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# Immunological and Biochemical Studies on Polyvalent *Pasteurella* Vaccine in Camels

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## ABSTRACT

Camel pasteurellosis is a bacterial disease affecting respiratory system of camels caused by *Pasteurella* species. In Egypt, there are no recommended vaccination programs of camel against pasteurellosis. Therefore, evaluation of the efficacy of a polyvalent *Pasteurella* vaccine in camels is the main target of this research focusing the attention on its immunological and biochemical patterns of response. The humeral immune responses in two different camel groups (vaccinated and control) were evaluated by indirect haemagglutination test (IHA) and Passive Mouse Protection Test. The biochemical profiles of different camel groups were estimated. In addition to, the assessment of lipid peroxidation and Matrix Metalloproteinase enzymes (MMP2 & MMP9) activity of camels under this vaccination trial. Significant positive humeral responses against different vaccinal *Pasteurella* species were recorded in the vaccinated camel group and reached their peaks (1:64-1:256) around the 3$^{th}$ month post vaccination. An evident passive mouse protection against the challenge of virulent strains of *P. multocida* type A, B and D and *Mannheimia haemolytica* types A & T were also recorded. Biochemically, there were no significant differences in the biochemical profiles, lipid peroxidation and Matrix Metalloproteinase (MMP) enzymes activity between the vaccinated and control group. It's recommended to include camels in the epidemiological studies and periodical vaccination programs (every 6$^{th}$ months) in parallel with other contact animal species to perform a high protection and good control managements of pasteurellosis in Egypt.

## 1. INTRODUCTION

Pasteurellosis is a bacterial disease affecting the health status and productivity of camels. Clinically, infected camels showed signs of depression, loss of appetite with respiratory manifestations as serous then mucopurulent nasal discharge and at late stages the camel becomes recumbent to death, due to edema, congestion and septicemia (Rana et al., 1993; Bekele, 1999). *Pasteurella multocida* and *Mannheimia haemolytica* are gram negative rods that can cause pneumonia either alone or with other organisms (Bekele, 1999; Al-Rawashdeh et al., 2000 and Azam and Zaki, 2006). Both strains were found to be normal inhabitant in the respiratory tract of camels; they are opportunistic bacteria causing diseases when the body's defense mechanisms are impaired (Quinn et al., 2002; Seleim et al., 2003 and Radostitis et al., 2007). In some reports, *Pasteurella* spp. were the main isolate obtained from pneumonic camel lungs, at a rate of 56% (Al-Ani et al., 1998 and Al-Rawashdeh et al., 2000). Tigani et al., (2007) and Wareth et al., (2014) recorded isolation rate from affected camel lungs, at low percentages of 1.07 and 2.85% respectively. El-Deeb (2015) recorded 0.04% as isolation rate for *Pasteurella* spp. from nasal swabs of pneumonic camel calves. The total recovery of *Pasteurella multocida* from pneumonic camel lung lesions were 2.9% (Abo-Elnaga and Osman, 2012), 4.4% (Abubakar et al., 2010) and 10.7% (Chitgar et al., 2014), while *Mannheimia haemolytica* was isolated from pneumonic camel lungs, at rates of 0.3% (Abubakar et al., 2010), 1.4% (Abo-Elnaga and Osman, 2012), 6.6% (Al-Tarazi, 2001) and 7.4% (Mahmoud et al., 2005). Nasal swabs and blood specimens from clinical cases and contact apparently healthy camels showed similar isolation patterns. *Pasteurella multocida* subspecies *multocida*

