PRELIMINARY TRIAL FOR PREPARATION OF VACCINE AGAINST LISTERIA MONOCYTOGENES IN RABBITS

Salma A. Shoulah[1], Mohamed G. Abdelwahab[1], Zakaria, I.M.[2] , Abdelmoneim M. Moustafa[1] and Faysal K. Hamouda[1]

[1]Department of Animal Medicine (Infectious Diseases) –Faculty of veterinary Medicine- Benha University, [2]Researcher in Animal Health Research Institute –Dokki-Giza-Egypt

A B S T R A C T

This study was applied on 631 sheep with ages ranged from 3month to 2 year, from which 60 animals were suffering from nervous manifestation. A total 45 brain samples were collected from recently dead and emergency slaughtered sheep and send to the lab. For bacteriological and histopathological examination. The bacteriological examination results 22 positive samples for *L. monocytogenes* (48.8%) with maximum results at 3-6 months of age (57.1%). Moreover, the result showed that the highest percentages for *L. monocytogens* isolation was at autumn and winter. Histopathological examination revealed that there were micro abscesses in the brain. In the same study, trial for preparation of listeria bacterin from the local strains isolated from the sheep was performed in rabbits as laboratory animal. Blood samples were collected from the rabbits groups for deferential leucocytic count and ELISA technique for detection of antibodies after challenge test. The results showed that there was elevation in antibody titer after vaccination and challenge. In addition, there was elevation in WBCs, Lymphocytes, Monocytes and Granulocytes in challenged group compared with vaccinated group. The results of ELISA were highly statistically significant.

KEY WORDS: *Listeria Monocytogenes*, vaccine, Rabbits

(BVMJ-26(1):134-144, 2014)

1. INTRODUCTION

Listeriosis caused by *L. monocytogenes* an infectious disease affecting wide range of animals including ruminants, monogastric animals and man (Smith, 2002). The reason for this broad degree of interest of listeriosis is due, in large part, to the fact that this facultative intracellular pathogen is highly amenable to experimental manipulation and has a broad range of relevant biologic activities ranging from its growth in the environment, infection of many different animal species, and as an important human pathogen (Portney, 2007). *L.monocytogenes* is a small, motile, non-sporulating Gram-positive bacillary to *coccobacillar bacterium*. It is high resistant microorganism. Optimal growth temperature is 30-37°C (Holt *et al*., 1994). *L.monocytogenes* can grow at temperatures as low as -2ºC in laboratory media broth (Bajard *et al*., 1996). The clinical manifestations of infection with *L. monocytogenes* include sepsis, meningitis/encephalitis, abortion and gastroenteritis (Drevets *et al.,* 2008). The affected animals may move in a circle towards the affected side, there is profuse, almost continuous, salivation, with food material impacted in the cheek, drooping ear, deviated muzzle, flaccid lip and lowered eyelid on the affected side (Scott, 2013). Attack rates are higher in sheep than in cattle suggesting the greater

susceptibility of ovines to listeriosis (Wesley, 2007). Bacteriological testing and histological examination are the classical methods used for the laboratory diagnosis of listeriosis in animal specimens (Quinn *et al.*, 1999). On histological examination, all the cases showed multiple microabscesses in the brain (Campero *et al.*,2002). A variety of serodiagnostic techniques exist (serum agglutination test, complement fixation test, ELISA test) can be used in diagnosis of Listeria (Low *et al.*, 1997). During the early phase of infection Listeria infected cells induce an intense infiltration by blood cells as monocytes resulting in the formation of microabscesses, then rapid inactivation of bacteria involving mainly T-cells over the next day occure (Bortolussi et al., 1984). There were trials for in-activated vaccine preparation for the control of listeriosis in sheep which recommended to perform boosterisation 2 weeks after initial vaccination which lead to highly significant increase of antibodies titers ((Bacic et al., 2012)

Therefore, the aim this research is to investigate the epidemiological situation of *L. monocytogens* in Kalubia Govenorate and try to approach a solution for this disease by preparation of bacterin applied on laboratory animals as a step for listeriosis control.

