Bacteriological And Pathological Studies On Listeria Infection In Rabbits

El-Banna, H.I; Elwan, I.F.; Dina, M. Mahady and El-Shafie, A.A.
Animal Health Research Institute, Zagazig branch

ABSTRACT

Sixteen *Listeria monocytogenes* isolates were recovered from 88 diseased and freshly dead rabbits aged 1-6 month-old collected from different localities at Sharkia Governorate. *Listeria monocytogenes* were isolated with high percentage from liver, spleen and uterus. Arteriogram of isolated *L. monocytogenes* to common antimicrobial agents was performed. The isolates were sensitive to Gentamycin, Ampicillin, Amoxycillin and Ciprofloxacin and high sensitivity to Ofloxacin. The isolated *L. monocytogenes* was standardized as 0.5 x 10^3 CFU/ml and orally inoculated to 40 one-month-old rabbits 20 left as positive infected control and 20 rabbits were treated 24hrs. post infection for 3 days with 10mg Ofloxacin/kg b.wt. by the intramuscular rout; another 20 rabbits were used as negative control (non-infected and non-treated). The obtained results concerning clinical signs, mortality rate, reisolation of *L. monocytogenes* and the body weight showed that Ofloxacin at a dose 10mg/kg b.wt. was highly effective agent in the control of the experimental infection and improve the general health status. Histopathological findings of the examined organs from exprimentally infected rabbits revealed intense lesions of the liver, spleen, lungs, brain and kidneys.

INTRODUCTION

Rabbit listeriosis is an infectious, highly contagious bacterial disease of both rabbits and human beings. The pathogene is incriminated by many workers for several disease conditions e.g. bovine abortion *(1)*, encephalitis in cattle, sheep and horses *(2)* and bovine mastitis *(3)*. The disease is economically important in rabbit breeding as causing sudden death and/or abortion or delayed parturition or infertility; severe emaciation and nervous manifestations *(4)*.

Rabbit listeriosis is a zoonotic disease, where in 1986, the Council of State and Territorial Epidemiologists recommended that listeriosis be remand a reportable disease in the U.S.A. and so the disease became reportable in the USA by the health authorities *(5)*.

Listeriosis is a world wide in distribution, it caused by *Listeria monocytogenes*, which is a gram positive, facultative, intracellular bacterium of animal origin. Six species of listeria were recognized, *L. monocytogenes, L. innocua, L. welshimer, L. seeligeri, L. ivanovii* and *L. gray (6)*. Three species of them were pathogenic. The most pathogenic and economic important pathogen was *Listeria monocytogenes (7)*.

Prevention measures of rabbit listeriosis should be paid to both feed, specially silage and live attenuated vaccines which have been developed for use in animals and it is claimed that this offers some protection *(8)*. *Listeria monocytogenes* was controlled in pregnant rabbits by using a three days course of 11-15mg tetracycline per animal, repeated after 7 days *(9)*.

This study was planned to study the isolation of listeria from rabbits, antibiogram of isolated *L. monocytogenes* and trial for treatment of experimentally infected rabbits.

MATERIAL AND METHODS

Materials

(1) Samples and cultivation

Two hundred and ten specimens from brain, liver, lungs, heart, kidneys, uterus and lungs were collected from 88 diseased and freshly dead rabbits with 1 to 6 months average age received by Zagazig Vet. Research Institute, from private farms at different localities in Sharkia Governorate. The specimens were cultured on Nutrient broth for 24hrs at 37°C, then loopfull was streaked onto listeria selective agar base (Oxoid). Bacterial colonies were selected and further

morphological, biochemical identification and heamolytic activity of listeria were carried out *(10, 11).*

(2)Media: Nutrient broth, Listeria selective agar base, motility test medium and blood agar base*(11, 12).*

(3)Drug: Ofloxacin solution was obtained from United Co. for Chem. and Med. Preparations (UCCMA), Cairo, Egypt, each ml contains 100mg of Ofloxacin.

Methods

(1) Experimental infection:

Sixty, one-month old, apparently healthy rabbits were obtained from a private farm. The rabbits were divided into 3 equal groups. The first group remained as negative control (non-infected, non-treated). The second and third groups were infected orally with 0.5×10^9 CFU/ml *L. monocytogenes*. The third group treated with 10mg Ofloxacin/kg b.wt. by intramuscular route for three successive days, the treatment started 24hrs. post infection. The clinical signs, postmortem findings, histopathological findings, reisolation trials of inoculated *L. monocytogenes* and body weights of experimental rabbits were recorded.

