رقم البحث (4)

EXPERIMENTAL EVALUATION OF LIVE LISTERIA MONOCYTOGENES VACCINE IN SHEEP AND RABBIT

BY

M.A.A. El-Beskawy[1], E.E. Younis,[1] Soumaya E. A[2]., and A.A El-Sawalhy[3]

[1] Faculty of Veterinary Medicine , Mansoura University, Mansoura, Egypt

[2] animal health research institute, Dokki, Giza, Egypt

[3]African Union/ Interafrican Bureau for Animal Resources (AU/IBAR), Nairobi, Kenya

ABSTRACT

This study was carried out on apparently, healthy 20 lambs (4-6 months old) were selected from private flock in Dakahalia governorate without previous history of nervous manifestations and 30 rabbits were used for experimental vaccination with live Listeria monocytogenes vaccine and were classified into three groups(10 in eachgroup). The vaccine was evaluated humerally by detection of antibodies titer and cellular activity through measuring of Nitric oxide and lysozyme in sheep and rabbit. While the protection aganist challenge and reisolation were used in rabbits

Immune response of sheep vaccinated with *Listeria monocytogenes* vaccine showed increase in serum neutralizing antibody titers which gradually increased after 2nd dose of injection till reach maximum level at 15 days of the second dose compared with non vaccinated control sheep.

This result stated that the Vaccination of rabbits and sheep with live *Listeria monocytogenes* (1/2a and 1/2b) vaccine induce cellular and humeral response and protect the vaccinated rabbits against challenge with *Listeria monocytogenes*.

Key words: Listeriosis , Sheep, *Listeria monocytogenes*, Listeriosis vaccination

INTRODUCTION

Sheep and goats are the main livestock in the Middle East **(El- Sukhon and Nareman 2004)**. In Egypt sheep represent an important source of meat and thrift; their number was estimated in 1996 to be 3.491 million sheep **(FAO 1996).**

Listeriosis is one of the most lethal bacterial diseases for fetuses and infants **(Hardy,et,al;2012)**. *Listeria monocytogenes* is a zoonotic agent, and the decisive role in the prevention of food-borne listeriosis in humans is the reduction of the presence of *Listeria monocytogenes* in all the critical stages of the food production and the distribution chain, including the epidemiological surveillance of livestock **(Fthenakis et al., 1998; Wagner et al., 2000).**

Among farm animals, sheep appear to be particularly susceptible to listeriosis. The organism is spread world-wide in nature (decaying herbage, soil, feces, sewage) and occurs usually in low numbers **(Fenlon 1988 and Woolford 1990).** listeria is an intracellular organism, only live vaccines can confer adequate immunity to effectively protect animals from the disease (**Linde et al., 1995)**.**Gudding et al., (1989)** Immunized against listeriosis in sheep using a live attenuated vaccine showed that the incidence of listeriosis decreased from approximately 4.0 per cent before the introduction of the vaccine to 1.5 percent after vaccination started. The incidence of abortions was 0.7 percent in vaccinated flocks compared to 1.1 percent in unvaccinated flocks. As Listeria is an intracellular organism, only live vaccines can confer adequate immunity to effectively protect animals from the disease.

Vaccinology is the most effective and efficacious of medical interventions for eradication or management of many infectious diseases, the generation of cellular immune response is key to survival against a variety of viral and intracellular bacterial pathogens, therefore the development of save vaccine capable in inducing strong cellular immunity continues to be a pressing challenge for medicine **(Zhongxia et al., 2005)**.

The first positive step in elimination of human listeriosis is the elimination of animal listeriosis so; this work was designed to throw some lights on evaluation of local prepared live *Listeria monocytogenes* vaccine in experimentally vaccinated sheep and rabbit as trial for control.

II- MATERIALS AND METHODS

1- Animals

Apparently, healthy 20 **lambs** (4-6 months old) were selected from private flock in Dakahalia governorate without history of nervous manifestation and 35 *Tyland* **rabbits**, one month old (800-900 gm weight) used for evaluation of local prepared live *Listeria monocytogenes* vaccine.