2. MATERIAL AND METHODS

2.1. Sample collection:

Throughout June 2013 to January 2014, 631 sheep aged ranged from 3 months to 2 years located in Kalubia Governorate scattered in small flocks examined clinically for any clinical manifestation of Listeriosis as circling, head twisting in one side and recumbancy. A total 45 brain samples were collected from recently dead sheep and emergency slaughtered. Each sample was kept in plastic pages labeled with full case history and kept in ice box and delivered to the laboratory for isolation and identification of isolates. Samples for r histopathological examination were preserved in formalin 10%.

2.2. Histopathological examination.

2.3. Isolation of L. monocytogenes :

It was carried out according to Curtis *et al*., (1989).

2.4. Identification of isolates :

The identification carried out according to Anneschuchat *et al*., (1991) and Donnelly (1992).

2.5. Biochemical examination:

Were carried out according to CruickShank *et al*., (1975):

2.6. Isolate preparation:

The isolated strain of *L. monocytogenes* was prepared as a concentration of 1×10^6 / ml by using McFarland nephelometer barium sulphate standard according to Baily and Scott (1990). The prepared isolate was used for infective dose and vaccine preparation.

2.7. Preparation of L. monocytogenes bacterin;

It was done according to Bacić *et al*., (2012) .

2-8- Rabbit vaccination: Thirty male rabbits of 3 month age were divided into three groups (10 rabbits each) as follow:

2-8-1- Group 1: 10 rabbits were inoculated subcutaneously with 1ml each of prepared listeria bacterin .

2-8-2- Group 2: It was kept as non-vaccinated but challenged (control positive).

Vaccinated (group 1) and non vaccinated (group 2) rabbits were kept under clinical observation. Serum samples were collected from vaccinated rabbits (group 1) after 2 weeks post 1st dose of vaccination and then 2 weeks post 2nd dose of vaccination.

2-8-3-Challenging of vaccinated rabbits with Listeria isolated strains:

Group 1 and group 2 were challenged by intranasal rout administration of 1ml

(1×10^6 / ml) of Listeria monocytogenes suspensions 15 days after second dose of vaccination of (group 1). Serum samples were collected from (group 1) 4 weeks post challenge for ELISA test.

2-8-4- Group 3: It was kept as non vaccinated and non-challenged (negative control).

2-8-5- For blood parameters blood samples collected from the 3 groups at (0,24,48,72,96 and 120 hours) after experimentally infection of group 1and 2 due to death of (group 2) within few days after intranasal infection and the brains were kept in 10% formalin for histopathological examination

2.9. Indirect Enzyme-Linked immunosorbent assay (ELISA):

The test was applied according to Engvall and Perlmann (1971)

2-10- Blood parameters:

Hematological analysis of peripheral blood specimens was performed automatically with a hematology cell counter in clinical pathology department at Faculty of Veterinary Medicine at Benha University.

3-RESULTS

3.1. Clinical examination:

Clinical examination revealed that out of 631 sheep located in different localities of Kalubia Governorate 60 (9.5%) were suffering from nervous manifestation. The clinical signs were dullness, depression, head tilt, animal rested their heads on the ground, facial asymmetry due to unilateral facial nerve paralysis, ear drooping, nostrils were covered with food and hay hanging from mouth. In advanced stages of disease, animals become recumbent show convulsions, paddling movement and death as shown in photo (1).

3-2-Epidemiological analysis:

The epidemiological analysis was illustrated in table (1,2,3,4)

3-3-Histopathological examination of brain of sheep:

The main microscopic changes in brain of sheep naturally infected with listeriosis comprised of asymmetrical meningoencephalitis with severe hyperemia and parenchymal degeneration centered in the pons and medulla oblongata. The cerebellum was also affected in most of the examined cases. The meninges showed moderate non-suppurative meningitis characterized by congested blood vessels and mononuclear inflammatory cellular infiltration mainly lymphocytes and fewer macrophages (photo. 2). Blood vessels in all brain stem sections and the cerebellum had mononuclear perivascular cuffs (photo. 3), most severe in the caudal brain stem sections. The perivascular cuffs consisted mainly of lymphocytes and macrophages (photo. 4)

3-4- Clinical observation of the rabbits:

The challenged rabbits with L.monocytogenes strain show nervous manifestation as paralysis in hind limb and erected ear while the vaccinated group did not exhibit these nervous signs. And histopathological examination of challenged group revealed micro abscess in brain as shown in (photo.) 5 and necrotic foci in liver as shown in (photo.6).