(PMM) (serotype B) was isolated in 85.9%, 65.6% and 30.4%, 8.1% respectively, while *Pasteurella multocida* subspecies *septica* (PMS) (serotype A) was isolated from clinical cases only by 3.1% and 1.6% respectively. *Pasteurella multocida* subspecies *multocida* (PMM) serotype (A) was also isolated from nasal swabs of contact apparently healthy camels by 3% (Seleim et al., 2003). Also, *Pasteurella multocida* subspecies *multocida* was isolated from representative clinical and morbid specimens during an outbreak of a respiratory disease occurred in the dromedary population in Pakistan (Fraz Munir Khan, 2011). The bacterium was also reported to affect a wide range of animal species as cattle, buffalo, sheep, goat, pig, wild animals as well as poultry and ducks (Bekele, 1999; Sunder and Kumar 2001). There are different types of *Pasteurella* vaccines, one of them is (Pneumo-bac®, VSVRI) which used to protect livestock against pasteurellosis in Egypt, while there is no studies about its evaluation trials in camels. The Pneumo-bac® vaccine is an inactivated oil adjuvant polyvalent *Pasteurella* vaccine used for active immunization of cattle, sheep and goat against pneumonic pasteurellosis. In areas where the disease occurs seasonally, animals should be vaccinated at 4 or 5 weeks before the season. When outbreaks of the disease occur, all contact stock must be vaccinated as soon as possible (VSVRI, 2014). In order to avoid epidemics, vaccination of dromedary population on a routine basis should be coupled with an efficient and percipient disease reporting and surveillance system (Fraz Munir Khan, 2011). Serum protein measurement constitutes a vital component of laboratory diagnostic evaluations in animals. Albumin is an acute phase protein and its concentration decreases in inflammation (Mohamed et al., 2010). As The main functions of albumin are the transport of several molecules and the maintenance of blood oncotic pressure (Melillo, 2013). Albumin fraction increased at a rate roughly matching total protein increase, while globulin fraction increased even more. This led to decreased albumin to globulin ratio after vaccination, which can be an indicator of antibody production. Globulin elevation is correlated with the body's immune response against infection (Agag et al., 1992). The immune responses and protection in vaccination are mediated through interaction of reactive oxygen free radicals and antioxidant system. These can be assessed by various oxidative stress and antioxidant biomarkers (Kumar et al., 2017). The main lipid peroxide byproduct is malondialdehyd (MDA) (Heiderpour et al., 2013) which used as a good marker for oxidative damage (Kandemir et al., 2011). Matrix metalloproteinases (MMPs) are enzymes very likely to have a central role in destructive pulmonary diseases where excess proteolytic activity causes aberrant degradation of the lung extra cellular matrix (ECM). In Egypt, Many *Pasteurella* vaccines are available for livestock including cattle, buffalo, sheep and goats without considerable data about the evaluation of these vaccines in camels. Therefore, the aim of this research work is to evaluate the efficacy of a polyvalent *Pasteurella* vaccine (Pneumobac®) in dromedary camels focusing the attention on its immunological and biochemical patterns of response.

## 2. MATERIAL AND METHODS

### 2.1. Animals:

Eight camels (6-8 years old) were divided into two groups four in each. One group was vaccinated and the other was control.

### 2.2. Vaccine:

Pneumo-bac® vaccine is an inactivated oil adjuvant polyvalent *Pasteurella* vaccine contains *Pasteurella multocida* types A, B & D and *Mannheimia haemolytica* types A & T inactivated with formalin and emulsified in oil base. It was injected s/c in two doses (2ml each) three weeks interval in one camel group.

### 2.3. Serum Samples:

Blood samples from the two groups were collected periodically, from 0 time till 6 months post vaccination from the jugular vein of each camel and left to clot in a clear dry centrifuge tubes, then centrifuged at 3500 r.p.m for 15minutes. The serum was frozen at-20°C until subsequent analysis.

### 2.4. Indirect Hemoagglutination (IHA):

Different *Pasteurella multocida* types A, B & D and *Mannheimia haemolytica* types A & T antigens were kindly provided from VSVRI, Cairo, Egypt. Blood was collected from sheep. Erythrocytes were washed in phosphate buffer saline (PBS) pH 7.4 then synthetized with the different antigens. The antibody titers of the serum samples were determined according to (Carter, 1972).