(2) Antibiogram

Disc-diffusion method was carried out *(13)* using Mueller-Hilton agar plates and different antimicrobial agents including Oflatoxin. The results were interpreted according to Oxoid Manual Company *(12).*

(3) Histopathological examination

Specimens from brain, liver, lungs, spleen and kidneys were collected and fixed in 10% neutral buffered formalin for histopathological examination *(14).*

RESULTS

(I) Bacteriological examinations

Examination of 88 diseased and dead rabbits revealed the presence of 16 *Listeria monocytogenes* isolates with ratio 5.5%.

The rates of isolation were 100, 100, 100, 68.75, 37.5 and 37.5 percent from liver, uterus, lungs, spleen, kidneys and heart respectively (Table 1).

(II) Identification of the isolates

(1) Morphologically

The isolates were Gram-positive, small rod shaped, arranged in V and Y shape manner. It showed umbrella like motility between 20-25°C in semisolid agar, the maximum growth of which occurred 3 - 5mm below the surface.

On Listeria selective agar, it produced dark greenish colonies surrounded by black (dark brown) zone. The isolates showed heamolysis on blood agar base with 10% sheep blood.

(2) Biochemically

The isolates showed acid production from rhamonse fermentation, negative to broth mannitol and xylose fermentation, positive catalase, and negative nitrate reduction, urea, oxidase and indol test. This confirmed that the isolates strain, were *Listeria monocytogenes* strain.

(III) Result of sensitivity test

The isolates were highly sensitive to Ofloxacin, Gentamicin, Ampicillin, Amoxycillin, Norfloxacin and Ciprofloxacine and resistance to Cephalexin, Cefotaxime and Naldixic acid.

(IV) The clinical signs

The infected, non-treated rabbits (2^{nd} group), showed clinical signs manifested by depression, ruffling fur, diarrhea soiled hindquarters followed by emaciation. Postmortem examination of both freshly dead and sacrificed experimentally infected rabbits revealed severe congested liver, spleen, lungs, kidneys, brain and engorgement blood vessels with blood. In addition, retention of urine and pneumonia. The symptoms and P.M lesions were disappeared by medication with 10mg Ofloxacin.kg b.wt. for 3 successive days in 3^{rd} group. Infected and non-medicated rabbits (2^{nd} group) showed 40% mortalities, reduction in

body gain and reduction of feed consumption, in comparison with the medicated rabbits (3rd group) (table 3). The clinical signs and P.M. lesions were similar to that observed in naturally infected rabbits.

(V) Histopathological findings

Brain

The brain of experimentally infected rabbits with *Listeria monocytogenes* revealed numerous scattered micro abscesses associated with chronic inflammatory cell mainly lymphocyte, macrophages and glail cell represented as pyogranuloma (Figs. 1& 2).

Liver

The portal areas were infiltrated with round cells beside presence of proliferative bile ductules in liver of orally inoculated rabbit (Fig. 3).

Meanwhile, multiple area of necrotic changes in liver parenchyma were replaced by leukocyte, mainly lymphocyte, macrophage, and neutrophils (pyogrnuloma) were seen in the hepatic lobules (Fig. 4).

Lungs

Lungs of inoculated rabbit showed sever focal or diffuse pneumonic areas and focal alveolar emphysema, hyperplastic peribronchial lymphoid aggregation with oedema and leukocytic infiltration in some pulmonary areas could be seen (Figs. 5 & 6).

Spleen

The spleen revealed severe lymphoid depletion from the majority of white pulps with thickened splenic capsule and septa.

Kidneys

Kidneys of orally inoculated rabbits revealed focal suppurative interstitial nephritis scattered in renal cortex, the adjacent areas suffered from necrotic changes (Fig. 7) heavy lymphocytic infiltration, hyperemia with dilated capillaries in the renal cortex and medulla.