3-5-Hematological examination:

The hematological examination was illustrated in table (5).

3-6- ELISA:

The ELISA results illustrated at table (6).

Table (1): Morbidity, mortality and case fatality rate of sheep showing nervous manifestations of listeriosis in different ages.

Age by months	Sheep under investigations			Morbidity rate (%)	Mortality rate (%)	Case fatality rate (%)
	Total No.	Diseased No.	Dead No.			
3-6	81	25	21	30.8	25.9	84
6-9	160	18	13	11.25	8.1	72
9-12	240	11	9	4.5	3.75	81.8
Total	631	60	45	9.5	7.1	75

Table (2): Morbidity, mortality and case fatality rate of sheep showing nervous manifestations of listeriosis in different seasons.

Season	Month	Sheep under investigations			Morbidity rate (%)	Mortality rate (%)	Case fatality rate (%)
		Total No.	Diseased No.	Dead No.			
Summer	Jun.	26	2	0	7.7	0	0
	Jul.	35	3	3	8.6	8.6	100
	Aug	35	0	0	0	0	0
Autumn	Sep.	37	6	4	16.2	10.8	66.6
	Oct.	51	5	3	9.8	5.8	60
	Nov.	82	7	6	8.5	7.3	85.7
Winter	Dec.	160	17	14	10.6	8.8	82.3
	Jan.	205	20	15	9.7	6	75
Total		631	60	45	9.5	7.1	75

Table (3): Prevalence of listeria monocytogenes isolated from different ages

Age by months	Diseased No.	Samples No.	Positive samples	
			No.	%
3-6	25	21	12	57.1
6-9	18	13	7	53.8
9-12	11	9	3	33.3
Total	60	45	22	48.8

Table (4): Prevalence of listeria monocytogenes isolated in different seasons

season	Month	Diseased No.	Samples No.	Positive samples	
				No.	%
summer	Jun.	2	0	0	0
	Jul.	3	3	1	33.3
	Aug	0	0	0	0
Autumn	Sep.	6	4	1	25
	Oct.	5	3	1	33.3
	Nov.	7	6	4	66.6
Winter	Dec.	17	14	7	50
	Jan.	20	15	8	53.3
Total		60	45	22	48.8

Table (5): Mean ± S.E. of differential Leukocytic count in relation to rabbit infection by L. monocytogen:

Leucocytes	Time by hours	Group 1	Group 2	Group3
WBS	0	7.74±0.21[a]	7.78±0.03[a]	7.68 ± 0.06[a]
	24	8.02 ±0.25[b]	9.42±0.15[a]	9.08± 0.21[a]
	48	7.82 ±0.16[b]	10.76± 0.28[a]	9.94± 0.36[a]
	72	7.98 ± 0.18[c]	12.44± 0.34[a]	11.00± 0.17[b]
	96	8.22±0.39[c]	13.26±0.21[a]	11.74± 0.25[b]
	120	7.88±0.08[c]	13.90±0.38[a]	12.42±0.40[b]
LYMPHOCYTES	0	0.64±0.009[b]	0.67 ±0.004[a]	0.66±0.002[a]
	24	0.69 ±0.01[a]	0.58±0.01[b]	0.62± 0.03[ab]
	48	0.65 ±0.004[b]	0.93± 0.02[a]	0.94± 0.02[a]
	72	0.69±0.008[c]	1.33± 0.08[a]	.97± 0.01[b]
	96	0.65±0.002[c]	1.28±0.03[a]	1.04± 0.02[b]
	120	0.68±0.007[c]	1.27±0.04[a]	1.05±0.02[b]
MONOCYTES	0	0.18±0.008[a]	0.19 ±0.003[a]	0.18±0.002[a]
	24	0.19 ±0.009[c]	0.40±0.009[a]	0.31± 0.02[b]
	48	0.18±0.005[c]	0.93± 0.04[a]	0.53± 0.04[b]
	72	0.19±0.008[b]	0.87± 0.03[a]	0.82± 0.02[a]
	96	0.19±0.004[b]	0.79±0.03[a]	0.71± 0.03[a]
	120	0.19±0.003[b]	0.70±0.05[a]	0.66±0.07[a]
GRANULOCYTES	0	6.96±0.20[a]	6.89±0.02[a]	6.79±0.05[a]
	24	7.17±0.22[b]	8.43±0.14[a]	8.13± 0.19[a]
	48	7.00±0.15[b]	8.90± 0.25[a]	8.54± 0.31[a]
	72	7.11±0.17[c]	10.23± 0.25[a]	9.19± 0.15[b]
	96	7.04±0.12[c]	11.13±0.19[a]	10.02± 0.19[b]
	120	6.99±0.07[c]	12.18±0.19[a]	10.68±0.32[b]

