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Some studies on Listeria Monocytogenes infection in buffaloes

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The present study concerned with *Listeria monocytogenes* which were isolated from apparently healthy buffaloes, buffaloes suffered from abortion, bedding and silage, which were collected from different localities. From the total examined samples (375), (300 samples from apparently healthy buffaloes, bedding and silage, also 75 samples from buffaloes suffered from abortion , bedding and silage) the prevalence of *L. monocytogenes* was 11(14.7%), isolates from buffaloes suffered from abortion, bedding and silage. All isolates were motile at room temperature, also exhibited positive CAMP test and exhibited narrow zone of β-hemolysis. Serological identification of isolates revealed 6(54.5%) were serotype 4 while 5(45.5%) were untyped. There were differences in isolates susceptibilities to different anti-microbial agents. *L. monocytogenes* had 100% sensitivity to Ampicillin, chloramphenicol, erythromycin, tetracycline and ceftiofur sodium. Virulence of isolates was assayed by I/P inoculation of 10^9 CFU in mice, all inoculated mice died within 1-5 days. PCR assay for *L. monocytogenes* isolates revealed positive amplification of 827 bp fragment of act A gene.

Listeria monocytogenes is facultative intracellular Gram-positive coccobacilli that cause listeriosis. The feeding of silage, particulary silage of inferior quality, appears to predispose animals to the development of infection by *Listeria* (Gitter *et al.,* 1986). Listeriosis is a frequent cause of abortion in cattle and sheep. In cases of milkborne infection, the organism is excreted to milk in an intracellular state within bovine neutrophils and macrophages (Bunning *et al.,* 1988). Cattle and sheep shed *Listeria monocytogenes* in faeces and manure and these materials, along with spoiled silage have been used as fertilizer without benefit treatment which may be the most significant sources of transmission of *Listeria* infection to animals. Abortion, still birth, septicemia, meningitis, endocarditis, conjunctivitis, pharyngitis and flue-like illness are known to be manifestation of infection by *Listeria monocytogenes*.

The aim of the present investigation was directed to the following. Isolation of *Listeria monocytogenes* from apparently healthy buffaloes, aborted buffaloes, bedding and silage. Determination of the pathogenicity of the isolates in mice, motility test, CAMP test and hemolytic activity on blood agar. Serological identification of *L. monocytogenes* isolates. Determination of antibiogram of *L. monocytogenes* isolates. Characterization of *L. monocytogenes* isolates by PCR assay.

Materials and methods

Samples. A total of 375 samples were collected from buffaloes, bedding and silage as the following: 300 samples were collected from apparently healthy animals (vaginal swabs, nasal swabs, and swabs from eye), bedding and silage samples; 60 sample for each. 75 samples were collected from aborted buffaloes (vaginal swabs, nasal swabs, swabs from eye), bedding and silage, 15 samples for each. The samples were collected from different localities in clean sterile containers. All samples were sent to laboratory in an ice box with a minimum of delay.

Isolation and identification of isolates. It was done according to Quinn et al., (2002).

Media used for identification of *Listeria*. *Listeria* Oxford agar supplemented with CCFA., Palcam agar supplemented with *Listeria* Palcam antimicrobic (SR 150 E) and Trypticase soy medium with 0.6% yeast extract were used for cultivation, identification, detection of motility as well as for antibiogram assay. Blood agar was used for detection of hemolytic activity of isolates and in CAMP test.

Motility test. it was done according to Federal Register, (1988)

CAMP test. It was done according to Federal Register (1988). For detection of the synergistic reactions of hemolysim of *L. monocytogenes* with beta-toxin of S. aureus.

Pathogenicity in mice. It was done according to Federal Register, (1988). Three mice were used for each isolate. Each mouse was injected intraperitoneally (I/P) with 0.1 ml of the bacterial

suspension. Each mouse received approximately 10^9 bacterial cells; three mice were inoculated I/P with sterile saline solution and were kept as control. The death rate and post-mortem changes as well as the re-isolation of the organism from the internal organs and heart blood were recorded.

Serotyping of *L. monocytogenes* isolates. It was done according to Federal Register, (1988). *Listeria monocytogenes* antisera type 1, 4 and 1/2a (Behring werke AG) were used.

Antibiogram of the isolated strains. The antibiotic sensitivity test was done according to Finegold and Martin, (1982), and the following discs were used: amoxycillin, ampicillin, cephalexin, chloramphenicol, erythromycin, lincomycin, ofloxacin, penicillin G, rifampicin, streptomycin, tetracycline and ceftiofur sodium.

