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Attenuated Mycobacterium Farcinogenes Strain A24 As A Protective Vaccine Candidate Against Bovine Farcy

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Abstract: The present study was designed to develop a live vaccine against bovine farcy. A wild strain of *Mycobacterium farcinogenes* was subjected firstly to serial passage (20) in modified Sauton's broth then in guinea pigs. The result of the vaccination in calves revealed that 75% of calves in the vaccinated group were protected and withstanded the challenge infection with a virulent freshly isolated strain *M. farcinogenes*. In addition to pathological and clinical assessment, ELISA was used to monitor the humoral antibody response where the highest antibody titer was achieved at the 5th week in calves post-vaccination. We came to conclusion that the attenuated *M. farcinogenes* A24 could be used to induce a protective immunity against bovine farcy, and further evaluation of the vaccine should be carried under field condition.

Key words: Bovine farcy, Mycobacterium farcinogenes, Sudan, Vaccine

Introduction

Bovine farcy is a chronic infectious disease affecting Zebu cattle mainly in the tropical countries. The disease is characterized by tumification and suppurative granulomatous inflamation of the lymphoid and subcutaneous tissues and had been reported in many African countries: Nigeria, Chad, Senegal and Somalia Mustafa (1966), Chamoiseau (1979), Hamid *et al.* (1991). In the past the disease was thought to be caused by *Nocardia farcinica* but in 1979 *M. farcinogenes* and *M. senegalense* were proved to be the causal agents Chamoiseau (1979).

In 1958, Awad and Karib, showed a relationship between bovine farcy and bovine tuberculosis. They noticed cross-reaction between tuberculosis and bovine farcy. These cross-reactions may confuse the interpretation of tuberculin testing in countries where both diseases exist Awad (1958), Mostafa (1967).

Many aspects of bovine farcy have been studied during the last four decades. These included bacteriological, taxonomical, pathological and serological studies Mostafa (1967), El- Sanousi *et al.* (1979), Ridell *et al.* (1982), Hamid *et al.* (1991), Hamid *et al.* (1993a), Hamid *et al.* (1993b), Hamid *et al.* (1998). The major breakthrough has been done in the bacteriology and the taxonomy of the causal agent, but there was no work done on the immunity and/or the production of a protective vaccine. However *M. farcinogenes* and *M. senegalense* were found to be sensitive to some antibiotics, yet no effective treatment in vivo was achieved. This will especially be true in case of the nomadic cattle where the nature of the granulomatous mycobacteriosis requires several months for complete recovery Chamoiseau (1979), El-Sanousi *et al.* (1979), Hamid (1988).

The aim of this study was to test the immunogenicity of a non-virulent attenuated *M. farcinogenes* strain as a vaccine candidate in Zebu calves and to monitor its immune response.

Materials and Methods

Strains: M. farcinogenes Strain M62 had been previously isolated from a case of bovine farcy in Sudan Hamid *et al.* (1988). This strain was then subjected to serial passages in modified synthetic media and in guinea pigs and the resultant strain was labeled *M. farcinogenes* A24.

M. farcinogenes strain SD117, was isolated from a case of bovine farcy which was purchased from local market. This strain was used for challenge infection. The previously identified *M. farcinogenes* strains M39, M217 and M16 were included as controls Hamid *et al.* (1993a).

Vaccination: Eleven calves, (six month old) were used in the vaccination trial. Group I, which included 8 calves, was inoculated with 1ml of the prepared vaccine, at a concentration of ($1.5x10^8$ CFU/ ml). The inoculation was done subcutaneously at the left side of the abdomen. Group II, contained 2 calves, inoculated with 1ml normal saline, and used as negative control. Group III, consist of one calf, inoculated with 1ml normal saline but received neither vaccine nor challenge infection (control group).

The vaccinated and non-vaccinated calves received challenge infections after five weeks post vaccination with ($1.5x10^8$ CFU/ ml) from a young culture of the virulent *M. farcinogenes* SD117. 1ml was inoculated subcutaneously at the right side of the abdomen (opposite to the side used for vaccination).

Indirect Enzyme – Linked Immunesorbant Assay (ELISA): ELISA was done as described by Ridell (1982), Hamid *et al.* (1998) to monitor the immune responses.

Preparation of Antigens: 0.5mg of *M. farcinogenes* SD117 cell culturing , were harvested and kept in sterile Bijou vial, then washed three times with sterile distilled water and suspended in 1.5 ml sterile distilled water. One gram of glass beads (100μl, Sigma), was added, the mixture was vortexed for 15 minutes and centrifuged at 3000 rpm for 15 minutes. The supernatant was transferred into another sterile Bijou vial and stored at -20°C till used. Blood samples were aseptically collected from calves according to the following program zero time; 3rd week, 5thweek, 17th week, 32nd weeks, and 52nd weeks post vaccination. Sera were separated and transferred in sterile Bijou vials and stored at -20 °C.