Table (6): Comparison between ELISA results in different stages of experiment for vaccinated rabbits

Items	ELISA results			F	P
	Min.	Max.	Mean±SD		
1st dose of vaccine	0.176	0.246	0.179±0.021	833	<0.001
2nd dose of vaccine	0.180	0.228	0.212±0.01		
4 week after challenge test	0.209	0.259	0.230±0.034		

Highly significant: P <0.001

Photo (1): sheep showing dropping of the ear

4-DISCUSSION

A total of 631 sheep of different ages and localitis in Kalubia Governorate were included in this study during period of June 2013 to January 2014. Clinical examination of 631 sheep revealed that, there were 60 sheep (9.5%) were suffering from nervous manifestation such as a circling toward the affected side, head tilt, depression and animal moving aimlessly away from the herd with slightly deviated neck as shown in photos (1) these mainfestation due to formation of microabcess in brain and this result agreement with (Kumar *et al*.,2007, Brugère 2008, Scott 2013). With respect to epidemiological analysis our results showed that out of 631 examined sheep there were 60 diseased sheep with nervous manifestation with morbidity rate 9.5% as shown in Table (1), this result agree with (Kumar *et al*.,2007) who reported that the morbidity rate was 9.5% in an outbreak in sheep flock suffered from encephalitic Listeriosis. On the other hand these result disagree with (El-Sawalhy *et al*., 1999) who recorded that morbidity rate was 5.81% in affected sheep flock with meningeoencephalitis in Dakahilia Governorate and this difference may be due to different localities and the numbers and ages of investigated animal . The total case fatality rate and the mortality rate in this study was 75% and 7.1% respectively and these results are going in parallel with that of (Kumar *et al*., 2007) who reported that the case fatality and the mortality rate was 89.85% and 8.3% respectively in affected sheep flock with encephalitic Listeriosis in India.

The season effect on the prevalence of ovine Listeriosis as shown in table (2) in which the highest morbidity rate in winter season followed by Autumn season then summer season also the occurrence of Listeriosis allover the year as it can grow in wide range of temperature (5ºC- 37ºC) and these result contributed to sudden changes of weather to very cold and wet, overcrowding, ingestion of spoiled silage and insanitary condition. These result going in parallel to (Sanaa *et al*.,1993, Malik *et al* 2002, Hirsh *et al*.,2004) who recorded that Listeriosis in general is more frequent in winter season. The effect of age on the occurrence of Listeriosis in sheep showed that sheep aging from 3-6months old were show highest nervous manifestation as 25 diseased out of 81 examined sheep in percent of 30.8% followed by sheep aging 6-9 months

