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GRAM NEGATIVE AEROBIC BACTERIA ASSOCIATED WITH AN ACUTE COLITIS AND DIARRHEA IN HORSE FARM AND EVALUATION OF THE EFFICACY OF *SALMONELLA* NEWPORT AUTOGENOUS BACTERIN

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SUMMARY

Field problem in a governmental horse farm accompanied with a fever, acute colitis and diarrhea was investigated. A total, 58 fecal samples, 7 samples obtained from horses suffering from acute colitis and diarrhea and 51 fecal samples from horses had mild diarrhea. Bacteriological examination of 7 samples revealed isolation of *Salmonella* Newport, *Escherichia coli, Klebsiella oxytoca* and *Proteus* species with an incidence of 85.7%, 42.9%, 28.6% and 14.3% respectively while examination of 51 fecal samples obtained from horses had mild diarrhea revealed isolation of *E. coli, Pseudomonas aeruginosa, Klebsiella oxytoca, Citrobacter freundii, Proteus* species *Klebsiella pneumoniae* and *Salmonella* Newport with an incidence of 86.3%, 39.2%, 29.4%, 25.5%, 21.6%, 11.8% and 2%, respectively. Serological identification of *Salmonella* species and *E. coli* were carried out. *Salmonella enterica* serotype Newport was recovered from 6 out of 7 horses suffering of acute colitis and diarrhea while it could be isolated from a horse had mild diarrhea. *Salmonella* Newport was isolated from the colonic mucosa and mesenteric lymph node of 2 dead horse. No *Salmonella* species could be isolated from feed and water. Analysis of the questionnaires showed access to new arrival the source of *Salmonella* excretion on horse farm. An experimental approach to control spreading of *S.* Newport by using a prepared *S.* Newport autogenous bacterin was evaluated in mouse model. Immunogenicity and protection studies against *S.* Newport challenge were performed in Balb/C mice. Mice were immunized I/M and S/C with 2 doses of an autogenous bacterin. Antibody responses were determined by enzyme-Linked-immunosorbent assay (ELISA). Also, non-

specific immune responses including nitric oxide production (NO), catalase activity and hydrogen peroxide release (H_2O_2), have been measured. Immunization of mice with the autogenous bacterin resulted in a significant enhancement of humoral response following to vaccination and challenge as compared to control group. Additionally, this immunization succeeded in raising NO production, activating catalase and increase H_2O_2 release. Increasing survival was noticed in immunized mice (80% and 66.7%) being declined in challenged non-immunized group (6.7%). It was concluded that the prepared autogenous *S.* Newport bacterin could elaborate not only humoral immune responses but also host innate responses against *S.* Newport. However, more studies should be conducted under field condition to evaluate the efficacy of vaccine.

INTRODUCTION

Acute diarrhea caused by colitis in adult horses is a potentially life-threating disorder. A varity of infectious organisms has been identified as a cause of acute colitis (Cohen and Woods, 1999 and Oeliver and Stampsli, 2006). It characterized by hypersecretion of fluid, motility disturbance, altered microbial flora in the colon and impaired mucosal barrier caused by direct injury or inflammation, severe dehydration with profound electrolyte abnormalities, and systemic inflammation from absorption of endotoxin or other bacterial product through the compromised mucosa (Reed et al., 2004; and Estepa et al., 2005).

Salmonellosis is reported to be the most frequently diagnosed infectious cause of acute diarrhea in horses. Many serotypes have been reported to infect horses with those in group B including *S.* Typhimurium and *S.* Agona (Larsen, 1997). *S.* Newport has previously been isolated from diarrheic horses (Estepa et al., 2005) appearing to be associated more commonly with disease. Although some *Salmonella* infection are subclinical, clinical disease may precipitated by stressful events that compromise host immunity, as exposure to an overwhelming challenge dose or introduction of virulent serotype into a native population.

Studies of the development of an immune response against *Salmonella* infection in domestic animals provide some of the vital information needed by industry to deal with *Salmonella* problem on the farm. Innate or non-specific immunity including complement, polymorphnuclear cells, neutrophils, macrophages and natural killer cells provides of the early front-line defence against microbial invasion (Dietret et al., 1991; Sharma and Schat, 1991; Kogut et al., 1994).

Both humoral and cellular immunity appear to play a role in protection against *Salmonella* infection (Mastroeni et al., 1993), although the importance of each in the ultimate protection of the host still remains controversial. Most of our standing

of immunity of Salmonellosis arises from experimental work with typhoid like disease usually *S* Typhimurium in mice (Brennan et al., 1994).

Treatment of equine salmonellosis with antimicrobial drugs do not reduce *Salmonella* shedding in the feces even when antimicrobial sensitivity test suggest that the drug selection is approciate (VanDuijkeren et al., 1995). Vaccines including bacterins, subunite and attenuated modified live vaccines have evaluated using virulent challenge model until recently only *S.* Typhimurium and *S.* Dublin bacterins have been licensed in United State (Hous et al., 2001). It is common practice for manager of some farms with animals infected with serotypes other than *S.* Typhimurium and *S.* Dublin to vaccinate with autogenous *Salmonella* bacterins. Because of these concerns, the goals of the personal work carried out to:

(1)- Investigate the Gram negative aerobic bacteria cause of acute colitis and diarrhea on a horse farm and to identify the source of *Salmonella* infection.