PCR assay. for detection of actA gene in the extracted DNA of 3 *L. monocytogenes* type 4 isolates according to Xiaohui and Jiao, (2005).

Results and discussion

All isolates were motile at room temperature, CAMP positive and β-hemolytic. Serotyping of 11(100%) *L. monocytogenes* isolates which were recovered from examined samples, revealed that 6(54.5%) isolates were serotype 4 and 5(45.5%) isolates were untyped. The post-mortem examination of dead mice revealed the presence of multiple tiny foci of necrosis among spleen and liver as well as congestion of the subcutaneous tissues. Also, *L. monocytogenes* isolates could be recovered from internal organs and heart blood of dead mice.

Listeriosis is a serious infectious disease caused by *Listeria monocytogenes* which has been recognized as a significant pathogen, occurring worldwide, capable of causing animal and human infections leading to severe economic losses (Maja et al., 2003).

Table (1) showed the prevalence of *L. monocytogenes* in samples collected from aborted buffaloes as the following. 4(26.7%), 3(20%), 1(6.7%), 2(13.3%), and 1(6.7%) were positive and 11(73.3%), 12 (80%), 14(93.3%), 13(86.7%) and 14(93.3%) were negative samples for *L. monocytogenes* from vaginal swabs, nasal swabs, swabs from eye, bedding and silage respectively.

Capita et al., (2001) concluded that the Palcam medium was more selective than the modified Oxford medium for isolating *Listeria* species. Moreover, EL-Gedawy, (2009) indicated that the highest incidence of *L. monocytogenes* was recovered from silage 10%. But, he could not recover *L. monocytogenes* from bedding samples.

All isolates were motile at room temperature these results come in agreement with that of Hindy, (2006). All isolates were positive for CAMP test. Also, Mckellar, (1994) stated that CAMP test is used for distinguishing *L. monocytogenes* from other non pathogenic *Listeria*. In this investigation *L. monocytogenes* isolates formed narrow zone of β-hemolysis. Isome et al., (1995) stated that hemolysin (Listeriolysin 0) has been considered as a main component of virulence of *L. monocytogenes*.

Serotyping of *L. monocytogenes* isolates recovered from examined samples revealed that 6(54.5%) isolates were typed as serotype 4. Meanwhile, 5(45.5%) could not be typed by the available antisera. The obtained results coincided to large extent with that of Hindy, (2006) who typed 14 *L. monocytogenes* isolates and found that, 4 isolates were belonged to serotype 1 and 8 isolates were typed as serotype 4, while 3 isolates were untyped.

Antibiogram of 13 chemotherapeutic agents on 11 isolates were presented in Table (2) all isolates were completely resistant to lincomycin and streptomycin. All isolates were 100% sensitive to ampicillin, chloramphenicol, erythromycin, tetracycline and ceftiofur sodium. While, there wee differences in isolates sensitivity to the other chemotherapeutic agents. In this concern, Hindy, (2006) found that 100% of the examined isolates were sensitive to ampicillin, 86.7% to erythromycin, 80% to chloramphenicol and 73.3% to amoxycillin, while, 100% of the examined isolates were resistant to lincomycin and streptomycin.

The data presented in Table (3) indicated that the intraperitoneal injection (I/P) of mice with 10^9 c.f.u. *L. monocytogenes* caused 100% death with in 1-5 days. These results come in agreement with the findings of El-Gedawy, (2009). These results come in accordance with the findings of Parker and Collier, (1990). Results achieved in Table (4), Fig. (1) revealed positive amplification of 827 bp fragment of actA gene was observed from the extracted DNA of 3 (100%) *L. monocytogenes* isolates. In this concern, Wiedmann et al., (1997) indicated that actA gene providing us with a good opportunity to find new clonal group and to investigate the contamination pattern of *L. monocytogenes* in the environment. Also showing that act A is a highly polymorphic virulence protein.

Table (1): Prevalence of *Listeria monocytogenes* in buffaloes.

Type of samples	Animal status									
	Apparently healthy buffaloes					Aborted buffaloes				
	No.	+Ve	%	-Ve	%	No.	+Ve	%	-Ve	%
Vaginal swabs	60	0	0	60	100	15	4	26.7	11	73.3
Nasal swabs	60	0	0	60	100	15	3	20	12	80
Swabs from eye	60	0	0	60	100	15	1	6.7	14	93.3
Bedding	60	0	0	60	100	15	2	13.3	13	86.7
Silage	60	0.0	0.0	60	100	15	1	6.7	14	93.3
Total	300	0.0	0.0	300	100	75	11	14.7	64	85.3

* The percent was calculated according to the number of examined samples.