2- Preparation of *Listeria monocytogenes* vaccines

The vaccine is locally prepared in Microbiology Department, Veterinary animal Research Institute, Dokki, Giza. Pool of local isolated strain 1/2a and 1/2b of Listeria monocytogenes were used for preparation of live vaccine (equal volume). **(Gudding et al., 1989) and** equal volumes of diluted 8 x 10^9 CFU/ ml by using McFarland nephelometer barium sulphate standard *Listeria monocytogenes* 1/2a and 1/2b and saponine adjuvant (Riedel-Dde-Haën Batch No.16109) was mixed and emulsified together **(Baily and Scott 1990)**

Vaccine purity:

The listeria strain vaccine was checked for purity before use by microscopical examination of Gram's stained smears. It was recultivated on nutrient, blood agar and brain heart infusion agar for 24 – 48 hours at 37 °c. (**Cruickshank, et,al;1975)**

Vaccine safety test:

The safety of the prepared vaccine was checked by injection of 1/2 ml vaccine subcutaneously to five small rabbits (800 – 900g). These rabbits were inspected daily for seven successive days and survivability in comparison with the 2 control rabbits (**Cruickshank, et, al;1975)**

3 - Experimental design

Lambs Vaccination :

GroupI: Containing 10 lambs (4-6 months old) each one were twice subcutaneous injected by 2 ml with 2 weeks intervals by prepared live Listeria monocytogenes vaccine.

GroupII : Containing 10 lambs of the same age each one were twice subcutaneous injected by 2 ml with 2 weeks intervals by normal saline and kept as non-vaccinated control group .All the lambs were kept under the same condition of feeding and housing with clinical observation for one year post vaccination. Sera samples were collected from the all lambs at day 0, 14; 30 days post vaccination and monthly till reach the 4th month then at 6th, 9th and 12th months after the first dose to follow up the level of antibodies.

Rabbits Vaccination:

Group RI: Containing 10 rabbits were injected subcutaneously, each one with 0.5 ml S/C of prepared live Listeria monocytogenes vaccine.

Group RII: Containing 10 rabbits were injected subcutaneously, each one with 0.5 ml S/C of normal saline and kept as non-vaccinated positive control. Vaccinated and non-vaccinated rabbits were kept under clinical observation for 3 months post vaccination. Sera samples were collected from the vaccinated and control rabbits at day 0, 2 weeks post 1st dose, 2 weeks post 2nd dose and 2 weeks post challenge.

Group RIII: Containing 10 rabbits were kept as non-injected and unchallenged group (Negative control) Fecal samples were collected daily for 7successive days from vaccinated lambs and rabbits and examined bacteriologically to record the shedding of *Listeria monocytogenes* in feces.

4- Challenge of vaccinated rabbits:

Group RI and group RII were challenged subcutaneously with 1/2 ml of Listeria monocytogenes strains suspensions 2×10^2, 10 days after second dose of vaccination Rabbits were clinically observed for 3 weeks after challenge, serum samples were collected at different times before vaccination, before challenge, 2 weeks after challenge **(Soumaya S.A. et.al; 2002)**.The dead rabbits were postmortem examined and brain, liver, spleen, and kidney tissues were collected from all dead rabbits. The selected samples were divided into two parts, one was bacteriological processed for isolation of listeria species and the other part was preserved in formalin 10% for histopathological examination

5- Reisolation of L. monocytogenes from vaccinated lambs and rabbits.

Fecal samples were collected from vaccinated lambs and rabbits and examined bacteriologically to record the shedding of Listeria monocytogenes in feces and from organs of dead rabbits.

6 – Histopathological examination:

Tissue specimens (brain stem, pons, and medulla oblongata, spleen, liver) collected from dead, sacrafied sheep and challenged rabbits were fixed in 10% formalin, after fixation the tissue specimens were processed by convential paraffin embedding techniques and stained by hematoxylin and eosine for routine histopathological examination **(Harris, H.E. 1998)**

7-Estimation of nitric oxide assay

100 µl of serum samples were mixed with an equal volume of freshly prepared Greiss reagent (0.5% sulfanilamide in 2.5 % phosphoric acid and 0.05 % N (1-naphthyl ethylene

diamine dihydrochloride) incubated for 10 minutes at room temperature and absorbency measured at 570 nm using a microtiter plate reader. The nitric level in serum samples was calculated by comparing the optical density against the nitrite standard curve of sodium nitrite in distilled water. **(Green, et al; (1982)**

8- Lysozyme estimation in sera of sheep and rabbits:

Lysoplates were prepared by dissolving 1% agarose in 0.0067 M PBS at pH 6.3 at 100ºC. Lower temperature to 60ºC then 500 mg /L of lyophilized Micrococcus lysodeikticus (Kindly obtained from Immunity Department- Animal Heath Research Institute. Dokki Giza.Egypt) in 5 ml saline was added.