### 2.5. Passive Mouse protection test:

Six groups of white Swiss mice weighing 20 to 25 g were used. *Pasteurella multocida* types A, B & D and *Mannheimia haemolytica* types A & T cultures were used for challenge (VSVRI). Trypticase soy agar (BBL) was used for their slant cultures and for poured plates. For the determination of LD50, The challenge inoculum was prepared by suspending in saline the growth on a 21-hr slant culture and adjusting the suspension to a standard optical density.

Decimal dilutions of this suspension were made in saline containing 0.1%c gelatin. The final dilution for inoculation was made in 5% gastric mucine (VSVRI) which had been adjusted to pH 7.2 to 7.4 after autoclaving. The actual number of colony-forming units injected was determined by plate counts of appropriate dilutions of the standardized suspension in gelatin-saline. Six groups of mice were inoculated intraperitoneally with 0.5 ml of mucin suspension containing graded numbers of bacteria and were observed for 72 hr. The majority of deaths occurred within 48 hr. The number of organisms required to kill half the mice (LD50) and the dilution of test material required to protect half the mice for 72 hrs (ED50) were estimated by the method of (Reed and Muench, 1938). For the determination of ED50, Mice were injected subcutaneously with 0.25 ml of fivefold dilution of serum fractions containing antibody. 21 hours later, they received a challenge intraperitoneal injection of live *Pasteurella multocida* types A, B & D and *Mannheimia haemolytica* types A & T suspended in mucine. The challenge dose was calculated to be between 1,000 and 2,000 LD50. The actual dose in each experiment was determined by plate count.

**2.6. Biochemical analysis:**

Total protein and albumin levels were estimated according to the methods described by (Doumas, 1975 and Doumas et al., 1971) respectively. Serum globulin was calculated according to (Latner, 1975).

**2.7. Assessment of lipid peroxidation:**

Malondialdehyde (MDA) concentration was used as the index of lipid peroxidation as described by (Ohkawa et al., 1979). MDA was determined by measuring the thiobarbituric acid reactive species. The absorbance of the resultant pink product was measured at 534 nm.

**2.8. Assessment of Matrix MetaloProteinase (MMP) enzymes activity:**

The activity of MMP-9 was detected in gelatin zymography by a method described by (Hawkes et al., 2010). Briefly, serum samples were separated by SDS/PAGE on 7.5% (w/v) gels, containing 1 mg/ml gelatin under non-reducing conditions. Then, it was washed twice for 15 min each in 2.5% (v/v) Triton X-100 and incubated in development buffer (0.05 M Tris/HCl, pH 8.8, 5 mM $CaCl_2$, 0.02% $NaN_3$) for 15 min to overnight incubation. Gels were stained with 0.1% Coomassie Brilliant Blue R250 in methanol:acetic acid:water (4.5:1:4.5, v/v/v). The zymograms gels were scanned in true colour and then analysed using commercially available software (myImageAnalysis Software; Thermoscientific ™) after conserving to grey scale.

**2.9. Statistical analysis:**

The obtained data were statistically analyzed using M.S. Excel 2010 software to get significance ($P < 0.005$).

