder, lungs, aborted foeti and uteri as well as ocular swabs and heart blood, were collected from these cases and subjected to bacteriological examination.

## **2- Media:**

A) Liquid: *Listeria* enrichment broth, tryptose broth, peptone water, semisolid agar, 1% peptone broth, sugars (dextrose, maltose, sucrose, sorbitol, xylose, lactose and mannitol).

B) Solid: *Listeria* enrichment agar, tryptose agar, blood agar, triple sugar iron agar and urea agar base.

3- Reagents, chemicals and stains used were Kovac's, urea, Andrade's indicator, H<sub>2</sub>O<sub>2</sub>, Gram's stain (Cruickshank *et al.*, 1975 and Stanley, 1986).

4 - Experimental animals : 5 native rabbits, 6-8 week old from local markets and 5 mice from the Animal House of Assiut University were used for Anton's test and pathogenicity test respectively.

## **Methods:-**

### **Isolation and identification of *Listeria monocytogenes*:-**

One gram of every specimen was macerated with one gram of sterilized sand in a clean sterilized mortar to which 10 ml sterilized distilled water was added. The emulsion was transferred to a test tube containing *Listeria* enrichment broth and tryptose broth with nalidix acid and incubated at 37°C for 24 hours. followed by subculturing on blood agar, *Listeria* enrichment agar and tryptose agar at 37°C for 24-48 h. The colonial morphology was examined using hand lens, the colonies appeared with blue-green discoloration. "B" haemolysis was noticed on blood agar. Morphological identification was undertaken by making smears stained with Gram's stain from the suspected colonies which appeared as small Gram +ve bacilli or coccobacilli which were occasionally pleomorphic. The isolates examined for motility, gave umbrella-like growth in semisolid agar. The suspected *Listeria monocytogenes* isolates were identified biochemically using sugar fermentation and other special biochemical tests.

### **Anton's test:-**

To study the biological characters of *Listeria monocytogenes* isolates, "Anton's test" was done. This test was adopted after Anton (1934) as follows:-

Instillation of 2-3 drops of *Listeria monocytogenes* suspension into the conjunctiva of 5 rabbits.

### **Pathogenicity test:-**

To study the pathogenicity of *Listeria monocytogenes* isolated from rabbits, intraperitoneal inoculation of 5 mice with 0.1-0.25 ml of *Listeria monocytogenes* broth culture was done.

### **Histopathology:-**

Tissue specimens from the affected rabbits were taken from liver, uterus, spleen, lungs, kidneys and brain, then fixed in neutral buffered formalin, washed, dehydrated with alcohols and embedded in paraffin. The paraffin blocks were sectioned at 7 $\mu$ m thickness. Tissue sections were stained with hematoxylin and eosin stain (Bancroft and Stevens, 1982).

### **Sensitivity test:-**

Study on the sensitivity of the isolates to antimicrobial agents was done using the paper disc technique adopted after *Finegold and Baron*, (1986) in order to determine their antibiogramme.

## **RESULTS**

### **Isolation and identification of *Listeria monocytogenes*.**

The obtained results of the positive cases are shown in Table 1. The biochemical characters of suspected *Listeria monocytogenes* isolates from rabbits are illustrated in Table 2.

### **Anton's test:-**

The applying of this test produced a purulent conjunctivitis within 24-48 hours followed by keratitis in all rabbits.

### **Pathogenicity test:-**

The applying of this test lead to deaths of the mice within 24-48 h. with severe congestion of parenchymateous organs and brain.

### **Gross pathology:-**

The post mortem findings were small whitish foci in the liver surface extend to the liver parenchyma, cystitis, enteritis, smallness of the spleen, endometritis, bloody vaginal discharge and congestion of the meningeal blood vessels.

### **Histopathology:-**

#### **Liver:**

Several necrotic foci were seen in the hepatic parenchyma. The necrotic foci consisted of necrosed hepatocytes infiltrated with heterophil cells. (Fig. 1) in addition to congestion of the vasculature.



**Brain:**

The brain showed multiple heterophilic cellular aggregations in the cerebrum with evidence of neuronal degeneration, manifested by chromatolysis in the nerve cell body and axonal degeneration (Fig.2). Congestion of the meningeal blood vessels were also seen in the cerebrum and cerebellum.

**Uterus:**

The non gravid uteri showed mild endometritis manifested by severe congestion of the endometrial blood vessels and presence of serous exudate in the subepithelial tissue with heterophilic cellular infiltration . Foci of endometrial epithelium showed necrobiotic changes (Fig.3).

The gravid uteri and the uteri of the aborted animals had a diffuse or focal necrotic endometritis which extended to the muscular and serosal layers in the aborted uteri. Necrotic areas were infiltrated by both intact and degenerated heterophils. The heterophil cells were also seen in the endometrial glands (Fig.4) The placentation site had a similar inflammatory response.

**Spleen:**

Severe exhaustion and necrosis of the lymphoid elements were observed in white pulp. Hemorrhage was extensively seen in the red pulp. The fibrillar splenic stroma was devoid from the lymphocyte ( Fig. 5 ) .