Plates were poured with 4 mm depth. Adding 25 µl of each serum sample and standard lysozyme solution were poured in each well and incubated at room temperature for 12-18 hr. Clear zone ring diameter was measured. The lytic zones were proportional to the concentration of lysozyme **(Osserman and Lawlor; 1966)**.

8-Indirect Enzyme-linked immunosorbent assay (ELISA):

The test was applied in diagnosis of *L. monocytogenes* in diseased and apparently healthy sheep and in measuring the titer of antibody in vaccinated, and unvaccinated control sheep and rabbits after **Engvall and Perlmann. (1971)**

9-Statistical analysis:

The obtained data in ELISA test were statistically analyzed. According to **Snedecor and Cochran (1973)**

RESULTS AND DISCUSSION

The present work was undertaken to study the humeral immune response to *Listeria monocytogenes* in sheep and rabbits and its response to vaccination with live prepared vaccine in sheep and rabbits.

Clinical closely observation of vaccinated lambs and rabbit revealed the safety of the prepared vaccine as the animal don't showed any clinical signs after using the vaccine either or after 1st dose or 2nd dose and this supported by **Low and Donachie (1991)** which recorded that the live *Listeria monocytogenes vaccine* are necessary for the production of immunity to listeric infection and the killed strains are incapable of stimulating any protection.

The antibodies titers in rabbit serum vaccinated with the live prepared vaccine was observed that there were a significant increase in antibodies level in post vaccinated rabbits than control ones and also there was no significance difference between titers post vaccination and post challenge **Table (1)**. These results were in agreement with **Gudding et al., (1989)** and **Soumaya and Amer (2002)** who reported the efficacy of the prepared live vaccine in sheep.

Measurements of some serum parameters can detect the activity of macrophage and neutrophils against the antigens as Nitric oxide (NO) and lysozyme level in the serum, as shown in **Table (2)** ,estimation of nitric oxide and lysozyme mean level in sera of rabbits we observed that Significant difference nitric oxide level between control group and post challenged group no significant difference between control and vaccinated group, and significant difference lysozyme level between control group and other groups but no significant difference between vaccinated group and after challenge.

Table (3) and (Photos 1,2,3,4,5 and 6) revealed the number of survived rabbits after challenge of the vaccinated and nonvaccinated rabbits, where one of vaccinated rabbits died after challenge by one week and was negative bacteriologically and histopathological lesions revealed that death may due to other cause than *Listeria monocytogenes*. While all the unvaccinated ones died within 7 days of challenge and by bacteriological examination *Listeria monocytogenes* was reisolated from internal organs of dead rabbits. These results revealed the efficacy of live prepared vaccine of pooled *Listeria monocytogenes* (1/2a and 1/2b) in protecting both rabbits and sheep. These results coincided with **Gudding et al., (1989)** and **Soumaya and Amer (2002)** who succeded in using prepared live vaccine from Egyptian local strains in protection of experimental infected pregnant ewes and their offspring from listeriosis.

Table (4) showed the titer of antibodies against *Listeria monocytogenes* in vaccinated and control sheep. It was clarified that the significant high level of antibodies titer were recorded after poster dose of vaccination by two weeks and the titer still high till 9^{th} month post the second vaccination dose and there was significance variance between the antibodies at two weeks post the second dose of vaccination and 9^{th} month post the second dose and the titers were decreased after one year of vaccination. These results revealed the efficacy of the vaccination with live prepared vaccine is suitable until one year. **Mietinen et al., (1990) and Gudding et al., (1989)** who reported that the specific immunological protection against

listeriosis is primarily based on the cell-mediated immune system and the cell-mediated immunity is most effectively stimulated by live vaccine

Table (5) revealed significant variance between vaccinated sheep and other groups, and significant variance between vaccinated group and contact apparently healthy sheep.

Unfortunately, it is difficult to study if the high level in antibodies titers against *Listeria monocytogenesis* efficient to prevent the infection by challenge in sheep. These pointed to apply the experiment in the rabbits to study the efficacy of live prepared vaccine when actually the animal exposed to infection.