Table 1. Showing isolation results of L. monocytogenes from different localities and different organs

Localities	*No. of case*	*No. of positive case*	*Liver*	*Uterus*	*Lungs*	*Spleen*	*Kidneys*	*Heart*
Belbis	21	6	6	6	6	4	2	2
Abohamad	14	3	3	3	3	2	1	1
Diarb Negm	10	2	2	2	2	2	1	1
Zagazig	11	1	1	1	1	1	0	1
Abo-Kabeer	12	2	2	2	2	1	1	0
Kafr-Saqr	15	1	1	1	1		1	1
Kanayat	5	1	1	1	1	1		
Total	88	16	16	16	16	11	6	6
Percentage		5.5	100	100	100	68.75	37.5	37.5

Table 2. The sensitivity of L. monocytogenes to some anti-microbials

Chemotherapeutic agent	*Symbol*	*Concentration (Mg)*	*Diameter inhibition zone unit (mm)*
Ofloxacin	OFX	5	23
Gentamicin	CN	10	20
Amoxycillin	AMC	10	17
Ampicillin	AM	10	17
Nor Floxacin	NOR	10	15
Ciprofloxacine	CIP	5	17
Cephalexin	CL	30	12
Cefotoxime	CTX	30	12
Naldixicacid	NA	30	12

Table 3. Mortality rate, re-isolation of L. monocytogenes and body weight from one month-old infected and Ofloxacin Treated rabbits

Groups	*No.*	*Morbidity*		*Mortality*		*Reisolation*	*Mean body weight after 3rd week post experimental infection*
		No.	*%*	*No.*	*%*		
Control	20	0	0	0	0	0/5	1680 ± 60.5^{a}
Infected and non treated	20	16	80	8	40	5/5	1130 ± 70^{c}
Infected and treated with Ofloxacine	20	2	10	0	0	0/5	1650 ± 65^{a}

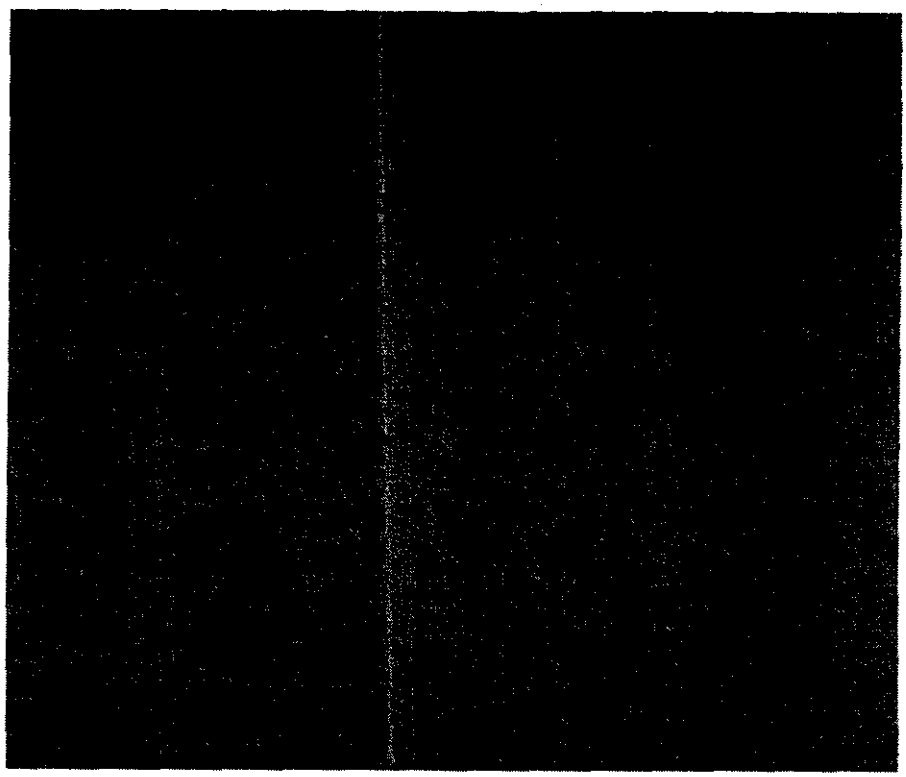


Fig. 1. Photomicograph of the brain of rabbit experimentally infected with Listeria monocytogenes, showing multiple micro abscesses (H & E x 120)