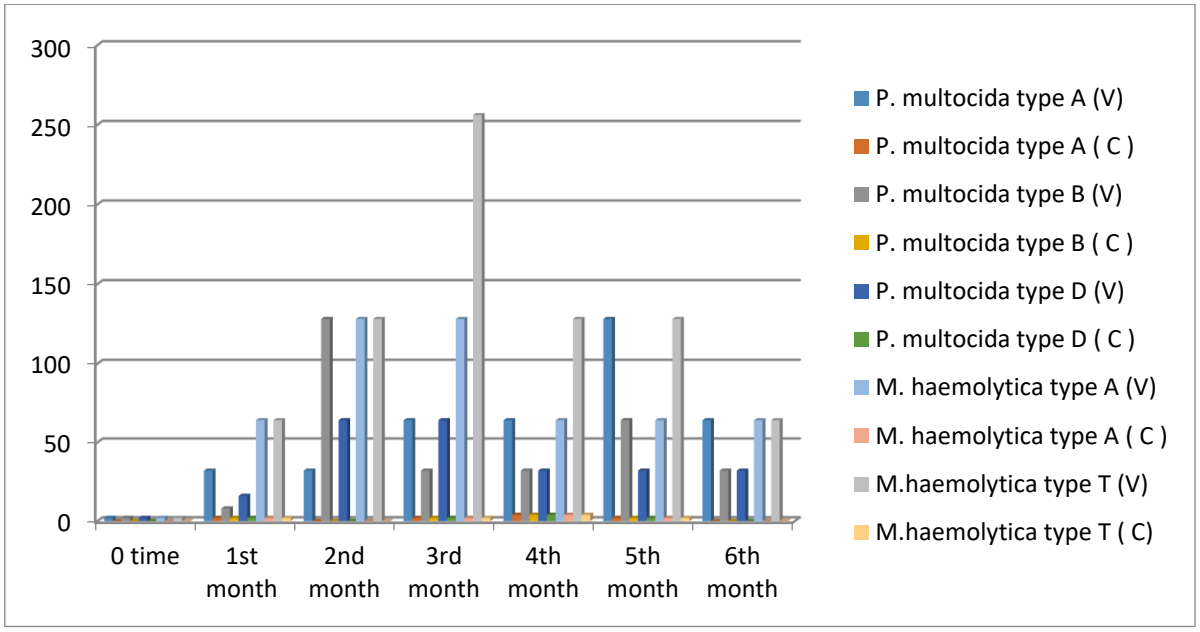
## 3. RESULTS AND DISCUSSION

There is a general lack of epidemiological investigations explaining camel diseases patterns in relation with the different prevalent husbandry systems (Baumann and Zessin, 1992), this may be attributed to the migratory system of camel production in remote areas with harsh living conditions that make such studies difficult and expensive to be executed (Khalafalla, 1998). Haemorrhagic septicemia is one of these epidemiological diseases which is widely spread and mainly affect cattle, buffaloes and sheep causing major economic losses and need more studies in camels from an epidemiological, management and control points of view in Egypt (Abd El Tawab et al., 2016). The disease occurs commonly in many countries (De Alwis, 1992). There are few studies about its control and vaccine evaluation in camels. Camels can be clinically affected or act as a carrier of the disease to other animal species. Septicemia in camels caused by *Pasteurella species* leading to fever, swelling in the throat region, pulmonary edema, fibrinous pneumonia and death (Saber, 2006). Data illustrated in table and figure (1), expressed the different humeral immune responses against the vaccination process of the polyvalent *Pasteurella* vaccine (pneumo-bac®) in camels evaluated by indirect hemagglutination test (IHT). Higher antibody agglutinating titers against different types of *Pasteurella multocida* and *Mannheimia haemolytica* were detected in the vaccinated camel group without any considerable titers in the control one. It begins to increase from the 1st month reaching its peak around the 3th month (1:64- 1:256) then begins to decline gradually till the 6th month. The obtained results of antibody levels were in agreement with that obtained by Effat et al., (2011) in calves who found that, the mean agglutination titer increased by the 4th week post vaccination, reached the maximal level at 16th then began to decrease**.** Similar result to some extent was also reported by Eman El-Rawy et al., (2003) in sheep who found that, the circulating antibodies against *Pasteurella multocida and Mannheimia haemolytica* were firstly detected two weeks post the primary vaccination, continued till 9 months and reached to peak in a period from 2 to 16 weeks post the first vaccination.

**Table (1):** Levels of antibody titers against different types of *Pasteurella multocida* and *Mannheimia haemolytica* in vaccinated camel sera by indirect haemagglutination test (IHA).