Regarding to immune secretory molecule As shown in **Table (6)** estimation of nitric oxide and lysozyme mean level in sera of sheep we observed that there is a significant difference in nitric oxide level between control group and vaccinated and diseased groups and no significant level between vaccinated group and diseased group . There is significant difference in lysozyme level between the three groups of sheep.

The increase in nitric oxide may be attributed to the macrophage respond to bacterial antigen by synthesize and releases of nitric oxide which in turn enhances macrophage mediated cytotoxicity against variety pathogen including intra-cellular bacteria in addition Nitric oxide is generated during immune and inflammatory responses. It is involved in innate immunity as a toxic agent towards infectious organisms, but can induce or regulate death and function of host immune cells as mentioned by **Coleman (2001).** Nitric oxide (NO) is a critical molecule in host defense to infection and a necessary component of non-specific defense mechanisms against several pathogens, including bacteria as reported by **Cifone et al., (2001)**. Moreover, produced at high levels by macrophages through activation of the inducible enzyme iNOS and its production is triggered as non-specific immune mechanism, this activation occurs after infection or vaccination **Aouatef et al., (2002).**

Lysozyme is a major component of the specific granules in phagocytic cell and has a role in defense system due to its antimicrobial activity **Ellison and Giehl (1991).** Lysozyme is a cationic enzyme that attacks the β-1, 4 glycosidic bond in the peptidoglycan of bacterial cell walls. This enables lysozyme to lyse certain Gram-positive bacteria **Paulsen, et al., (2001).**

Our result can be explained by that lysozyme was actively secreted by polymorphonuclear cell and monocyte into the external environment as this cell releases the content of its secondary granules as supported by **Ellison and Giehl (1991).**

This results revealed that the vaccination of rabbits with live *Listeria monocytogenes* vaccine and challenge gave good results for protection against listeriosis, while in sheep recorded high titer of antibodies in vaccinated lambs

Table (1): Mean antibody titers against *L. monocytogenes* in vaccinated rabbits

Groups	Prevaccinated	2 weeks post 1st dose	2 weeks post 2nd dose[a]	2 weeks post challenge[b]
Group RI	**43.4**	**660**	**844.3**	**2160**
Group R II	**45.4**	**55**	**58**	**58.2**
Group RIII	**48.2**	**52.4**	**40**	**56**

a: significant increase at 2 weeks post 2nd dose in vaccinated group (RI) in compared with prevaccinated and control groups.

b: no significant increase at 2 weeks post 2nd dose and 2 weeks post challenge

Table (2): Comparison of nitric oxide and lysozyme mean level in sera of rabbits Post-challenged

Group	Nitric oxide(µmol/ml)*	Lysozyme (µg/ml)**
Vaccinated (G. RI)	**6.987 ± 1.082^{AB}**	**329.700 ± 9.721^{B}**
Control positive (G. RII)	**8.178 ± 0.966^{B}**	**355.850 ± 22.155^{B}**
Control negative (G. RIII)	**5.110 ± 0.220^{A}**	**267.890 ± 7.129^{A}**

A-Significant difference nitric oxide level between control negative group and control positive group, no significant difference between control negative and vaccinated group.

B- Significant difference lysozyme level between control negative group and other groups but no significant difference between vaccinated group and control positive

Table (3): Rabbits challenge experiment after vaccination .

Percent of survivors after challenge					
Vaccinated rabbits		**Control rabbits (20)**			
Group RI (10)		**Group R II (10)Control positive**		**Group RIII (10)Control negative**	
NO.	**%**	**NO.**	**%**	**NO.**	**%**
9	**90**	**1**	**10**	**10**	**100**

Table (4): Mean of Anti-*Listeria monocytogenes* antibody titers in vaccinated sheep

	Prevaccination	Mean antibody titer in vaccinated sheep							
		2 weeks post 1st dose	2 weeks post 2nd dose*	2nd month	3rd month	4th month	6th month	9th month	12th month
Group I	**45.77**	**754.18**	**2913.3**	**2557.5**	**2525**	**2477.5**	**2392.5**	**1055**	**500**
Group II	**45.77**	**57.23**	**83.12**	**76.34**	**63.21**	**89.36**	**76.34**	**74.35**	**70.22**
SD	23.8	300.7	765.4	2045.660	1958.686	2025.861	2103.643	141.421	358.614

- Significance variance between 2 weeks post 2nd dose in vaccinated group and other weeks The peak of antibodies titer was at 2 weeks post the second dose of vaccination and still high till the end of the 8th month of the experiment.