(2)- Evaluate the possible protective immunity of the prepared autogenous *Salmonella* bacterin by measuring some parameters of innate and acquired immune response in mice model and assessment the protection induced by vaccination challenge inoculation system.

MATERIALS AND METHODS

Sampling:

A total of sixty samples were examined, 58 fecal samples were taken from the rectum of diarrheic horses. Seven horses were suffering from acute colitis (fever, depression and abdominal pain) followed by a perfuse watery diarrhea while the remainder 51 horses suffering of mild diarrhea. During the work 2 horses suffering of acute diarrhea were died. Post-mortum culture of colonic mucosa and mesenteric lymph node were examined. Samples were collected from a governmental horse farm, Cairo Governorate and were transferred to the laboratory in ice box with a minimum of delay.

2- Bacteriological examination:

A loopfull of fecal samples obtained from diarrheic horses were cultivated directly onto MacConkey bile salt agar media and incubated at 37°C for 24 hrs. Also ten gram of sample was inoculated into 90 ml selenite F broth and incubated at 37°C for 16 hrs then plating on *Salmonella* Shigella agar (S.S) and xylose lysine Desoxycholate (XLD) media. The suspected isolates were purified and identified according to Quinn et al. (2002). Serological identification of suspected *Salmonella* isolates were carried out according to the Kauffmann white scheme as described by Kauffmann (1997) using sera product Denka Sieken Co., LTD, Japan.

Biochemically identified *Escherichia coli* isolates were serologically investigated by the slide agglutination technique. The diagnostic O sera (polyvalent and monvalent) seiken product code

312002, Japan Denka seiken Co. LTD were used. Examination of food samples was carried out after pooling according to the procedures of international commission on microbiological specification for food, ICMSF (1978).

Water samples were collected and examined bacteriologically for pathogenic bacteria and coliform counts according to standard method for examination of water and waste water, APHA (1989).

3- Preparation of *S.* Newport bacterin:

S. Newport recovered from diarrheic horse was used to prepared inactive whole cell vaccine (bacterin) as described by Xu et al. (2007). Briefly an over night culture of *S.* Newport grown in shaker water bath at 200 rpm in 200 ml of Luria broth was inactivated with 1.0% final concentration of formalin. The broth culture was centrifuged at 9000 rpm for 15 min. at room temperature and pellet washed three times with sterile phosphate buffered saline (PBS). The final pellet was re-suspended in 50 ml sterile PBS equivalent to a bacterial count of 1.0 X 10^{10} CFU/ml and diluted by using Macfrland tube to 9 X 10^{8} CFU/ ml prior to vaccination in mice.

The prepared bacterin was mixed with equal volume of Freunds incomplete adjuvant safety and sterility test were carried out before use.

4- Experimental animals:

A total of 60, six week old female Balb/C mice were purchased. One week acclimatization period was allowed. Fecal examination were done and mice were prescreened for the presence of antibodies to *Salmonella* by ELISA to ensure no prior *Salmonella* infection before vaccination.

5- Experimental immunization of mice and challenge study:

60 mice were separated into 4 groups (15 mice/ each) in experimental design as follows:

Group (I) was immunized I/M with 1ml of the autogenous *S.* Newport bacterin, group (II) was immunized S/C with the autogenous bacterin at the same inoculum dose. While group (III) and (IV) were kept untreated. Fifteen days post primary immunization, mice of group (I) and (II) were secondary immunized with the same vaccine dose they received previously. Two weeks after 2nd immunization, mice in group (I), (II) and (III) were I/ P challenged with a dose of 1ml (3.0 X 10^{6} CFU/ml) *Salmonella* Newport whereas group (IV) kept as negative control.

Mice were observed for 7 weeks whereas mortality, clinical symptoms, bacteriological examination of feces and re-isolation of *S.* Newport from internal organs of dead mice were recorded.

Serum samples were collected on weekly basis after vaccination and post challenge and kept at

-20°C till used for estimation of humoral immune response by ELISA.

6- Preparation of Salmonella antigen and measurement of antibody titre by Enzyme Linked Immunosorbent assay (ELISA)

It was performed according to Xu et al., (2007).

S. Newport was grown as described previously. After washing three times with sterile PBS, the final pellet was re-suspended in 10 ml of sterile carbonate bicarbonate buffer coating buffer pH 9.6 followed by sonication on ice, six times with 20 seconds bursts at 60 duty cycle, out-put 7 with 20 second pauses to ensure that greater than 95% of the bacterial cell suspension had been lysed, the efficacy of cell disruption was checked by gram staining of the lysate. The cell lysate was further diluted with sterile carbonate bicarbonate buffer. The protein concentration in the preparation was estimated by the method of Lowry et al. (1951).