| | Level of antibody titers | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***P. multocida* type A** | | ***P. multocida* type B** | | ***P. multocida* type D** | | ***M. haemolytica* type A** | | ***M. haemolytica* type T** | |
| | **V** | **C** | **V** | **C** | **V** | **C** | **V** | **C** | **V** | **C** |
| **0 time** | 2 | 0 | 2 | 0 | 2 | 0 | 2 | 0 | 2 | 0 |
| **1st month** | 32 | 2 | 8 | 2 | 16 | 2 | 64 | 2 | 64 | 2 |
| **2nd month** | 32 | 0 | 128 | 0 | 64 | 0 | 128 | 0 | 128 | 0 |
| **3rd month** | 64 | 2 | 32 | 2 | 64 | 2 | 128 | 2 | 256 | 2 |
| **4th month** | 64 | 4 | 32 | 4 | 32 | 4 | 64 | 4 | 128 | 4 |
| **5th month** | 128 | 2 | 64 | 2 | 32 | 2 | 64 | 2 | 128 | 2 |
| **6th month** | 64 | 0 | 32 | 0 | 32 | 0 | 64 | 0 | 64 | 0 |
| **Over all mean of AB titers** | 55.14 | 1.42 | 42.57 | 1.42 | 34.57 | 1.42 | 73.42 | 1.42 | 110 | 1.42 |

V: vaccinated camel group. C: control camel group




V: vaccinated camel group. C: control camel group

**Figure (1):** Levels of antibody titers against different types of *Pasteurella multocida* and *Mannheimia haemolytica* in vaccinated camel sera by indirect haemagglutination test (IHA).

It was found that, the mean of the agglutinating antibody titers against *Mannheimia haemolytica* is higher than that of *Pasteurella multocida* in agreement with Rad et al., (2009) who also concluded that, *Mannheimia haemolytica* is a significant cause of septicemia and mortality with acute pneumonia.As the Passive mouse protection test is an essential test for the evaluation of the efficacy and potency of the polyvalent *Pasteurella* vaccines against challenge with virulent types of *Pasteurella multocida* and *Mannheimia haemolytica* we can recorded that, Pneumo-bac® as a polyvalent *Pasteurella* vaccine is effective and potent as it can stimulate immunological humeral immune response in camels and protect 100% against virulent challenge with different types of *Pasteurella multocida* and *Mannheimia haemolytica* in mouse protection test. Similar findings were reported by Fatma (2012) in sheep.

From biochemical point of view as presented in Table (2), there was no significant difference in protein profiles (total protein, albumin and globulin) with in control and vaccinated group in agreement with Barkakati *et al.*, (2015) who compared the average value of total serum protein in FMD affected, recovered and apparently healthy animals and found no significant variation between the recovered and apparently healthy animals, also with Hamada *et al.*, (2013) who found no significant difference in albumin and total protein between brucellosis-

affected and healthy ewes. Wolf *et al.*, (2008) also found no statistically significant changes in β- and γ-globulin fractions after vaccination with two types of commercial equine vaccines against West Nile virus infection. However, El-Boshy *et al.*, (2009), Al-Kaysi *et al.*, (2010) and Al-Hussary *et al.*, (2010) reported a significant decrease in serum albumin between brucellosis-affected and healthy camels.

**Table 2:** Total protein, albumin and globulin concentration (gm%) in Clinically Healthy control Camel and vaccinated camel groups within 6 months.

| Groups | Control | Vaccinated group | | | | | |
|---|---|---|---|---|---|---|---|
| | | **1st month** | **2nd month** | **3rd month** | **4th month** | **5th month** | **6th month** |
| Total protein | 6.5±0.7[a] | 5.7±0.8 [a] | 6.1±0.4 [a] | 5.5±0.7 [a] | 6.6±0.2 [a] | 6.2±0.3 [a] | 5.3±0.3 [a] |
| Albumin | 3.5±02 [a] | 3.2±0.2 [a] | 3.3±0.2 [a] | 3.2±0.2 [a] | 3.7±0.1 [a] | 3.6±0.1 [a] | 3.4±0.2 [a] |
| Globulin | 2.9±0.5 [a] | 2.4±0.6 [a] | 2.8±0.2 [a] | 2.2±0.7 [a] | 2.8±0.3 [a] | 2.5±0.3 [a] | 1.9±0.5 [a] |