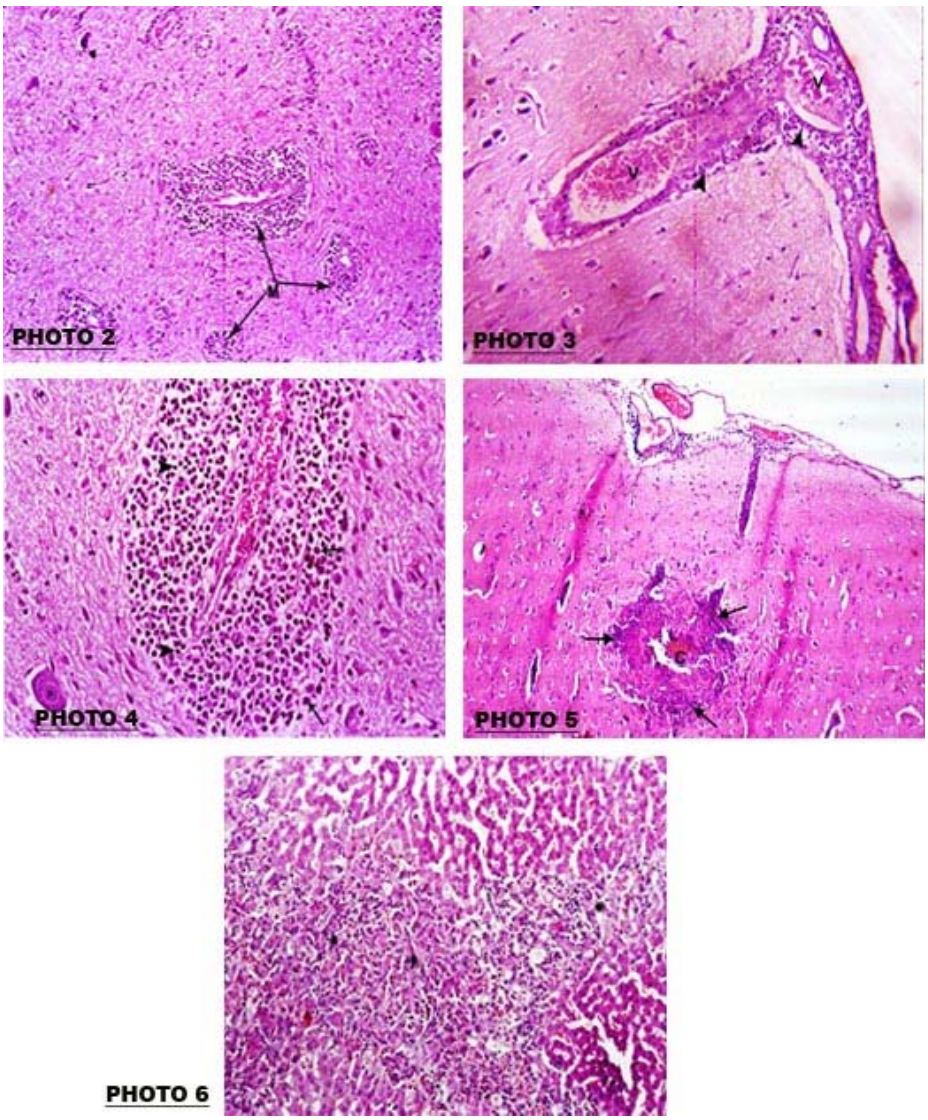

Photo.2 Brain of sheep naturally infected with listeriosis showing mononuclear perivascular cuffs (M). H&E stain x 100. Photo.3 Brain of sheep naturally infected with listeriosis showing congestion of the meningeal blood vessel (V) and mononuclear inflammatory cellular infiltration (arrow head). H&E stain x 200. (Fig. 3) Photo.4 Brain of sheep naturally infected with listeriosis showing severe mononuclear perivascular cuff consisted mainly of lymphocytes (arrow head) and macrophages (arrow). H&E stain x 400. Photo. (5): Brain of rabbit experimentally infected with listeriosis showing microabscess with necrotic center (C) and surrounded by microglial reaction (arrow). H&E stain x 100. Photo. (6): Liver of rabbit experimentally infected with listeriosis showing hepatocellular necrosis (asterisk) infiltrated by neutrophilic inflammatory cells. H&E stain x 200.