ELISA plates were coated with 10µg protein in 100µl of coated buffer per ml incubated overnight at 4°C and washed with PBS (pH 7.4) containing 0.05% Tween 20 (PBST). 200µl PBS containing 1% bovine serum albumin (BSA) block buffer was added and blocked for 1 hr at room temperature then washed three time with PBST. Series of two fold dilution of mouse sera in PBS containing 1% BSA (50µl) were added. A positive control anti-*Salmonella* Newport high titre serum was included on each plate as an internal standard. Plates were incubated for 1hr at 37°C, washed 3 times with PBST. Antibodies were detected using 50 µl/ well diluted horse radish peroxidase (HRP)-conjugated goat anti-mouse IgG (Sigma) diluted 1:1000 in PBS containing 1% BSA. The plates were incubated for 1hr at 37°C and washed. Then the plates were developed with 100µl of 1.0 mg/ml OPD (Sigma) substrate in dark at room temperature for 30 min., plates were read at 450 nm on the BioRad 550 microplate reader.

7- Nitric oxide assay (NO assay):

72 hr following challenge serum samples were collected from all groups 100 µl of each sample was mixed with 100µl of freshly prepared Griess reagent (Sigma) in flate bottom 96 well plates, the plates incubated for 15 min at room temperature and the optical density measured at 540 nm. Nitrite concentration was determined using standard curve generated with sod. nitrite according to Green et al. (1982).

8- Determination of Catalase activity:

72 hr following challenge serum samples were collected for estimation of catalase activity. Catalase solution was obtained from bio-diagnostic. Catalase activity in serum of all mice groups was assayed as described by Aebi (1984) expressed as:

$$\text{Catalase activity } (\mu/l) = \frac{\text{A standard - A sample}}{\text{A standard}} \times 1000$$

9- Determination of hydrogen peroxide production:

72 hr following challenge serum of all mice groups was tested for H_2O_2 relieve by using solutions obtained from biodiagnostic, assessed as mentioned by Aebi (1984) and expressed as:

$$(\mu M/L) = \frac{\text{A sample}}{\text{A standard}} \text{ X } 500$$

10- Statistical Analysis:

The obtained data were computed and expressed as Mean ± SEM. All studied parameters were statistically analysed by analysis of variance using a model that including the different parameters in each individual experiment (SPSS version 11).

RESULTS

Bacteriological examination of 7 fecal samples obtained from horses suffering of fever, acute colitis and prefuse fetid odour diarrhea revealed isolation of *Salmonella* Newport, *Escherichia coli, Klebsiella oxytoca* and *Proteus* species with incidence of 85.7%, 42.9%, 28.6% and 14.3%, respectively. While examination of 51 fecal samples obtained from horses had mild diarrhea revealed isolation of *E. coli, Pseudomonas aeruginosa, Klebsiella oxytoca, Citrobacter freundii, Proteus species, Klebsiella pneumoniae* and *Salmonella* Newport with an incidence of 86.3%, 39.2%, 29.4%, 25.5%, 21.6%, 11.8% and 2%, respectively (Table, 1). Mixed infection is present in almost cases.

Serological identification of *Salmonella* species using O and H sera revealed presence of one serovars *S.* Newport (6, 8: e, h: 1,2).

Salmonella Newport was also isolated from the colonic mucosa and mesenteric lymph node of the 2 dead horses Photo (1) illustrated that the presence of congestion, petchial hemorrhage and dilation of intestine.:

Serotyping of *E. coli* re covered from horses had acute colitis and diarrhea revealed the presence of 3 serogroups O164, O27 and O168 (33.3%) each. While 9 serogroup were identified (O164, O159, O125, O78, O27, O126, O168, O8 and O142) from horses had mild diarrhea with an incidence of 18.27%, 13.64%, 11.4%, 9.1%, 6.8%, 6.8%, 4.5%, 4.5% and 2.3%, respectively (Table, 2).

Concerning bacteriological examination of the feed stuffs {barley, wheat straw and green food (barseem)} revealed the occurrence of *E. coli* (027) in the barley, 0164 and 0125 in wheat straw while green food contaminated with *E. coli* (0126) and *P. aeruginosa*. The results of coliform counts in chlorinated water supplied to animals by automatic pump were negative in all tubes by using 5 tube method most probable number. There was no pathogenic bacteria could be isolated from water.

Measurement of antigen antibody responses in mice immunized with autogenous *S.* Newport bacterin by ELISA.

Serum immunoglobulins (Igs) were determined by ELISA at first and second week post primary and secondary immunization in both group (I) and (II) and has been illustrated in Table (3).

Mice immunized I/M & S/C with the autogenous bacterin began to produce antibody titre at 1st week post primary immunization followed by initial increase which could be seen especially at 2nd week post primary immunization as shown in Table (3).

Mice immunized I/M with the autogenous bacterin generate noticeable antibody responses at 1st week post second immunization this was continued and subsequently increased at 2nd week post second immunization. Similarly mice immunized S/C with the autogenous bacterin expressed an increase antibody levels at these times but not as much as I/M immunized group when compared with control negative group.

Measurement of antigen antibody response in immunized mice challenged with *S.* Newport by ELISA

Table (4) showed serum antibody titres produced by groups (I, II and III) after being challenged with *S.* Newport. Monitoring of immunoglobulin responses was evaluated for the next 3 weeks. However, at 1st week post challenge, there was a significant elevation of antibody titres in the I/M immunization group over S/C one. Furthermore, the I/M route of immunization was clearly the most efficient inducing Antibody response as indicated by a significantly elevated humoral response in the 2nd week post challenge which nearly remain persisted till 3rd week post challenge. Although, I/P challenged non- immunized group showed antibody response at the same time points but was considered lower than both immunized- challenged groups.