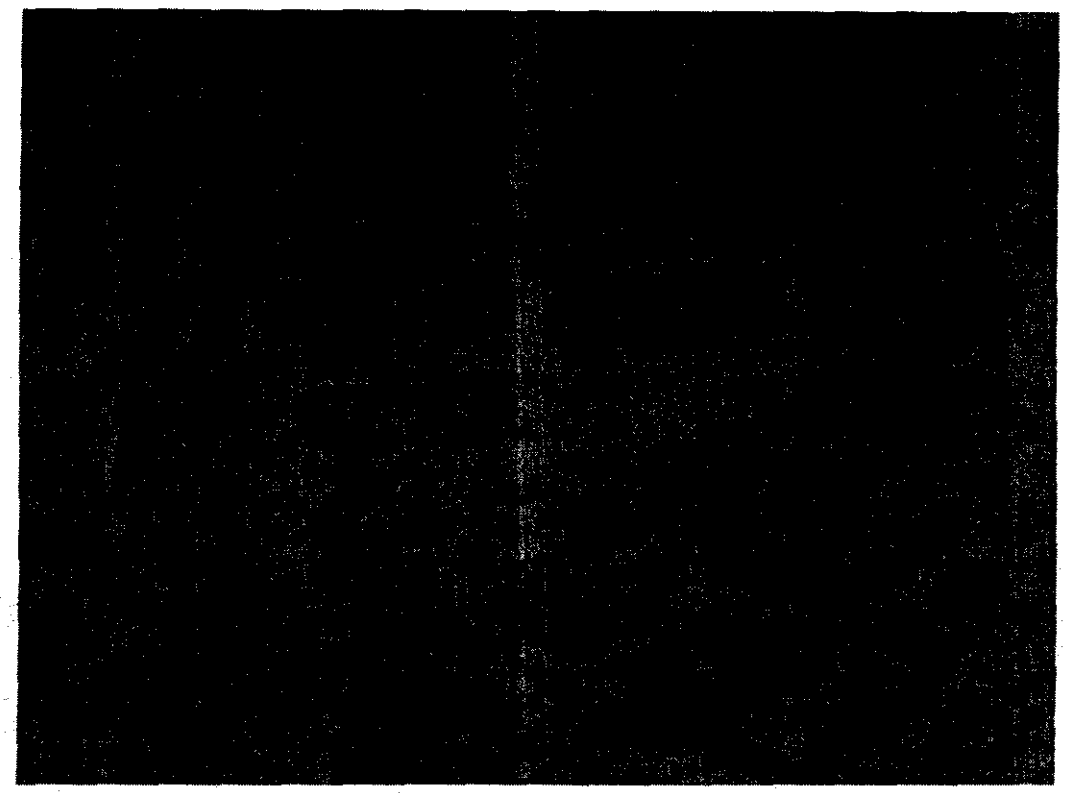
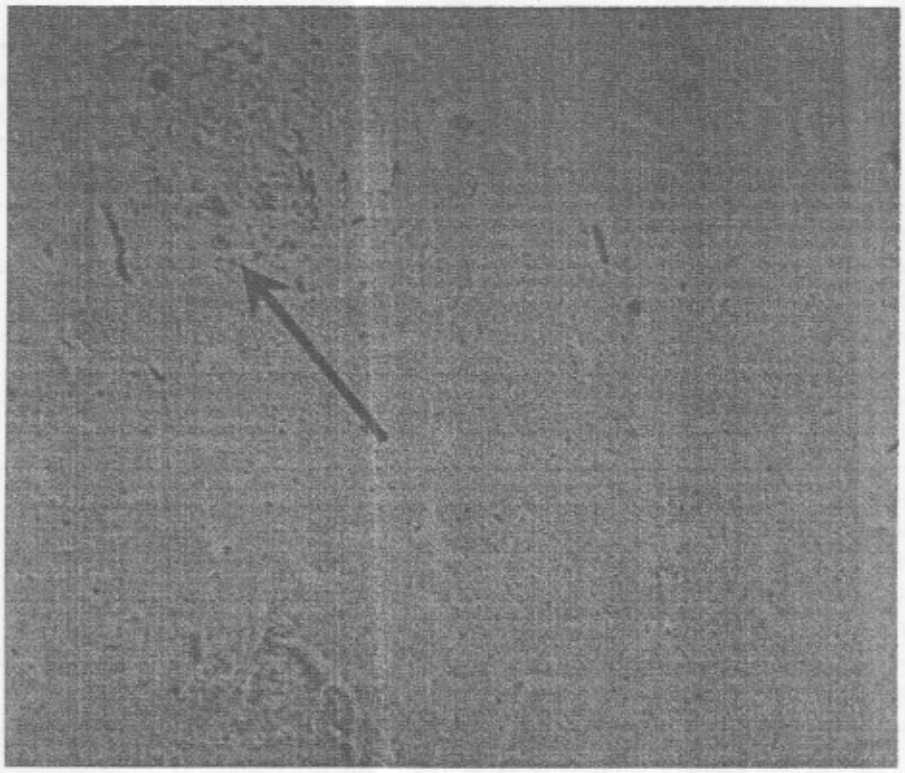