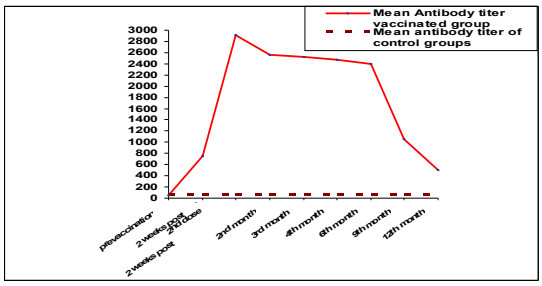

Figure (4): Anti-*Listeria monocytogenes* antibody titers in vaccinated sheep compared by non vaccinated control

Histopathological picture of tissues from challenged control positive rabbits ((GRII)

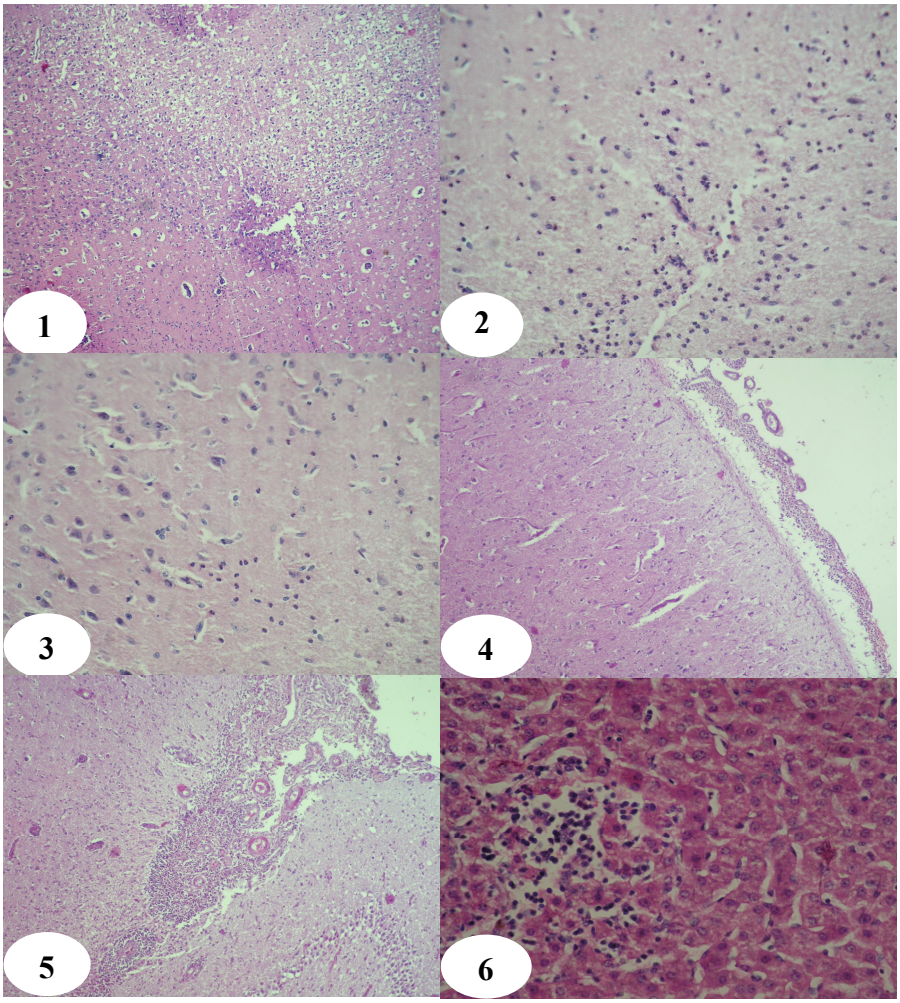

Photo (1) Brain showed diffuse degeneration of neurons with pericellular edema
Photo (2) Brain showed microabscess in white matter
Photo (3) Brain showed microabscess in grey matter
Photo (4) Brain showed submeningeal aggregation of round cells.
Photo (5 Brain showed perivascular cuffing particularly round cells.
Photo (6) Liver showed focal replacement of necrotic hepatocytes with round cells.