as 18 diseased sheep out of 160 examined sheep in percent of 11.25% then sheep aging 9-12 months as 11 diseased sheep out of 240 examined sheep in percent of 4-5% and sheep aging 1-2 year showed that 6 diseased sheep out of 150 examined sheep in percent of 4%. These result showed that young sheep more susceptible to Listeriosis than adult sheep and the mortality rate in young sheep higher than adult sheep. And these result agree with (Green *et al*.,1994 , Börkü *et al*.,2006) who recorded that Listeriosis frequently occur in lambs from 6 to 12 weeks Also agreement with (Ryster *et al*.,2007) who recorded that Listeriosis was higher in young ages due to changes in dentation and other lesions in the oral cavity as well as on the Lips, nostrils or conjunctiva. With regarding to the hematological examination of the blood samples which collected from the infected and vaccinated rabbits our results showed that there were significant increase in lymphocytes count in infected (group2) compared to vaccinated (group1) at 72, 96 and 120hours. Also significant increase in monocyte count in infected group compared to vaccinated group at 24 and 48hours and also granulocytes count showed significant increase in granulocytes count in infected (group2) compared to vaccinated (group1) at 72, 96 and 120hours as shown in table (5). These result agreement with (Bortolussi *et al*.,1984) who recorded that During the early phase of infection Listeria infected cells induce an intense infiltration by blood cells as monocytes resulting in the formation of microabscesses. Then occure rapid inactivation of bacteria involving mainly T-cells over the next day. The results of ELISA in table (6) showed that there was slight increase in antibody titers after 4 weeks (1 month) post challenging of vaccinated animals and these result agreement with Mitsuyama et al., 1978 who recorded that Listeria is faculitative intracellular parasite and protection depend on monocytes, macrophages and lymphocytes. On the other hand the elevation of antibodies titers in our experiment after second dose of vaccine and post challenging of laboratory animals were statically highly significant and this reflect the probability of its value and permit further studies on preparation of field vaccine for listeria monocytogenes and this is agree with (Bacic *et al*.,2012) whom concluded that antibody titers were significantly higher after boosterisation ($P<0.001$) and protective levels could be detective in the sera of vaccinated animals during the next 6 months The result of histopathological examination of brain of sheep (photo. 2,3 and 4) reveled The most pathognomic lesions for the disease were a combination of suppurative parenchymal lesions (microabscesses) and necrosis. Microabscesses were occasionally observed in the cerebellar parenchyma and brain stem; characterized by structureless necrotic center with neutrophils infiltration. In severe cases, microabscesses may coalesce to large areas of suppuration infiltrated by degenerate and non-degenerate neutrophils and surrounded by moderate numbers of glial cells admixed with lymphocytes and macrophages. And these results were agreement with (Wagner *et al*., 2005) whom recorded that main feature is meningoencephalitis microabscesses necrotic and liquefactive changes with infiltration by neutrophils and mononuclear cells located either in the gray and/or white matter.

The results of histopathological examination of rabbits reveled that there was The characteristic parenchymal lesion was a microabscess characterized by tiny collection of neutrophils as shown in figure (5) the liver had variably sized, randomly distributed foci of intense hepatocellular necrosis infiltrated by principally neutrophilic inflammatory cells . Irregular coalescent areas of coagulation necrosis characterized by hepatocellular

hypereosinophilia with little cellular infiltration were also present. Our result is going paraelle to these of (Percy and Barthold 2007) who recorded that In the encephalitic form the predominant lesion includes focal microabscesses focal hepatic abscesses and myocardial degeneration are common. Bacteria may be found in Kupffer cells of liver or in the periphery of brain lesions infiltration of plasma cells, lymphoid cells, and macrophages are characteristic of the lesion miliary foci of necrosis on the liver focal suppurative hepatitis and marked infiltration with heterophils.

From these results we conclude that: Liseriosis occur throughout the year in sporadic cases. Highest morbidity and mortality rate of Listeriosis in sheep at age varying from 3-6 months. Highest morbidity and mortality rate of Listeriosis in sheep at Autumn and winter season. Immunity of Listeriosis depends mainly on cell mediated immunity. Since the studied vaccine showed efficient protection of rabbits after experimental infection, further research and evaluation on larger number of experimental lab. Animals as well as sheep flocks should be applied to produce local economic vaccine to reduce the economic losses of seep through out the year.

5-REFERENCES

Anneschuchat, Bala, S.T., Broome, C.V. 1991. Epidemiology of human Listeriosis, Clin. Microb. Rev. april, 169-183.