Nitric oxide production

The host defence response in the group (I) and group (II) assessed by induction of NO which was relatively higher when compared with control negative group. Furthermore, there was no significant differences in the NO level between the (I) and (II) groups as being shown in Fig. (1).

Catalase activity and hydrogen peroxide release

In general, the group (I) and group (II) produced high level of H_2O_2 with activation of catalase when compared with negative control. On the contrary, this elevation was not apparent in the non-vaccinated ones as shown in Tables (5 & 6).

Challenge trial post-immunization

Mice in group (III) developed clinical symptoms characterized by depressed attitude and diarrhea. The severity of these symptoms was greater in group (III) than group (I) and (II). *S.* Newport

was isolated from feces in the litter of group (III) for successive 3 weeks post challenge while it could be isolated from group (I) and (II) for one week post challenge it may be due to recycling and shedding of *Salmonella* microorganism. The protective rates were 80% & 66.7% in group I & II while being 6.7% in the non-immunized challenged group (Table, 7).

Table (1): Incidence of bacterial isolates recovered from fecal samples of horses.

Isolates	Diarrheic horses				Total (58)	
	Acute (7)		Mild (51)		No	%**
	No	%*	No	%*		
Salmonella Newport	6	85.7	1	2	7	12.1
E. coli	3	42.9	44	86.3	47	81.0
Klebsiella oxytoca	2	28.6	15	29.4	17	29.3
Klebsiella pneumoniae	0	0	6	11.8	6	10.3
Pseudomonos aeruginosa	0	0	20	39.2	20	34.5
Citrobacter freundii	0	0	13	25.5	13	22.4
Proteus species	1	14.3	11	21.6	12	20.7

* The percentage was calculated according to the number of examined horses.

** The percentage was calculated according to the total number of examined horses.

Table (2): Serogroup of *Escherichia coli* recovered from diarrheic horses.

Serogroup	Horses had acute colitis and diarrhea		Horses had mild diarrhea	
	No.	%	No.	%
O164	1	33.3	8	18.2
O159	0	0	6	13.6
O125	0	0	5	11.4
O78	0	0	4	9.1
O27	1	33.3	3	6.8
O126	0	0	3	6.8
O168	1	33.3	2	4.5
O8	0	0	2	4.5
O142	0	0	1	2.3
Untypable *E.* coli	0	0	10	22.7
Total	3	100	44	100

The percentage is calculated on the basis of the total number of isolates in each cases

Table (3): Anti *S.* Newport serum Igs titres of mice parentrally immunized with an autogenous *S.* Newport bacterin measured by ELISA.

Weeks post-primary immunization	I/M (group, i)	S/C (group, ii)	Control –ve (group, iv)
1st	0.5227 ± 00055^{Aa}	0.4157 ± 0.0068^{Ba}	0.1627 ± 0.0032^{C}
2nd	0.5810 ± 0.0012^{Ab}	0.4760 ± 0.0079^{Bb}	0.1650 ± 0.0040^{C}
Weeks post-secondary immunization	I/M (group, i)	S/C (group, ii)	Control –ve (group, iv)
1st	0.7147 ± 0.0144^{A}	0.6110 ± 0.0023^{Ba}	0.1633 ± 0.0030^{C}
2nd	0.7573 ± 0.0095^{A}	0.6470 ± 0.0098^{Bb}	0.1653 ± 0.0045^{C}

Means with different capital superscript in the rows and small superscript in columns are significantly different at least at P<0.05.

Table (7): Humoral immune response in different groups after challenge with *S.* Newport.

Weeks post-challenge	I/M group (I)	S/C group(II)	Challenge group (III)	Control –ve group(IV)
1st	0.8213 ± 0.0152^{A}	0.7030 ± 0.0042^{Ba}	0.4387 ± 0.0049^{Ca}	0.1621 ± 0.0033^{D}
2nd	0.9446 ± 0.0454^{Aa}	0.7597 ± 0.0137^{Bb}	0.4640 ± 0.0036^{Cc}	0.1645 ± 0.0043^{D}
3rd	0.8693 ± 0.0578^{A}	0.7500 ± 0.0047^{Bb}	0.4693 ± 0.0046^{Cb}	0.1640 ± 0.0047^{D}

Means with different capital superscript in the rows and small superscript in columns are significantly different at least at P<0.05.

Fig. (1): Effect of autogenous *S.* Newport bacterin on nitric oxide level in differnt mice groups