REFERENCES

Aouatef, D.; Eugene, M.; Nelly, B.; Yves, V. and Pascale, Q. (2002): Similar pattern of ions expression, nitric oxide production and cytokine response in genetic and vaccination-acquired resistance to Marek`s disease. Vet. Imm. & Immnopathol., 85: 63-75

Baily and Scott (1990): Diagnostic microbiology 8th ed. Ellent, Baron and Syndney, M.Fineold. St.Louis, Baltimore.Philadelphia, Torento

Boerlin, P., E.; Bannerman, T. Jemmi; and J. Bille. (1996): Subtyping Listeria monocytogenes isolates genetically related to the Swiss epidemic clone. J. Clin. Microbiol. 34:2148-2153

Cifone, M. G.; Ulisse, S. and Santoni, A. (2001): Natural killer cells and nitric oxide. Intern. Immunopharm. 1513 –1524

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

Coleman, J. W. (2001): Nitric oxide in immunity and inflammation. Inter. Immunopharmacology 1: 1397-1406

Ellison, R. T. and Giehl, T. J. (1991): Killing of Gram negative Bacteria by lactorferrin and lysozyme. J. Clin. Invest. 88: 1080-1091.

El-Sukhon, S. N. and Nareman, M. Abu-Kuhail (2004): The association of pasteurella species and oestrus ovis in frontal sinuses in Awassi sheep in northern Jordan. Alex. Vet. Sci. (21): 1; 151 – 160.

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

FAO (1996): Annual reports, production year book. FAO/U.N; Rom 50, 193.

Fthenakis, G.C., Saratsis, Ph., Tzora, A., Linde, K., (1998): Naturally occurring subclinical ovine mastitis associated with Listeria monocytogenes. Small Ruminant Research 31, 23–27.

Fenlon D.R. (1988): Listeriosis. In: Silage and health. BA Stark & JM Wilkinson (Eds.). Chalcomb Publications , Marlow, Bucks, U.K. p.7-17.

Green, L.C.; Wagner, D.A.; Glogowsk. J.; Skipper,P.L.;Wishnok, J.S. and Tannenbann, S.P. (1982): Analysis of nitrate and nitrite in biological fluids. Anal. Bioscem. 26: 131-138.

Gudding, R.; Nesse, L.L.; and Gronstol, H. (1989): Immunization against infections caused by Listeria monocytogenes in sheep. Vet. Rec. 125, 5, 111-114

Hardy J, Kirkendoll B, Zhao H, Pisani L, Luong R, Switzer A, McConnell MV, Contag CH(2012): Infection of pregnant mice with Listeria monocytogenes induces fetal bradycardiaPediatr Res. May;71(5):539-45

Harris, H.E. (1998): Veterinary pathology. Cited by Carlton, Dury, Wallington and Cameron, 4th ed. Oxford, unipress, New York,Torento.

Irene V. Wesley; David J. Larson; Karen M. Harmon; John B. Luchansky; Ann Ramos Schwartz (2002): A case report of sporadic ovine listerial menigoencephalitis in Iowa with an overview of livestock and human cases J. Vet. Diagn. Invest. 14:314–321

Low, J.C., and Donachie, W., (1991). Clinical and serum antibody responses of lambs to infection by Listeria monocytogenes. Res. Vet. Sci. 51, 189–192.

Miettinen, A., Husu, J., and Tuomi, J. (1990): Serum antibody response to Listeria monocytogenes, Listerial excretion, and clinical characteristics in experimentally infected goats. J. Clin. Microbiol., 28: 340-343.

Osserman, E. F. and Lawlor, A (1966): serum and urinary lysozym (murimidase) in monocytic and monomyelocytic leukemia. J. Exp. Med. 124: 921 – 952.

Paulsen, S. M.; Engstad, R. E. and Robertsen, B. (2001): Enhanced lysozyme production in Atlantic salmon (Salmo salar L.) macrophages treated with yeast glucan and bacterial lipopolysaccharide. Fish & Shellfish Immunology. 11: 23-37.