Fig. 2. High power of previous figure showing neutrophils, lymphocytes and macrophages (H & E x 300)



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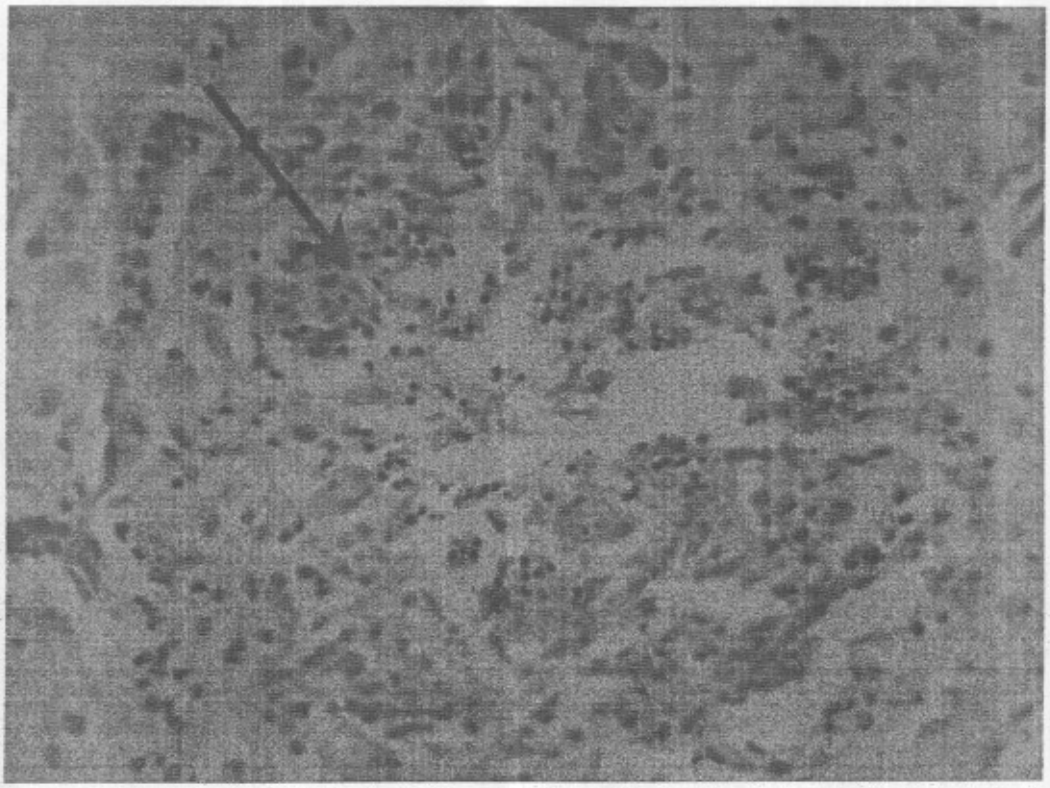