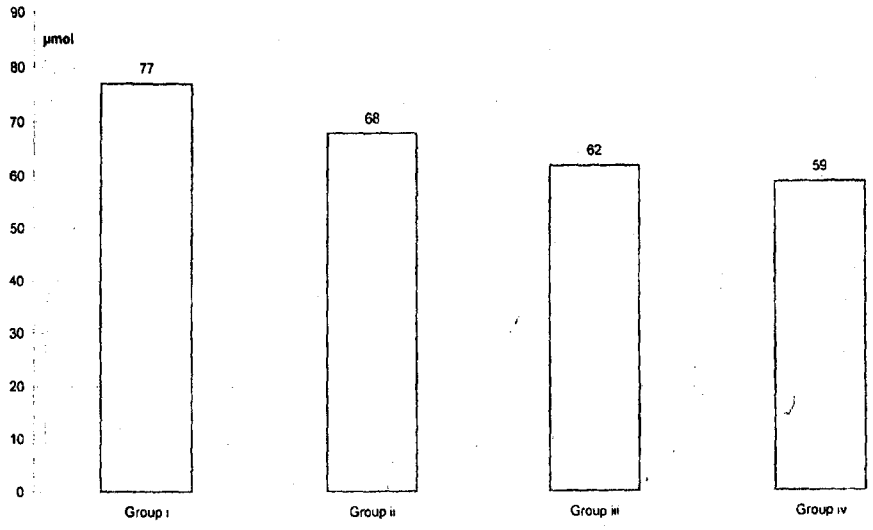

Table (5): Effect of autogenous *S.* Newport bacterin on catalase activity in different mice groups.

Group I	Group II	Group III	Group IV
357.5	276.1	240.6	200.5
392.4	305.2	220.5	211.4
427.3	334.3	233.4	209.4

Table (6): Effect of autogenous *S.* Newport bacterin on H_2O_2 release in different mice groups

Group I	Group II	Group III	Group IV
184.2	194.1	412.2	207.1
187.7	271.9	307.1	210.5
185.5	236.8	359.5	198.5

Table (7): The protection percent of mice immunized with *S.* Newport bacterin after challenged with homologus virulent strain.

Weeks / Groups	Number of dead mice			
	1st week post-challenge	2nd week post-challenge	3rd week post-challenge	Survival rate
Group I	2	1	0	80%
Group II	3	2	0	66.7%
Group III	6	5	3	6.7%

Photo (1) shows dilatation of the intestine with petchial haemorrhage and congestion

DISCUSSION

Acute colitis is a debilitating condition that can affect horses of different breed, age or gender. Colitis is associated with inflammation of the colonic mucosa which leads to the development of diarrhea (Atherton, 2007). *Salmonella* Newport was isolated from 6 out of 7 cases suffering of acute colitis and diarrhea. *Salmonellosis* is typically characterized by an acute septic colitis resulting in prefuse diarrhea (Estepa et al., 2005).

S. Newport has previously been isolated from horses (Traub-Dargatz et al., 1990; Lyytikainen et al., 2000), from carcasses (Hofer et al., 2000) and from horse meat (Espie et al., 2005).

Salmonella species could not be isolated from a horse may be due to mixed infection masked its isolation or *Salmonella* isolation required two or three successive samples for several days. Diarrhea as field problem is caused by multifactor, including the interaction between bacteria (Table, 1). *Salmonella* species was recovered from a case had mild diarrhea in this concern (Atherton, 2007) stated that *salmonella* infection was detected in four syndrome 1) inapparent infection (carrier), 2) acute colitis, 3) depression, fever, 4) septicemia with or without diarrhea. The results obtained in table (1) for incidence of *Klebsiella* species of isolated from diarrheic horse is in agreement to large extent with that of Rennie et al. (1990) who isolated *K. pneumoniae* from horses had watery diarrhea and cramps while concerning *E.coli* isolates our result in the same line of with Holland et al., (1996) who identified different serogroup of *E. coli* from the diarrheic horses.

S. Newport was isolated also from the colonic mucosa and mesenteric lymph node of the 2 dead horses. Larsen (1997) reported that *Salmonella* enterocolitis induced disruption of the host defences and colonization of the distal small intestine and colon was the first step of pathogenesis. Endogenous bacteria and toxins translocated from the gastrointestinal tract into tissue and circulation leading to endotoxemia.

No *Salmonella* species could be isolated from feed and water that were introduced to the animals. The histories of horses showed access to new arrival horses that was a significant risk factor for *Salmonella* excretion. Rodent also may play a role in the spreading of infection. Development of effective strategies to prevent *Salmonella* infection of livestock is important not only for animal welfare but also to reduce losses and the risk of human disease. In Egypt Safwat et al. (1986) prepared formalized alum precipitated vaccine for the local S. Abortus equi and suggested to inject booster doses to ensure long lasting immunity.

Induction of memory by most vaccines is more important from their ability to induce effectors re-

sponses and is most effectively assessed by challenge in the natural host. The humoral responses obtained in this investigation revealed that immunization of mice with autogenous bacterin could enhance anti-*Salmonella* Newport antibody responses which increased rapidly following challenge. This suggest that B cells are needed for the development of antibody responses to *Salmonella* proteins and for isotype switching of antibody response against different *Salmonella* antigens and also, strong serum antibody responses could be detected against cell wall components (Hassan et al., 1993; and Mastroeni et al., 1993; Gray et al., 1996 and Sinha et al., 1997). This might be explained as there is a possible activation of an immunological memory state by this bacterin which is characterise by an accelerated recall response and it was practiculaly evident in the early post challenge periods (between 1st and 2nd week).