ELISA Procedures

Antigen: The antigen was diluted with Phosphate Buffer Saline (PBS) at PH 7.2 as a coating buffer in the ratio of 1:2 (antigen: PBS). 100 μl of antigen were delivered into the wells of microtetire ELISA plates and incubated at 37°C overnight. Plates were then washed three times for three minutes with PBS-Tween 20 (washing solution).

Test serum: 100 μl volumes of the test sera diluted at 1:100 in PBS (PH 7.2) were delivered into the wells of the coated plates and incubated at 37°C for one hour, and then washed with Tween 20 as mentioned previously.

Conjugate: 100μl volumes of anti-bovine IgG (whole molecule) alkaline phosphatase conjugated, were freshly diluted at the rate of 1:1000 in PBS and added to the wells of the plates, and incubated at 37°C for one hour.

Substrate: 100 μl volumes of a freshly prepared p-nitrophenyl phosphate (p-NPP) tablets in diethanolamin buffer (pH 9.2 at conc. 1mg/ml) were added to the wells of the plates and incubated at 37°C for one hour and then read at 450 nm.

Statistical Analysis: Student ?s t-test was used to compare the means of the optical density in the vaccinated cohort and the non-vaccinated cohort of calves against time (in weeks). The data were screened for consistency, independence and normality and it were found independent and approximately normally distributed. SPSS (version 10) statistical software was used to carry out the analysis

Results

Vaccination of Calves: The results of calves vaccination using *M. farcinogenes* strain A24 and the challenge infection with *M. farcinogenes* strain SD117 were as follows:

Seventy five percent (75%) of the calves' in-group I (vaccinated group) produce no lesions at the site of inoculation neither after three weeks nor after 52 weeks post inoculation. Twenty five percent (25%) of the calves showed small nodules at the site of inoculation, which were smaller than those, produced in the non-vaccinated group. All the calves in group 2, which contained the negative control, showed different size nodules at the site of inoculation (Fig. 1). One large nodule of them (1-2cm in size) was found to contain *M. farcinogenes* when examined microscopically.

ELISA: This test was carried out to detect humoral antibody titers in vaccinated and non-vaccinated calves. The mean optical densities for the vaccinated calves were as follows: 0.153 nm at zero weeks; 0.777 nm at 3rd week; 2.72 nm at 5th week; 0.64 nm at 17th week; 0.45 nm at 32nd week and 0.24 nm at 52nd week. The mean optical density for the non-vaccinated calves was found to be 0.20 nm at zero weeks; 0.229 nm at 3rd week; 0.229 nm at 5th week; 0.78 nm at 17th week; 0.577 nm at 32^{nd} week, and 0.175 nm at 52^{nd} week. The results were shown in Fig. (2). The antibody titre reached the peak at the 5th weak and then began to decline progressively till it disappeared at the 52nd weak post vaccination.

Comparison of Antibody Titres Between Vaccinated and Non-Vaccinated Calves: No significant difference ($P>0.05$) between the antibody titre was observed at the zero weeks, 17th week, 32^{nd} and 52nd week between vaccinated and non vaccinated group. However a significant difference ($P<0.05$) was observed between the two groups at the 3rd and 5th week.

Comparison of the OD Between Vaccinated Calves at Different Intervals of the Experiment: One-way analysis of variance (ANOVA) was used to test the difference in antibody titre between week intervals. No significant difference

($P > 0.05$) in antibody titre was observed in zero weeks, 17th week, 32nd week and 52nd week, between the vaccinated and non-vaccinated calves. However a significant difference ($P < 0.05$) was observed between the two groups at the 3rd and 5th week post vaccination.

Discussion

To the best of our knowledge, there are no reports in literatures concerning usage of vaccine against bovine farcy, but there is some debates in vaccination against some other mycobacteria. The treatment and control of bovine farcy was not successful. The aim of this study was to test an attenuated strain of *M. farcinogenes* and to monitor the humoral antibody responses in vaccinated and non-vaccinated calves using ELISA technique in order to help in the control of an important disease of cattle in Africa.