Bacic, D., Obrenovic Sonja, Dimitrijevic, B., Jonic, B., Ẑutic Jadranka , Aṡaninn 2012. A Study on the inactivated bivalent vaccine preparation from serotypes 1/2a and 4b *Listeria Monocytogenes* for the control of Listeriosis in sheep, Acta Veterinaria (Beograd), (62) 5-6, 531-541.

Bajard, S., Rosso, L., Fardel, G., Flandrois, J.P. 1996. The particular behaviour of *Listeria* monocytogenes under sub-optimal conditions. Int. J. Food Microbiol. 29, pp. 201– 211.

Baily, Scott 1990. Diagnostic microbiology 8th ed. Ellent, Baron and Sydney, M. Fineold. St. Louis, Baltimore. Philadelphia ,Toronto.

Börkü, M.K.,Ural, K., Gazyağcl, S.,Özkanlar, Y., Babür, C. , Kllç, S. 2006. Serological detection of Listeriosis at a farm. Turk J Vet Anim Sci, 30, 279-282.

Bortolussi, R., McGregor, D.D., Kongshavan, P.A.L., Galsworthy, S., Albiritton, W., Davies, J.W. , seeliger , H.P.R. 1984. Host defense mechanisms for perinatal and neonatal Listeria monocytogenes infection. Surv. Synth. Pathol. Res. 3:311-332.

Brugère-Picoux, J. 2008. 'Ovine listeriosis', Small Ruminant Research, (76) pp:12-20.

Campero, C.M., Odeon, A.C.,Cipolla, A. L.,Moore, D.P. , Poso, M.A. , Odriozola, E. 2002. Demonstration of Listeria monocytogenes by immunohistochemistry in formalin-fixed brain tissues from natural cases of ovine and bovine encephalitis. J. Vet. Med. B Infect. Dis. Vet. Public Health 49, 379–383.

Cruickshank, R., Duguid, J.P., Marmion, B.P., Swain, R.H.A. 1975. Medical Microbiology. 12th Ed. (2). Churchill, Livengstone, LTD, Edinburgh, London, UK.

Curtis, G.D.W., Mitchell, R.G., King, A.F., Emma, J. 1989. A selective differential medium for isolation of listeria monocytogenes. App. Microb. 8, 95-98.

Drevets, D.A., Bronze, M.S. 2008. Listeria monocytogenes: epidemiology, Pathogenesis and mechanisms of brain invasion. FEMS Immunology and Medical Microbiology, 53, 151-165.

Donnelly, C.W. 1992. Listeria, In compendium of methods for microbiological examination of foods,

3rd Edn. (Editors: vanderzant, C. and spliyy sttoesser, D.F.) pp: 637-663.

Engvall, E., Perlmann, P. 1971. Enzyme – linked immunosorbent assay (ELISA): quantitative assay of immunoglobulin G. Immunochemistry 8:871-874.

Green, L.E., Morgan, K.L. 1994. Descriptive epidemiology of listerial meningoencephalitis in housed lambs. Prev. vet. Med., 18 (2), 79-87.

Hirsh, C.D., Maclachlan, J.N., Walklers, L.R. 2004. Veterinary Microbiology. 2 ed, Black well publishing, USA. pp: 185-189.

Holt, J.G., Noel, R., Krieg, Peter, H.A., Sneath, James, T., Staley, Stanley T., Williams 1994. Bergey's manual of determinative bacteriology Ninth Edition, 566-570.

Kumar, H., Singh, B. B., Bal M.S. ,Kaur, K.,Singh, R., Sidhu, P. K., Sandhu, K,S. 2007. Pathological and epidemiological investigations into listerial encephalitis in sheep. Small Rumin. Res. 71, 293-297.

Low, J.C., Donachie, W.A. 1997. Review of *Listeria monocytogenes* and listeriosis *The Veterinary Journal*, 153, (1), 9-29.

Malik, S.V., Barbuddhe, S.B., Chaudhari, S.P. 2002. Listeric infections in humans and animals in the Indian subcontinent: a review. Trop. Anim. Health Prod. 34, 359–381.