The present study showed that immunization by I/M route induces higher antibody titres when compared to the S/C route. This may be due to short period of antigen expression in the epidermis of mice and be insufficient to provoke strong immune response. In our work, the level of protection against S. Newport challenge afforded by parental immunization with autogenous bacterin was effective when compared to the I/P challenged non-immunized group. This finding was similar to others whose mentioned that serum antibody response following immunization against *Salmonella* infection in which individuals possessing antibody responses to whole bacterial cells exhibited decreased intestinal shedding (Gast et al., 1993), reduced extra-intestinal dissemination to different organ sites (Timms et al., 1990 and Charles et al., 1994) and protection against lethal challenge (Aitken et al., 1982).

From the present work, it is cleared that, the immunized mice with autogenous bacterin provoke noticeable innate immune responses as measured by nitric oxide production, catalase activity and hydrogen peroxide production. Nitric oxide is a gaseous inorganic free radical molecular species produced by many types of cells such as leukocytes. Several lines of evidence have suggested a critical role of nitric oxide as an endogenous antimicrobial mediator. In the present trial, we observed that the immunized mice were capable to induce high out put of nitric oxide which has a beneficial effect in host defence against pathogenic bacteria in which activation of nitric oxide production resulted in inhibiting microbial proliferation and decreased mortality. This would met with previously mentioned reports (Granger et al., 1988; James, 1995 and Nathan and Shiloh, 2000).

The experimental immunized mice model in this work expressing an increase of catalase activity and hydrogen proxide as well. Catalase is very efficient enzyme in catalyzation H_2O_2 by influencing its rate of decomposition and capable to regulating the intra-cellular hydrogen peroxide steady state concentration. In particular, hydrogen peroxide has been demonstrated to play role in medi-

ating cell differentiation, proliferation and bactericidal activity in preventing infection. This finding is in agreement to those obtained by Bonini et al. (2007). The increased protection rate amongst vaccinated mice was associated with an elevation of antibody titres at the time of challenge and correlated with an increase of NO production, catalase activity, H_2O_2 release as well. Our results were concided with these reported by DeRose et al. (2002). It was concluded that an awareness of carriers is important because cases of mild disease (diarrhea) in a population of horses caused by *Salmonella* species might otherwise be misdiagnosed. As a result they would not be recognized as a risk for the spread of infection to more susceptible population which may developed severe typical cases. Thus the present trial was clearly illustrate the protective efficiency of autogenous *Salmonella* Newport bacterin and its beneficial enhancement of both humoral and non specific immune responses. However, extensive field trials should be undertaken in order to evaluate this vaccine under stress conditions and current situation in the field of animal husbandry.

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REFERENCES

A P H A American Public Health Research Association (1989): Standard Methods for examination of water and waste water 17th New York.

Aebi, H. (1984): Methods Enzymol., 105:121-126.

Aitken, M. M.; Jones, P. W. and Brown, G. T. (1982): Protection of cattle against experimentally induced *Salmonellosis* by intra-dermal injection of heat killed *Salmonella* Dublin. Research in Vet. Sci., 32: 368-373.

Atherton, R. P. (2007): Efficacy of hyper immunized plasma in the treatment of horses with acute diarrhoea. Master of Science in biochemical and Veterinary Sciences. Faculty of Virginia, Polytechnic Institute, Lees bury, Virginia.

Bonini, M. G.; Siraki, A. G.; Atanassov, B. and Mason, R. P. (2007): Immunolocalization of hydrochlorite induced, catalase bound free radical formation in mouse hepatocytes. Free Radical Biol. Med., 42: 530-540.

Brennan, F. R.; Oliver, J. J.; Baird, G. D. (1994): Differences in the immune responses of mice and sheep to an aromatic dependent mutant of Salmonella Typhimurium. J. Medical Microbiology, 41: 20-28.

Charles, S. D.; Hussain, I.; Choi, C. U.; Nagaraja, K. V. and Sivanandan, V. (1994): Adjuvant subunit vaccines for the control of *Salmonella enteritids* infection in Turkeys. Am. J. Vet. Res., 55: 636-642.

Cohen, N. D. and Woods, A. M. W. (1999): Characteristics and risk factors for failure of horses with acute diarrhoea to survive 122 cases (1990-1996). J. Am. Vet. Assoc., 214 (3): 382-389.

De Rose, R.; Tennet, J.; Mcwaters, P. Chaplin, P.; Wood, P. R.; Kimpton, W.; Cahill, R.; Scheerlinch, J. P. Y.

(2002): Efficacy of DNA vaccination by different routes of immunization in sheep. Vet. Immunology & Immunopathology, 90: 55-63.

Dietert, R. R.; Golemboski, K. A.; Bloom, S. E. and Qureshi, M. A. (1991): The avian macrophage in cellular immunity In: Sharma, J. M. (ed.) Avian Cellular Immunology. CRC Press, Boca Raton, Florida, pp. 71-95.

Espie, E.; Valk, H. DE.; Vaillant, V.; Quelauejen, N.; Querec, F. LE and Weill, F. X. (2005): An outbreak of mulit-drug resistant *Salmonella* enterica serotype Newport infections linked to the consumption of imported horse meat in France. Epidemiol. Infect., 133: 373-376.

Estepa, J. C.; Lopez, I.; Valor, R. M. and Aguilera-Tejero, E. (2005): What is your diagnosis? J. Am. Vet. Med. Assoc., 7 (1): 1081-1082.