Ralovich, B. S. (1987): Epidemiology and significance of listeriosis in the European countries, p. 21-55. In A. Schonberg (ed.), Listeriosis: joint WHO/ROI consultation on prevention and control. Institute fur Veterinarmedizin des Bundesgesundheitsamtes, Berlin.

Snedecor, G. W. and Cochran, W. C. (1973): Statistical methods.6th Ed. Iowa State University Press Iowa, USA

Soumaya S.A. Shafii and H.A. Amer (2002): An attempt to control the major manifestations (abortion & nervous symptoms) attributed to Listeria monocytogenes infections in sheep. Vet. Med. J Giza Vol 50, 4 639-656

Wagner, M., Podstatzky-Lichtenstein, L., Lehner, A., Asperger, H., Wagner, M., Podstatzky-Lichtenstein, L., Lehner, A., Asperger, H., Baumgartner, W., and Brandl, E. (2000): Prolonged excretion of Listeria monocytogenes in a subclinical case of mastitis. Milchwissenschaft. 55: 3–6.

Woolford MK (1990): The detrimental effects of air on silage (review). J. Appl. Bacteriol., 68, 101-116.

Zhongxia, L. I.; Darren, E. H. and Fred, R. F. (2005): Conditional lethality yields anew vaccine strain of Listeria monocytogenes for the induction of cell mediated immunity. Infect. Imm. 37(8): 5065 – 5073

التقييم التجريبي للقاح الليستريا مونوسيتوجين الحي في الأغنام والأرانب

د.محمد البسكاوى[١]، د.عماد يونس[١]، د.سمية الشافعي[٢] ، د.أحمد الصوالحى[٣]

١- قسم الأمراض الباطنة والأمراض المعدية والأسماك- كلية الطب البيطري –جامعة المنصورة

٢-قسم البكتريولوجى –معهد بحوث صحة الحيوان –الجيزة

٣-مكتب الاتحاد الافريقى للاوبئة نيروبى –كنيا

الملخص العربي

تعتبر الاغنام مصدر هام للتوفير والربح السريع للفلاح المصرى كما تعتبر مصدر للبروتين الحيوانى حيث يزيد الاقبال عليها وخاصة فى المناسبات الدينية ومرض الستيريا هومرض خطير يصيب المغترات وخاصة الاغنام ويسبب خسائر اقتصادية تتمثل فى النفوق او الذبح الاطرارى والاجهاض وعادة ما تفسل محولات العلاج فى مواجهة المرض او تقليل خسائره لذا استهدفت الدراسة محاولة تجريبية لتقيم لقاح حى محضر من عترات الستيريا مونوسيتوجين المعزولة من الاغنام المصرية فى الحملان والارانب واستخدم فى هذة التجربة ٢٠ حمل مقسمة الى مجموعتين الاولى حقنت مرتين بالقلح (٢ملى تحت الجلد) بينهم اسبوعين والاخرى حقنت فى نفس الوقت بمحلول ملحى معقم وحفظت كمجموعة ضابطة واستخم ايضا عدد ٣٠ ارنب لتقيم اللقاح مقسمة الى ٣مجموعات تم حقن الاولى بالقاح ٢/١ملل تحت الجلد فى جرعتين والثانية حقنت بمحلول ملحى معقم وحفظب كضابط ايجابى والمجموعة الخير لم تحقن وتم التحفظ عليها كضابط سلبى تم تقيم المناعة السائلة عن طريق مستوى الاجسام المناعية فى الدم للحملان والارانب وكذالك المناعة الخلوية عن طريق قياس مستوى نيترك اوكسيد واليزوسوم فى مصل الحملان والارانب وتم قياس الحماية فى الارانب عن طرية التحدى باستخداو اللستريا مونوسيتوجين ووجودت زيادة معنوية في نسبة الاجسام المضادة كانت اعلاها في الاسبوع الثاني من بعد الحقنة الثانية واتسم بارتفاع الأجسام المناعية علي مايقرب من نهاية الشهر الثامن واستطاعت مجموعة الارانب المحصنة ان تصد العدوى فى حين فشلت المجموعة الاخرى الغير محصنة وتم عزل الميكروب منها وتشخيص التاثير الباثولوجى عليها ،لذلك نوصي بإمكانية استخدام اللقاح الحي من الليستيريا مونوسيتوجين في الأماكن الموبوءة بالمرض للحد من انتشاره و الحد من الخسائر الناجمة عنه.