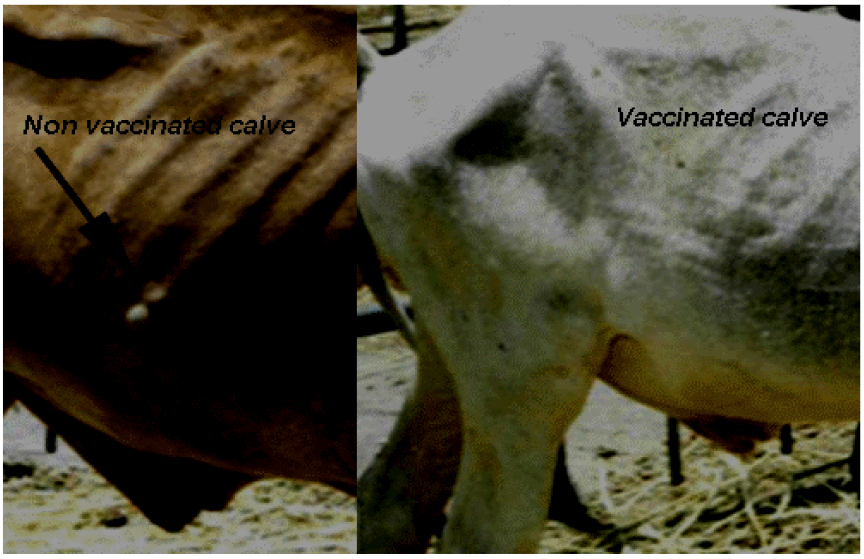

Fig. 1: Vaccinated calve (right) showing no lesions at the site of the challenge infection neither after three weeks nor after 52 weeks post inoculation. Notice calve in the non-vaccinated group (left) showing different size bovine farcy nodules (arrow) at the site of challenge infection.

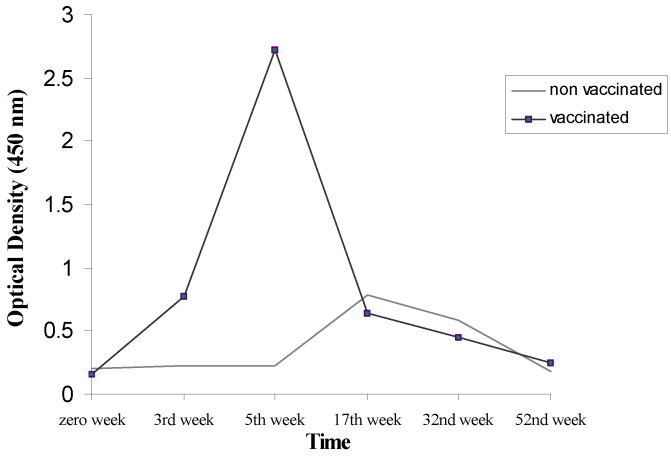

Fig. 2: Humeral antibodies response in vaccinated and non-vaccinated calves

Frerichs (1973) vaccinated guinea pigs with *M. jahnei* in a dose equal to 1\3 that of cattle; he found that the concentration of the antigen determines the size of the local reaction. Such lesions were also seen in some mycobacteria such as *M. bovis* which produced tuberculin hypersensitivity reaction when injected intradermallay William (2001). This was in agreement with Saxegaarad (1985) who noticed local infiltration at the site of inoculation in goats vaccinated against paratuberculosis. In the present study on challenge infection we noticed that all the non-vaccinated calves and guinea pigs showed local nodules at the site of inoculation. The size of nodules was bigger comparable to that of the vaccinated group; this is because the non-vaccinated animals had not been exposed to the antigen before and hence severe local reaction occurred when subjected to the challenge infection. Seventy five percent (75%) of the calves vaccinated with *M. farcinogenes* strain A24 were protected when exposed to challenge infection. Our live attenuated vaccine was found to be superior to the bacterin of *M. avium* used by Hines *et al.* (1998) that offered no protection but only reduced the severity of both gross and microscopic lesions. The same authors used conjugated MIF-A3. Subunit vaccine, but the vaccine also failed to prevent infection and development of lesions.

Complement fixation test (CFT) and enzyme linked immuno-sorbent assay (ELISA) were used successfully as serological methods to test *M. paratuberculosis* Babiker *et al.* (1983), El-Hussein (2001). For this reason we used ELISA to detect antibody titres, because it is more sensitive, more specific and simple.

The results of sera collected from calves during one whole year showed that there was a considerable increase in the titre, which reached its maximum at the 5th weak, and then it started to decline. This result does not agree with Gilmour *et al.* (1971) who found that the peak of antibody titre in cattle infected with *M. avium,* was reached at the 8th week. We think that this difference might be due to the use of different animal species, and of course *M. farcinogenes* is different in its antigenic and virulent factors from *M. avium*. Statistically, there was a significant difference between vaccinated and non-vaccinated calves using the student's t-test. ($P \leq 0.05$). This finding came in line with El Hussein (2001) who used humeral antibody titre for diagnosis of bovine farcy.

The killed bacterin of *M. avium* used by Hines (1998) in controlling swine paratuberculosis failed to prevent infection but it reduced the severity of gross and microscopic lesions. The same author used conjugate macrophage inhibitory factor –A3 (MIH-A3) subunit vaccine but it did not prevent infection or lesion development. However, the adjuvant vaccine with live *M. paratuberculosis* offered a high degree of protection against paratuberculosis in goats Saxegaard and Fodstad (1985).

In conclusion, we believe that *M. farcinogenes* 24 attenuated strain is a potential candidate and, therefore, recommended it for the production of attenuated vaccine against bovine farcy which can give up to 75% protection level. Further investigations in regard to the duration of immunity and field trials should be carried out.

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