Gast, R. K.; Stone, H. D. and Hoft, P. S. (1993): Evaluation of the efficacy of an oil emulsion bacterins for reducing faecal shedding of *Salmonella* by laying hens. Avi. Dis., 37: 1085-1091.

Granger, D. L.; Hibbs, B.; Perfect, j. R. and Durack, D. T. (1988): Specific amino acid (L-arginine) requirement for the microbiostatic activity of murine macrophages. J. Clin. Invest., 81: 1129-1136.

Gray, J. T.; Fedorka; Cary, P. J.; Stabel, T. J. and Ackerman, M. R. (1996): Influence of inoculation route on the carrier state of *Salmonella cholerasuis* in swine. Vet. Microbiol., 47: 43-59.

Green, L. C.; Awagnar, D. A.; Glogowski, J.; Skipper, P. L.; Wishok, J. S. and Tonnebaum, S. R. (1982): Analysis of Nitrate, Nitrite and (15N) nitrite in Biological fluids. Anal. Bioch., 126: 131-138.

Hassan, J. O.; Mockett, A. P. A., Catty, D. and Barrow, P. A. (1993): Infection and reinfection of chickens with *Salmonella* Typhimurium: Bacteriology and immune responses. Avi. Dis., 35: 809-819.

Hofer, E.; Zanora, M. R. N. and Lopes, A. E. (2000): *Salmonella* serovars in meat horses slaughtered in northeastern Brazil. Pesq. Vet. Bras., 20: 80-84.

Holland, R. E.; Schmidt, A.; Siranquanathan, N.; Crimes, S. D.; Wilson, R. A.; Brown, C. M. and Walker, R. D. (1996): Characterization of *Escherichia Coli* isolated from foals. Vet. Microbiol., 48 (3-4): 243-255.

Hous, I. K.; Ontiveros, M. M.; Blackmer, N. M.; Dheyer, E. L.; Fitchhorn, J. B.; Mcarthur, G. R. and Smith, B. P. (2001): Evaluation of an autogenous *Salmonella* bacterin and a modified live *Salmonella* serotype cholera suis vaccine on commercial dairy farm. Am. J. Vet. Res. 62(2): 1897-1902.

I C M S F, International Commission On Microbiological Specification For Food (1978): Micro-organism in food, their significance and methods of enumeration, 2nd ed., Toronto Press, Toronto.

James, S. L. (1995): Role of nitric oxide in parasitic infections. Microbiol. Rev., 59: 533-547.

Kauffmann (1997): White Schem Proff and Le Minor, WHO Center for References and Research on *Salmonella* Institute, Pasteur, France.

Kogut, M. H.; Tellez , G. I.; McGruder, E. D.; Hargis, B. M.; Williams, J. D.; Carrier, D. E. and Deloach, J. R. (1994): Heterophils are decisive components in the early responses of chickens to *Salmonella enteritidis* infections. Microbiol. Pathogenesis, 16: 141-151.

Larsen, J. (1997): Acute colitis in adult horses: A review with emphasis on aetiology and pathogenesis. Vet. Q., 19 (2): 72-80.

Lowry, C. H.; Rosebrough, N. J. and Far, A. L (1951): Protein measurement with the folin phenol reagent. J. Bio. Chem., 93: 265-275.

Lyytikainen, O.; Koort, J. and ward, L. (2000): Molecular epidemiology epidemiology of an outbreak caused by *Salmonella* enterica serovar Newport in Finland and the United Kingdom. Epidemiol. Infect., 24: 185-192.

Mastroeni, P.; Villarreal Ramos, B. and Hormaeche, C. E. (1993): Adoptive tranfere of immunity to oval challenge with virulent *Salmonella* in innately susceptible Balb/C mice requires both immune serum and T cells. Infect. Immun., 61:3981-3984.

Nathan, C. and Shiloh, M. U. (2000): Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogenesis. Proc. Nath. Acad. Sci. US. A., 97: 8841-8848.

Oeliver, O. E. and Stampsli, H. (2006): Acute diarrhoea in the adult horse: Case example and review. Vet. Clin. North American Equine Prac., 22 (1): 73-84

Quinn, P. J.; Markey, B. K.; Carter, M. E.; Donnelly, W. J. and Leonard, F. C. (2002): Veterinary Microbiology and Microbial Diseases, BlackWell science Publication Oxford, London.

Reed, S. M.; Bayly, W. M. and Sellon, D. C. (2004): Equine Internal Medicine (Inflammatory disease of the gastro-intestinal tract causing diarrhoea). 2nd ed, Saunders, Elsevier (USA) pp 1884-1908.

Rennie, R. P.; Anderson, C. M.; Wensley, B. G.; Albritton, W. L. and Mahony, D. E. (1990): *Klebsiella Pneumoniae* gastroenteritis masked by *Clostridium perfringens*. J. Clin. Microbiol., 28 (2): 216-219.

Safwat, E. E. A.; Shouman, M. T.; Awad, M. M.; EL-Bakry, M. and Gergis, S. M. (1986): Preliminary studies on formalized alum precipitated *Salmonella abortus equi* vaccine. J. Egypt Vet. Med. Assoc., 46 (2): 229-234.

Sharma, J. M. and Schat, K. A. (1991): Natural immune functions. In Sharma, J. M. Avian Cellular Immunology, CRC Press, Boca Raton, Florida, 51-70.