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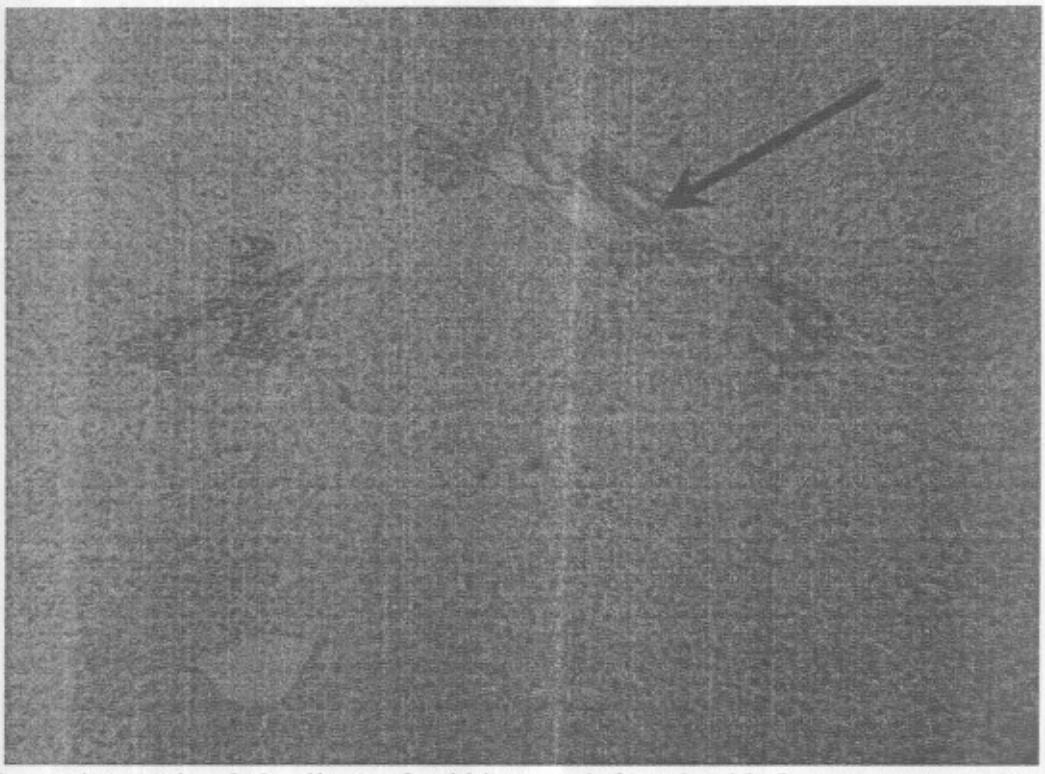

Fig. 3. Photomicograph of the liver of rabbit exp. infected with L. monocytogenes showing leukocytic aggregations in the portal area (H & E x 120)

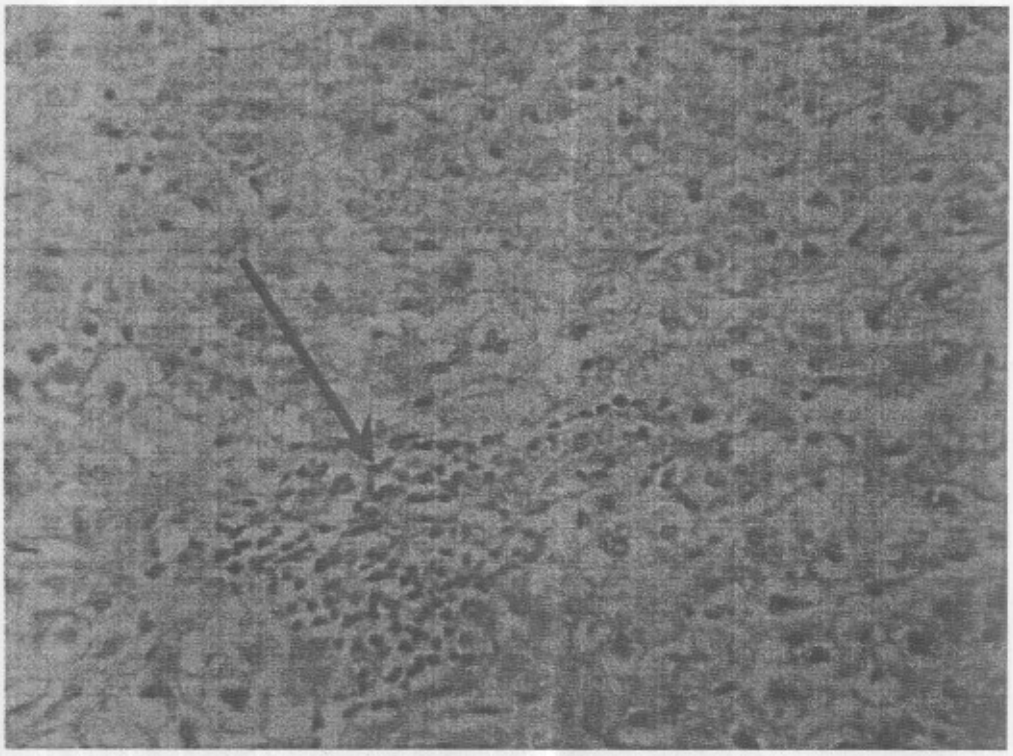

Fig. 4. Photomicograph of the liver of rabbit exp. infected with L. monocytogenes showing focal replacement of the hepatic parenchyma with lymphocytes, macrophage and neutrophils (H & E x 300)