Table (2): Antibiogram of the isolated strains.

Antibiotic disc used (mg/disc)	Sensitive		Resistant	
	No.	%	No.	%
Amoxycillin	8	72.7	3	27.3
Ampicillin	11	100	0.0	0.0
Cephalexin	7	63.6	4	36.4
Chloramphenicol	11	100	0.0	0.0
Erythromycin	11	100	0.0	0.0
Gentamycin	9	81.8	2	18.2
Lincomycin	0.0	0.0	11	100
Ofloxacin	8	72.7	3	27.3
Penicillin G	*10	90.9	1	9.1
Rifampicin	7	63.6	4	36.4
Streptomycin	0.0	0.0	11	100
Tetracycline	11	100	0.0	0.0
Ceftiofur sodium	11	100	0.0	0.0

* The percent was calculated according to the total number of isolates (11).

Table (3): Mice pathogenicity test of *L. monocytogenes*.

No. of examined isolates	No. of inoculated mice	No. of dead mice		Death within
		No.	%	
11	33 (3 per isolate)	33	100	1-5 days
-Ve control	3	0	0	No deaths

* The percent was calculated according to the number of inoculated mice.

Conclusion. It is an important to mention that this investigation aimed to characterize *L. monocytogenes* isolates from Egyptian buffaloes which may contaminate carcasses during slaughter and processing, thereby transmit infection to man and cause human disease. Poor-quality silage should not be fed to pregnant animals. Also, feeding methods which minimize direct ocular contact with silage should be implemented. Also, we recommended early and rapid treatment of diseased animals with drug of choice. Moreover, good management should be follow and separation of diseased animals to avoid spread of infection.

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Table (4): Characterization of *L. monocytogenes* isolates by PCR assay

Examined isolates	ActA gene
Isolate No.1	+
Isolate No.2	+
Isolate No.3	+

+: gene present

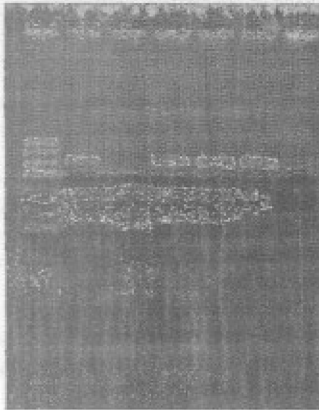

Fig. (1): Shows agarose electrophoresis of PCR amplification of 827 bp Fragments of actA gene from extracted DNA of L. monocytgenes type 4 isolates. Lane M shows 100 bp ladder marker. (1= + Ve control, 2 = - Ve control. 3, 4 and 5=examined isolates).

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بعض الدراسات على الإصابة بالليستريا مونوسيتوجينز في الجاموس

أجريت هذه الدراسة على ميكروب الليستريا مونوسيتوجين الذى تم عزله من عينات تم تجميعها من مناطق مختلفة من الجاموس السليم ظاهرياً والجاموس المصاب بالإجهاض والفرشة والسيلاج. وكان عدد العينات الكلى ٣٧٥ عينة (٣٠٠ عينه من الحيوانات السليمة ظاهرياً والفرشة والسيلاج، و ٧٥ عينة من الجاموس الذى أصيب بالإجهاض ومن الفرشة والسيلاج). تم عزل ١١ عتره من ميكروب الليستريا مونوسيتوجين بنسبة (١٤.٧%) من عينات الجاموس المصاب بالإجهاض والفرشة والسيلاج. وكانت كل المعزولات متحركة في درجة حرارة الغرفة وإيجابية لاختبار الكامب وأيضاً حققت منطقة ضيقة من تحليل الدم (ب).

كما جاءت نتائج التصنيف السيرولوجي كما يلي:
(٥٤.٥%) إيجابية لنوع ٤ بينما ٥(٤٥.٥%) لم يتم تصنيفها سيرولوجياً. كما وجدت اختلافات في نسبة إستجابة المعزولات لاختبار الحساسية فكانت استجابتها ١٠٠% للأمبيسلين وكلورمفنيكول واريثروميسين وتتراسيكلين وسيفتيفيور صوديوم. وأيضا تم قياس كفاءة المعزولات بحقنها في الفئران داخل التجويف البريتوني بمقدار ١٠ ^١ خلية بكتيرية،نفقت كل الفئران المحقونة بالميكروب.
أثبتت نتائج اختبار البلمرة المتسلسل وجود actA جين في المعزولات التى تم اختبارها.