STUDIES ON SHEEP *LISTERIOSIS* IN SOME GOVERNORATES IN EGYPT

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Abstract

This work was carried out on 48 sheep animals (21, 15 and 12), collected from Ismailia , El-faoum and Giza Governorates, respectively, which were suffering from nervous manifestations as (circling, bilateral facial paralysis and drooling) .Clinical, postmortem inspection and brain tissue microbiological investigations were carried out. In addition, Pathogenicity of the isolated strains in mice, Anton's test in rabbit and antimicrobial sensitivity test were adopted. The results of bacteriological examination revealed that *L. monocytogenes* was recovered through direct plating from mid brains of 5 cases (10.4 %) with percentages of 14.3, 6.6 and 8.3 % in Ismailia, Elfayum and Giza Governorates respectively, while after cold enrichment procedure, the organism was recovered from 7 cases (14.6%) with percentages of 19.04, 13.3 and 8.3 % from the same governorates respectively All (100%) *L. monocytogenes* I/ P inoculated mice showed pathological signs in the brain , heart ,liver and spleen and all instillated rabbits with the same organism (100%) were positively affected as described by Anton's eye test .On the other hand , the antimicrobial sensitivity test of the *L. monocytogenes* isolates recoverd from sheep against different antimicrobial agents were studied .All tested (100%) strains were sensitive to Gentamicin, and Tetracycline but resisted Naldixic acid (0%) , while most (85.7%) of them were sensitive to Penicilline G then Erythromycin (71.4%) but resisted Amoxicillin, and trimethoprim sulfamethazol (14.3 % for both) . The study concluded that for the best diagnosis of *Listeriosis* in sheep and isolation of *L. monocytogenes*, specimens must include parts of brain stem and Medulla oblongata using the cold enrichment producer.

Keywords : Sheep *listeriosis*, *L. monocytogens*, Pathogenicity, Anton's test.

INTRODUCTION

Listeriosis is a specific bacterial disease of animals characterized, in some livestock species, by three distinct syndromes, meningeoencephalitis, abortion or stillbirth and neonatal septiceamia (**Low, & Renton, 1985, and Schneider, 1994)** that is known as circling disease when it occurs in sheep leading to some symptoms vary from circling behavior and drooling up to severe neurological impairments and ultimately death (**Jerry,1999 and Darian , 2009)** .It is caused by bacterium *Listeria monocytogenes (L. monocytogenes)* widely distributed in nature and has been isolated from a wide variety of healthy and diseased mammals and bird species as well as from soil, water, mud and silage (**Blenden, 1986).**

Otherwise, *L. monocytogenes* may invade sheep causing the enteric form of *Listeriosis* (Fairley, *et al*, 2012).

It is well known that *L. monocytogenes*infection resulting in high economic losses as it cause deaths of sheep within 2-5 day and of cattle within 7-10 days of infection (**Low and donachieuw, 1997**). Moreover, it is represented as a foodborne pathogensince it couldbe transmitted among either adults or young humans through consumption of the infected cheese, yoghurt (**Sabreen and Eman, 2001 ,and Sohir and Hanaa 2009)** unpasteurized milk **(Chinching, 1998)** or raw sea meals **(Yassin *et al* 2010).**

Since several cases of circling, bilateral facial paralysis and drooling were reported of sheep at Ismailia , El-faoum and Giza provinces and owing to the above mwntioned economic and zoonotic importances, the current study dealt with this field problem aiming establish the best isolation method of. the causative agent, its pathogenicity and its antimicrobial susceptibility testing.

MATERIALS AND METHODS

Animals: The present study was carried during winter on a total of 48 emergency slaughtered sheep aged between 3-6 months old which had nervous manifestations as circling, bilateral facial paralysis and drooling, collected from farms in Ismailia , El-fayum and Giza Governorates. The brains of the slaughtered animals were collected and prepared for bacteriological examination .

Bacteriological examination :-

Isolation:-

A- Direct plating method, Under complete aseptic conditions and disinfection of the outer surfaces of the collected brains, a loopful from the entire brain tissue samples was inoculated directly onto 5% sheep blood agar and Listeria selective agar (Oxford) Oxoid. Plates were incubated aerobically at 37°C for 24-48 hrs.