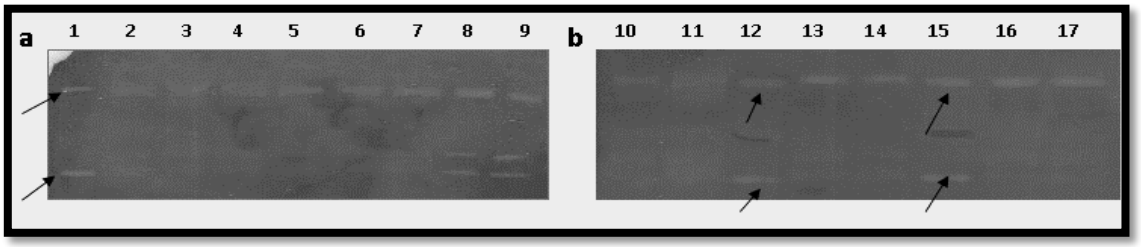
Data are presented as mean ±S.E.M.

**Table 3:** lipid peroxidation marker (Malondialdehyde, mM/gm protein) concentration and Matrix metalloproteinases activity (%) in clinically healthy control camel and vaccinated camel groups within 6 months.

| Groups | control | Vaccinated group | | | | | |
|---|---|---|---|---|---|---|---|
| | | **1st month** | **2nd month** | **3rd month** | **4th month** | **5th month** | **6th month** |
| MDA | 11.5 ±0.5 [a] | 11.4 ±0.8 [a] | 10.9 ±0.6 [a] | 11.9 ±0.6 [a] | 11.7 ±0.6 [a] | 11.1 ±0.5 [a] | 11.9 ±0.3 [a] |
| MMP-9 | 4 ±0.0 [a] | 3.9 ±0.1 [a] | 4 ±0.0 [a] | 4 ±0.2 [a] | 4 ±0.0 [a] | 4 ±0.2 [a] | 3.9±0.1 [a] |
| MMP-2 | 3±0.0 [a] | 3±0.0 [a] | 3±0.1 [a] | 3±0.2 [a] | 3±0.2 [a] | 3±0.1 [a] | 3±0.1 [a] |

**Data are presented as mean ±S.E.M.**

**Figure (2): Activity of MMP by gelatin zymography in clinically healthy camel and vaccinated camel groups within 6 months.**




**(a)** Lanes 2–3 = control; lanes 4-5 = 1st month ; lanes 6-7 = 2nd month ; lanes 8-9 = 3rd month Positive controls shown in lanes1 are from baby hamster kidney cells transfected with active MMP-9 (86 kDa) and MMP-2 (66KDa) that are indicated by arrows.

**(b)** Lanes 10-11 = 4th month; lanes 13–14 = 5th month; lanes 16-17 = 6th month. Positive controls shown in lanes12 and15 are from baby hamster kidney cells transfected with active MMP-9 (86 kDa) and MMP-2 (66KDa) that are indicated by arrows.

There is a general lack of available studies that describe blood antioxidant and lipid peroxidation status of camel diseases. In our present study, as showed in table (3) & fig. (2), there is no significant difference between clinically healthy and vaccinated camel within 6 months as the role of vaccination disappear the presence of disease. Although MMPs play important roles in normal pulmonary immunity, in excess they can contribute to immunopathology that leads to morbidity and mortality (Elkington and Friedland, 2006). In the present study There was no significant difference in gelatin form of matrix metalloproteinases activity % (MMP-9,MMP-2) between clinically healthy camel and vaccinated camel within 6 months.

The non-significant results of biochemical profiles between control healthy and vaccinated camel groups may indicate the wide safety profile of Pneumo-bac® vaccine when used in camels.

From the obtained results we can conclude that, a polyvalent *Pasteurella* vaccine is effective for the protection of camels against pasteurellosis for at least 6 months post vaccination as it can stimulate the humeral immune response to produce durable and

potent agglutinating antibody titers which can protect against challenge with different virulent types of *Pasteurella multocida* and *Mannheimia haemolytica*. Therefore, Camels should be included in vaccination programs in parallel with other contact animal species with a polyvalent *Pasteurella* vaccine every 6th months to ensure complete and restricted control measures against pasteurellosis in Egypt.

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