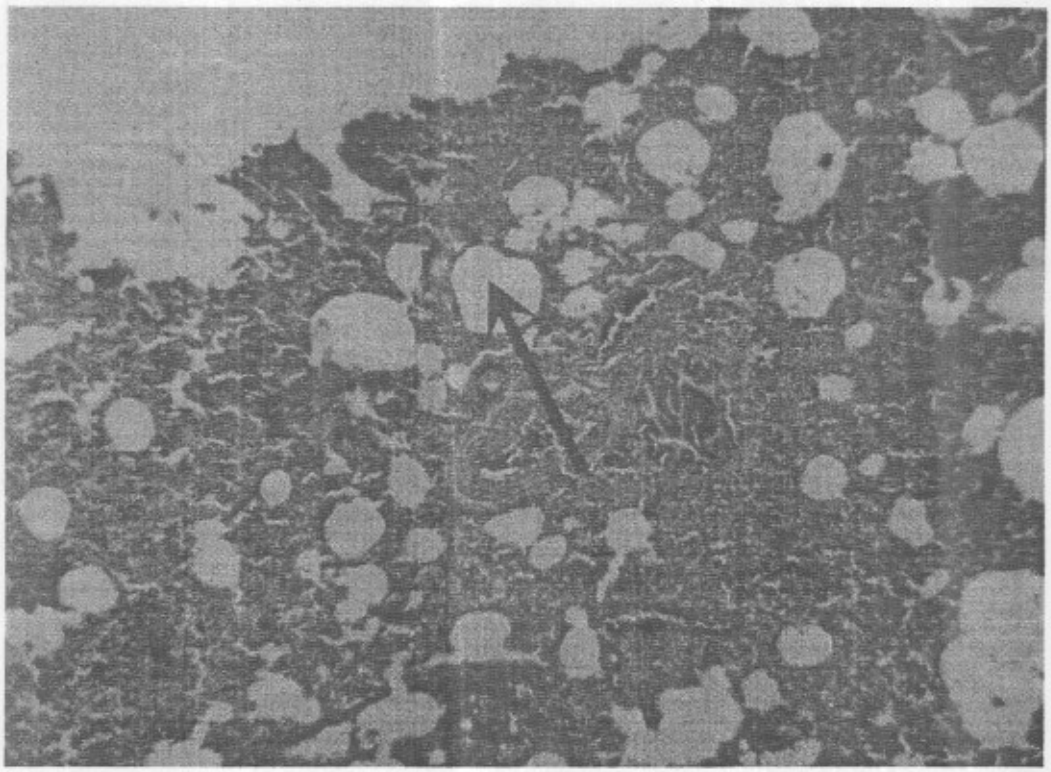

Fig. 5. Photomicograph of the lung of rabbit, exp. infected with *L. monocytogenes* showing pneumonic area and alveolar emphysema (arrow) (H & E x 120)