B- Cold-enrichementprocedure : also under complete aseptic conditions, small pieces of brain tissues (specimens must included parts of brainstem and medulla oblongata) were homogenized and a 10% suspension which was done in nutrient broth. The broth suspension was placed in the refrigerator at 4°C and subcultured onto blood agar once weekly for up to 12 weeks (**Quinn *et al.*, 1994).**

Identification:

1. Microscopicalexamination : Films from the pure suspected colonies were stained with Gram stain and (**Quinn *et al.*, 1994).**

2.Biochemical identification: Pure isolates were identified biochemical according to **(Quinn *et al.* 2002 and Warburton *et al.* 2003).**

3.Motility test : -Semisolid trypticase soya agar with yeast extract was stabbed . The isolates were incubated for 7 days at room Temperature (20-25 °C) and observed daily to detect the characteristic growth and the motility **(Quinn *et al.* 2002 and Warburton *et al.* 2003).**

4.Pathogenicity test :

a-Mice inoculation :- Forty mice were included, five mice were used for each isolate, where each mouse was injected I\P with 0.1 ml of the bacterial suspension (approximately 10^9 cfu / ml) and five mice were kept as control not inoculated... The death rate and P/M changes as well as the reisolation of the organism from the internal organ and heart blood were carried out **(Seelinger and Jones 1986).**

b-Rabbit instillation (Anton's test): Each rabbit's eye was instillated with 0.1 ml of the previously mentioned bacterial suspension into its conjunctiva. Conjunctivitis within 24 hrs was noticed. **(Anton 1934).**

Antimicrobial sensitivity testing:

*L. monocytogenes*isolates were tested against 9 different antimicrobial agents, Gentamicin (10 µg), Erythromycin (15 µg), Tetracyclin (30 µg).Chloramphenicol (30 µg).Trimethroprimsulfamethizol (25 µg), Naldixic acid (30 µg) and Ampicillin (10 µg) was tested by the agar disc diffusion method Quinn *et al.* (1994).the degree of sensitivity was determined and interpreted according to **Manual of Clinical Microbiology, (1999)**

RESULTS

Through P/M examination of the brains of emergency slaughtered sheep revealed congestion and thickening of meningial membrane.Bacteriological examination revealed isolation of *L.monocytogenes*using direct plating from mid braintissus (5 cases representing 10.4 %)in percentages of 14.2 %,6.6 % and 8.3 % in sheep belonged to Ismailia , El-faoum and Giza Governorates respectively, while by using enrichment procedure from brain stem and medulla oblongata (7 cases representating14.6 %).in percentages of 19.04 % 13.3 % and 8.3 % in the same

The Inoculated mice showed depression , off food shortly before deaths that occurred within 48 hrs post inoculation. P/M inspection revealed sever congestion of parenchymatous organs except lung and brain were observed in postmortem study (Table 2), as well as, *L.monocytogenes* could be reisolated from their parenchymatus organs (Table 3) .All isolated strains caused purulent keratoconjunctivitis within 24-36h post rabbit instillation (Table 4).The antibiogram of *L.monocytogenes* was tabulated in Table (5).

Table 1. Incidence of of*Listeria monocytogenes*isolates in relation to the method used.

Governorate	Number of samples	Positive samples			
		Direct plating method		Cold enrichment method	
		No.	%	No.	%
Ismailia	21	3	14.2	4	19.04
El-Fayum	15	1	6.6	2	13.3
Giza	12	1	8.3	1	8.3
Total	48	5	10.4	7	14. 6

Table 2. Death rate in mice subjected to the Pathogenicity test

Lab. animals	Injected dose	Death up to			Death rate	
		1st day	2nd day	3rd day	No	%
Inoculated mice (n=35)	0.1 ml of $1x10^9$cfu /ml	17	18	0	35	100
Control mice (n=5)	Not injected	0	0	0	0	0

Table 3. Reisolation ofL.monocytogenesfromdifferent organs of experimentally injected mice.

Site of reisolation	*L. monocytogenes*
Brain	+
Heart	+
Liver	+
Lung	-
Spleen	+

Table 4.Experimentally instillatedrabbit eyes with L.monocytogenes isolates (Anton's test)

Instillatedstrain	No. of isolates	Dose of instillation / eye	Positive Anton's test	
			No	%
L. monocytogens	7	$1x10^9$cfu / ml	7	100

Table 5. Antibiogram of L. monocytogenes(7 isolates).

Antimicrobial agent	Conc.	Sensitive (n=7)		Resistances (n=7)	
		No	%	No	%
Gentamicin	10 µg	7	100	0	0
Penicillin G	10ug	6	85.7	1	14.3
Erythromycin	15 µg	5	71.4	2	28.6
Tetracycline	30 µg	7	100	0	0
Chloramphenicol	30 µg	4	57.1	3	42.9
Trimethoprim sulfamethizol	30 µg	1	14.3	6	85.7
Naldixic acid	30 µg	0	0	7	100
Ampicillin	10 µg	1	14.3	6	85.7
Amoxicillin	10ug	5	71.4	2	28.6