**Sensitivity test:**

The results obtained are shown in Table (3).

## DISCUSSION

In Egypt, rabbit breeding is considered as an important source of good quality and economic animal protein because it needs simple requirements to start as well as its short production cycle and a large number of offsprings.

Our results indicate that *Listeria monocytogenes* was recovered from 18% of the examined rabbits. The same organism was isolated previously from rabbits (Galli, 1963; Vetesi and Kemenes, 1965 and Stanley ,1986).

Moreover, closely similar percentage of isolates was reported by Abd El-Waneas (1985) who recovered *Listeria monocytogenes* with an incidence of 20%. A much lower percentage was reported by Galli, (1969) who isolated only 2 isolates from dead cases.

The biochemical activities of the isolates recovered from the present study were similar to that reported by Cruickshank et al., (1975); Stanley, (1986). Experimental infection by instillation in the eye of rabbits and intraperitoneally in the mice indicated the pathogenic nature of the tested isolates, this were exactly similar to that reported by Abd El-Waneas, (1985) and Hassanein (1994).

The clinical signs and postmortem pictures of the diseased and dead rabbits reported in this study were agreed with those reported by Galli, (1963); Vetesi and Kemenes (1965); Abd El-Waneas (1985); Stanley (1986) and Abd El-Motelib *et al.* (1990) and disagree with those reported by Devois (1983) who recovered an out break of listeriosis without nervous signs.

The histopathological examination of the present positive cases clarified a typical lesions of listeriosis in rabbit which were described by Watson and Evans (1985) in the liver and uterus as well as by Taha *et al.* (1991) in the brain. So the presence of multiple necrotic foci infiltrated with heterophils in the liver and brain, in addition to necrotic endometritis were considered as differential diagnostic lesions of listeriosis in rabbit. Moreover, the obtained results in this study revealed that the gravid uteri showed severe necrotic endometritis while the non gravid uteri showed mild endometritis indicating that the gravid uterus was more affected than the non gravid one. Severe exhaustion of the lymphocytic elements in the spleen draw the attention to the effect of listeriosis on the lymphoid tissue of the affected rabbits, however this need further investigation

In vitro sensitivity testing of isolates to 11 antimicrobial agents revealed that the isolates examined were highly sensitive to gentamycin, tetracycline & spictrimycin, moderately sensitive to ampicillin, colistin sulfate, erythromycin & flumequine and weakly sensitive to furazolidone. None of isolates were sensitive to chloramphenicol, lincamycine and streptomycin. In this respect our results agree to some extent with those reported by Stanley (1986) and Abd El-Motelib *et al.* (1990). In conclusion, gentamycin and tetracycline are the most effective among all tested drugs, so they are recommended for treatment and controlling of such problem in rabbits. This in addition to the application of the usual hygienic measures with detection and elimination of the carrier animals.



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### LIST OF FIGURES

- Fig. 1:** Liver of rabbit showing heterophilic cellular infiltration in the necrotic area. Other hepatocytes undergo necrobiotic changes. H&E. 10 X 25.
- Fig. 2:** Brain of rabbit showing focal area of heterophilic cellular infiltration with evidences of neuronal degeneration. H & E. 10 X 40.
- Fig. 3:** Non gravid uterus showing mild endometritis manifested by congestion of the vasculature (c), oedema (O), heterophilic cellular infiltrations (H) and epithelial necrobiosis (N). H&E. 10 X 10.
- Fig. 4:** Gravid uterus showing necrotic endometritis with necrobiosis and sloughing of the epithelium (S), congestion of the blood vessels (C) and heterophilic cellular infiltration (H). H&E. 10 X 10.
- Fig. 5:** Spleen of rabbit showing necrosis (N) and exhaustion of the lymphocyte white pulp with presence of freely red blood cells in the meshes of the red pulp (R). H&E. 10 X 10.



Table (1): Percentage of positive cases of *Listeria monocytogenes* isolates.

No. of examined rabbits	No. of positive cases	%	No. of negative cases	%
50	9	18%	41	82%

Table (2): Biochemical activities of the suspected *Listeria monocytogenes* isolates

Sugar fermentation tests											
Dextrose	Maltose	Sucrose	Sorbitol	Xylose	lactose	Maltose	Irease	Catalose	Indole	H <sub>2</sub> S	motility

**Table (3):** Sensitivity of the isolates to antimicrobial agents in vitro.

<b>Chemotherapeutic agents</b>	<b>Sensitivity of <i>Listeria monocytogenes</i> isolates</b>
Gentamycin	+++
Tetracycline	+++
Spictinomycin	+++
Ampicillin	++
Colistin sulfate	++
Erythromycin	++
Flumequine	++
Furazolidone	+
Chloramphenicol	-
Lincomycin	-
Streptomycin	-

+++ = Highly sensitive  
++ = Moderate sensitive  
+ = Weakly sensitive  
- = Resistant