Sinha, K.; Mastroeni, P.; Harrison, J.; DeHormacche, R. D. and Hormacche, C. E. (1997): *Salmonella* Typhimurium aroA, htrA and aroDhtrA mutants cause progressive infections in a thymic (nu/nu) Balb/C mice. Infect. Immun., 65: 1556-1569.

Timms, L. M.; Marshall, R. N. and Breslus, M. F. (1990): Lab assessment of protection given by an *Salmonella entritidis* PT inactivated adjuvant vaccine. Vet. Record, 127: 611-614.

Traub-Dargatz, J. L.; Salman, M. D. and Jones, R. L. (1990): Epidemiologic study of *Salmonellae* shedding in the faeces of horses and potential risk factor for development of the infection in hospitalized horses. J. Am. Vet. Med. Assoc., 196: 1617-1622.

VanDuijkeren, E.; Flemming, C.; VanOldruitenborgh, M.; Kolsbeck, H. C. and Van derGiessen, J. W. B. (1995): Diagnosing *Salmonellosis* in horses, Culturing of multiple versus single faecal samples. Veterinary Quartely, 17: 63-66.

Xu, Y.; Chen, A.; Fry, S.; Barrrow, R. A.; Marshall, R. L. and Mukkur, T. K. S. (2007): Modulation of immune response in mice immunize with an inactivated *Salmonella* vaccine and gavaged with Andrographis pariculata extract or andrographolide.

البكتريا الهوائية سالبة الجرام المصاحبة لتقلصات حادة واسهال فى الخيول وتقييم كفاءة البكترين الذاتى لسالمونيلا نيوبورت

أمانى ابراهيم البيلى وحدة السيرولوجى- معهد بحوث صحة الحيوان- الدقى- الجيزة
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تم دراسة مشكلة حقلية فى احدى مزارع الخيول الحكومية التى تعانى من ارتفاع فى درجة الحرارة وتقلص حاد واسهال. تم فحص ٥٨ عينة براز (٧ عينات من خيول تعانى من حالات تقلص حاد واسهال و ٥١ عينة براز من خيول تعانى من حالات اسهال بسيط). بالفحص البكتريولوجى ل ٧ عينات تم عزل سالمونيلا نيوبورت، الميكروب القولونى، الكلبسيلا اوكسيتوكا والبروتيس بنسبة ٨٥,٧%،٤٢,٩%، ٢٨,٦%، ١٤,٣% على التوالى وبفحص ٥١ عينة براز من الخيول التى تعانى من حالات اسهال بسيط تم عزل الميكروب القولونى، السودوموناس ايروجنوزا، كلبسيلا اوكسيتوكا، وستربتوباكتر فريندى، بروتيس، كلبسيلا الرئوية وسالمونيلا نيوبورت بنسبة ٨٦,٣%، ٣٩,٢%، ٢٩,٤% ٢٥,٥%، ٢١,٦%، ١١,٨% و ٢% على التوالى. وقد أجرى التصنيف السيرولوجى للسالمونيلا والميكروب القولونى. تم عزل سالمونيلا نيوبورت من ٦ حالات خيول من ٧ تعانى من تقلصات حادة واسهال وعزلت من احدى الخيول التى تعانى من اسهال بسيط. كما تم عزل سالمونيلا بيوبورت من الغشاء المبطن للقولون والغدد الليمفاوية بالامعاء من حالتين نافقتين. لم يتم عزل سالمونيلا من الأعلاف أو الماء وقد وجد ان وصول احدى الخيول الجديدة هو السبب الرئيسى فى وجود السالمونيلا وانتشارها فى مزرعة الخيول.

تمْ عمل تجريبى لبكترين السالمونيلا نيوبورت الذاتى وتقييمه فى نموذج الجرذان للتحكم فى انتشار هذا الميكروب. حيث اجريت دراسات مناعية وحماية فى الجرذان بتحصين الجرذان بجرعتين من البكترين الذاتى بحقن عضلى واخر تحت الجلد. وقد حدتت الاجسام المناعية المضادة السائلة باستخدام اختبار الاليزا. كما تم قياس كل من انتاج اكسيد النيتريك، نشاط الكتاليز وانتاج بيروكسيد الهيدروجين كقياسات مناعية خلوية غير خاصة. وقد أدى التحصين بالبكترين الذاتى الى رفع مستوى الاستجابة المناعية السائلة بعد التحصين وخاصة بعد التعرض لميكروب السالمونيلا نيوبورت مقارنة بالمجموعة الضابطة. بالاضافة الى ارتفاع فى انتاج اكسيد النيتريك- نشاط الكاتليز وزيادة انتاج بيروكسيد الهيدروجين وقد أوضحت النتائج ان نسبة الحماية قد زادت فى المجموعات المحصنة بالبكترين (٨٠%، ٦٦,٧%) بينما قلت فى المجموعة الغير محصنة والمعرضة للميكروب.

وعلى هذا يتضح ان لهذا البكترين القدرة على تحسين كل من الاستجابة المناعية السائلة والخلوية الغير خاصة ضد العدوى بالسالمونيلا نيوبورت لذلك يجب عمل المزيد من الدراسات الحقلية لتقيمكفاءة اللقاح.