DISCUSSION

Sheep Listeriosis is an infectious,but not contagious,sincetranmission occurs occurs after the bacteria has proliferated in substances such as soil ,faeces of animals, and rotting vegetation, which ingested by sheep or where low quality silage bales become mouldy (Darian, 2009), then invade through oral mucosal abrasions (ChinChing, 1998) ascending via the trigeminal nerve to brain stem and medulla oblongata. So among all the transversal and longitudinal sections into the infected brain (Kahan, et al. 2006). In the present study, L. monocytogenes infection showed that the higher percentages of isolation from sheep belonged to Ismailia and El-fayum Governorates (19.04 and13.3 %, respectively) than that in Giza Governorate (8.3%). The variation may be explained as a results of several factors including feeding of large quantities of poor quality silage with pH in excess of 5.5 (Wilsemith and Gitter, 1986), resulted in growth of bacteria. Particularly in the top and side layers of the silage, (Arimi, et al. 1997). As Listeriosis tend to be seasonal disease (Czuprynsk, 1993). It was obvious that – through the current study – all infected sheep were emergency slaughtered during winter season and the bacteriological investigation revealed that cold enrichment procedure for L. monocytogenes isolation was better than direct plating since the organism grows readily at a temperature of 4°C for a period of time ranged from 5 weeks to 3 months (Coetzer, et al 1994).

In the present work,I/P inoculation of the mice with the isolated strains in the dose of 109 cfu / ml caused their death within 48 h to confirm the identification as L. monocytogenes as recoded (Schonberg, 1989, and Morsi, et al 2006), and could be successfully reisolated from brain, heart, liver and spleen of inoculated mice (Marco, et al., 1997, and Sohair and Hanaa, 2009). The production of an experimental purulent keratoconjunctivits (Anton's eye test) is another classical

This conjunctivitis usually heals spontaneously and the animal rarely dies but may be followed by keratitis (Parker and Collier, 1990).

Sensitivity of L. monocytogenes strains to Gentamicin, Tetracycline ,Penicilline G , Erythromycin and Amoxicillin and their resistance against Trimethoprim sulfamethazol, Naldixic acid and Ampicillin, through the present findings ascertained the concluded results of the previous studies (Al Said, et al., 1998 and Morsi, et al 2006). It was concluded that for the best diagnosis of Listeriosis in sheep, specimens must included part of brain stem and Medulla oblongata and use the cold enrichment producer for isolation of microorganism.

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دراسات فى الليستريا المعزوله من الخراف فى بعض محافظات مصر

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اجريت هذه الدراسه على ٤٨ من الخراف المريضه من محافظات الاسماعيليه والفيوم والجيزه وهذه الخراف كانت تعانى من دوران وحالات عصبيه مما أدى الى ذبحها اضطراريا وتم أخذ المخ منها تحت ظروف التعقيم الكاملة وقد اجرى الفحص البكتريولوجى لهذه العينات لعزل المسبب البكتيرى. وبالفحص البكتريولوجى بالزرع المباشر من المخ تم عزل ميكروب اللستريا مونوسيتوجينز من ٥ حالات أغنام(١٠,٤%) من محافظات الاسماعيليه والفيوم والجيزه بنسب ١٤,٢% &٦,٦% &٨,٣% على التوالى و بطريقة الزرع بالتحضين فى الثلاجه (الطريقه البارده) تم عزل الميكروب من ٧ حالات (١٤,٦ %) بنسب ١٩.٤% و ٣,١٣% و ٨,٣% على التوالى من نفس المحافظات . كما تم حقن الفئران معمليا بهذه العترات السبعة المعزولة فى السبطن حيث تأثرت كل الفئران المحقونة ونفقت فى المدة ٤٨ ساعة عقب الحقن وأظهرت آفات تشريحية فى المخ والقلب والكبد والطحال وبتقطير نفس العترات فى عين الارانب أظهرت التهاب الملتحمة الصديدى بنسبه ايجابيه (١٠٠%) فيما يعرف باختبار أنتو ن فى العين . وباجراء اختبار حساسيه هذه المعزولات (اللستريا مونوسيتوجينز) ثبت حساسية حميعها (١٠٠%) للجنتاميسين والتتراسيكلين ومقاومة جميعها أيضا للنالدكسك اسيد (صفر %) وحساسية معظمها للبنيسيللين (٨٥,٧%) ثم الارثرومايسبن (٧١,٤%) كذلك مقاومة معظمها للتراى ميثبريوم والامبسيللين (١٤,٣% لكل منهما). ومن هذه الدراسه يستخلص أن الطريقه الافضل لعزل هذا الميكروب هى بأخذ عينات من نسيج المخ بأجزاء من الخلايا الجذعية وباستخدام طريقة التحضين البارده فى الزرع البكتريولوجى حيث نسب العزل فيها أعلى من نسب العزل بطريقة الزرع المباشر .