Mitsuyama, M., Takeya, K., Nomoto, K.,Shimotori,S. 1978. Three phases of phagocyte contribution to resistance against Listeria monocytogenes. Journal of General Microbiology 106, 165-71.

Percy, D.H., Barthold, S.W. 2007. Pathology of laboratory rodents and rabbits. (third edition) pp: 278-280 .

Portney, D.A. 2007. A 20-Year Perspective on *Listeria monocytogenes* Pathogenesis, p. 1-25. In H. Goldfine and H. Shen. (ed.), *Listeria monocytogenes*: Pathogenesis and Host Response. Springer-Verlag New York, NY.

Quinn,P.J., Carter, M.E., Markey, B., Carter, G.R. 1999. The *streptococci* and related *cocci*. Clinical veterinary microbiology. Mosby International, Edinburgh, Scotland, pp:127-136.

Ryser, ET. , Marth, E.M. 2006. Listeria, Listeriosis, and Food Safety. 3rd edition. Boca Raton, Fl: Taylor & Francis Group; S: Serological detection of listeriosis at a farm. Turk J Vet Anim Sci, 30, 279-282.

Sanaa, M., Poutrel, B., Menard, J.L., Serieys, F. 1993. Risk factors associated with contamination of raw milk by Listeria monocytogene in dairy farms. J Dairy Sci, 76:2891-2898.

Scott, P.R. 2013. Clinical diagnosis of ovine listeriosis. Small Ruminant Research 110 (2013) 138– 141.

Smith, B.P. 2002. Large Animal Internal Medicine, 3rd ed, pp. 946–949.

Wagner,M., Melzner,D., Bago, Z., Winter, P., Egerbacher, M., Schilcher, F.,Zangana, A. , Schoder, D. 2005. Outbreak of clinical listeriosis in sheep: Evaluation from possible contamination routes from feed to raw produce and humans. Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health 52 (6): 278-283

Wesley, I.V. 2007. Listeriosis in Animals, p. 55-72. In E.T. Ryers and E.H. Marth (editors), *Listeria*, Listeriosis and Food Safety (3rd ed), FL: CRC Press, Boca Raton.

محاولة تمهيدية لتحضير لقاح ضد ميكروب الليستريا مونوسيتوجينز في الأرانب

سلمي عبدالرحيم شولح[1]، محمد جودة عبدالوهاب[1]، اسلام زكريا محمد [2]، عبدالمنعم محمد مصطفي[1]، فيصل خليل حموده[1]

[1]قسم طب الحيوان–الأمراض المعدية–كلية الطب البيطري–جامعة بنها، [2] قسم البكتريولوجي معهد بحوث صحة الحيوان–الدقي–الجيزة–مصر

الملخص العربي

أجريت هذه الدراسة على عدد 631 من الأغنام التي تتراوح أعمارها من ثلاثة أشهر الي سنتين. من بينهم ستين حيوان يعانوا أعراض عصبية. تم تجميع خمسة وأربعون عينة من مخ الحيوانات حديثة الوفاة والحيوانات المذبوحة اضطراريا وأرسلها للمعمل لإجراء الفحص البكتيري والهستوباثولوجي لها. الفحص البكتري أوضح أن هناك اثنان وعشرون عينة إيجابية بالليستريا مونوسيتوجينز بنسبة 48.8% وتتراوح أعمارهم من ثلاثة الي ستة أشهر بنسبة 57.1% وذلك خلال فصول الخريف والشتاء. الفحص الهستوباثولوجي أوضح أن هناك خراجات دقيقة بالمخ وهذه صفه مميزة لليستريا. وفي نفس هذه الدراسة كانت هناك محاولة لتحضير لقاح الليستريا الميت من العتره المعزولة من الأغنام تحت الدراسة باستخدام الأرانب كحيوانات تجارب. تم تجميع عينات دم من مجموعات الأرانب للفحص المقارن للخلايا البيضاء واجراء اختبار الإليزا لاكتشاف الأجسام المضاده. والتي اظهرت ارتفاع ملحوظ فى زيادة الاجسام المضادة وصد العدوى عن الارانب قيد التجربه.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(1):134-144, مارس 2014)