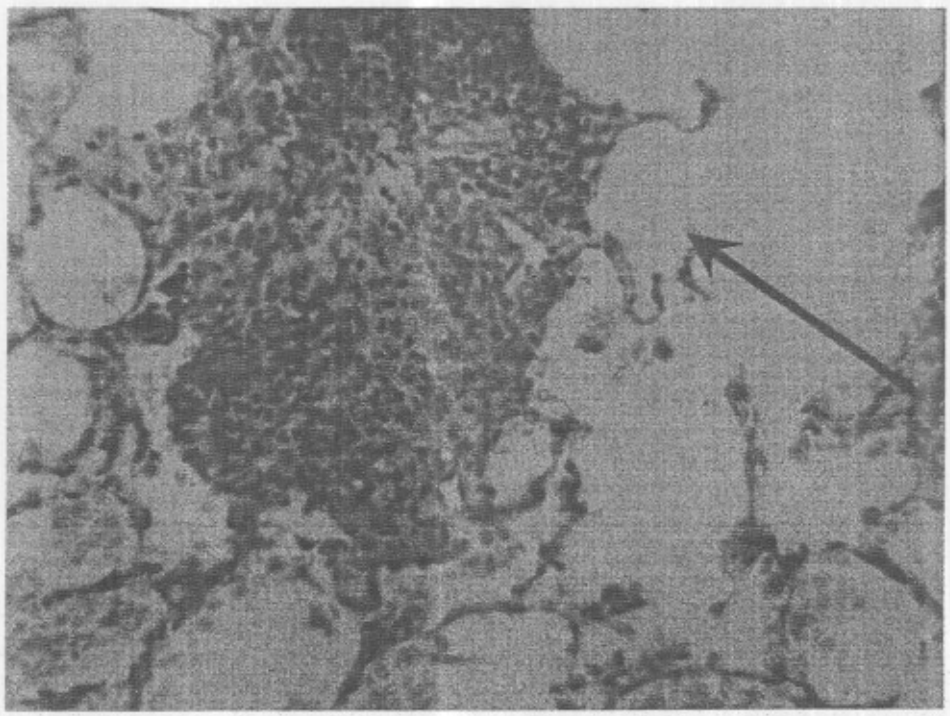

Fig. 6. High power of the previous figure to showing pneumonia and focal alveolar emphysema (arrow) (H & E x 300)

Regarding to histopathological finding of *L. monocytogenes* infected rabbits, the brain revealed numerous scattered micro abscesses associated with chronic inflammatory cell. These findings are similar to those reported by several studies *(20, 26).*

The liver of inoculated rabbits showed infiltration of portal area with round cells, beside presence of proliferative bile ductules. These findings were recorded previous *(18, 27, 28).*

The lungs of inoculated rabbits showed focal or sever diffuse pneumonic areas. These findings has been previously described *(27, 28).*

The spleen revealed severe lymphoid depletion in white pulps. The spleen in Listeriosis showed congestion and thrombosis in sinusoids and blood vessels *(27, 28).*

The kidneys of orally inoculated rabbits revealed multiple minute abscess scattered in renal cortex. Nearly similar lesions were cited *(27).*

It could be concluded that Listreiosis is an economic important disease affecting rabbits. The medication of *Listeria monocytogenes* infected rabbits with Ofloxacin, at a dose 10ml/kg b.wt for three days via I/M resulted in improvement in general health status and body weight.

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الملخص العربى

دراسات بكتريولوجية وباثولوجية عن عدوي الليستيريا في الأرانب

حلمي إبراهيم رجب البنا ، إبراهيم فكري علوان ، دينا محمد مهدي ، اشرف عبد الرحمن الشافعي
معهد بحوث صحة الحيوان – فرع الزقازيق

في هذه الدراسة تم عزل ١٦ معزولة بكتيرية من ميكروب الليستريا مونوسيتوجين من عدد ٨٨ أرنب مريض ونافق حديثاً عند عمر ١- ٦ أشهر جمعت من أماكن مختلفة بمحافظة الشرقية وكانت نسبة العزل عالية من الكبد والرئتين والرحم.

وبعمل اختبار حساسية لميكروب الليستيريا مونوسيتوجين المعزولة من الأرانب لبعض مضادات الميكروبات شائعة الاستخدام، اتضح حساسية الميكروب لـ: الاوفلوكساسين والامبيسيلين والاموكسيسلين والجينتاميسين.

وبالإضافة لما سبق تم إجراء عدوى صناعية لعدد ٤٠ أرنب عمر شهر بميكروب الليستريا المعزول عن طريق الفم (بجرعة ٠,٥ × ١٠^{٩}) وتم تقسيمها إلى مجموعتين متساويتين (٢٠ أرنب تركت كمجموعة ضابطة إيجابية العدوي وبدون علاج، ٢٠ أرنب عولجت بعد العدوي بـ ٢٤ ساعة لمدة ٣ أيام بعقار الأفلوكساسين ١٠مجم/كجم بالحقن العضلي) بينما ترك ٢٠ أرنب أخري كمجموعة ضابطة سلبية (لم تعدي ولم تعالج).

واستناداً إلى الأعراض الظاهرية للمرض ونسبة النفوق وإعادة العزل للميكروب من الأرانب المصابة (غير المعالجة) والمعالجة بالإضافة إلى الزيادة في الوزن كان الحكم بكفاءة الأوفلوكساسين بجرعة ١٠مجم/كجم وزن حي في الوقاية من العدوي وتحسين الحالة الصحية العامة للأرانب.

كما تم تسجيل نتائج الهستوباثولوجي لهذه الأرانب ووضح أن العدوى بالميكروب المعزول أدى إلى تغييرات باثولوجية شديدة في الكبد والطحال والمخ والرئتين والكلى.