ANTIBODY RESPONSE OF SHEEP AND GOATS IN SAUDI ARABIA TO THE LIVE ATTENUATED RIFT VALLEY FEVER VACCINE

By

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ABSTRACT

A field trial was performed on 30 female sheep and goats as a vaccine-ation group and 33 males as a control to evaluate the immunogenicity of the live attenuated RVF vaccine (Smithburn). Immunoglobulin M (IgM) antibodies to RVF virus were detected in 10%, 3% and 10% of vaccinated animals in the first, second and third week following inoculation respectively. Immunoglobulin G (IgG) antibodies were detected in 37%, 80% and 87% of vaccinated animals in the second, third and fourth week respectively. Regarding detection of IgM and IgG antibodies, significant differences were observed between vaccinated and control animals (p-value = 0.005 and 0.002 respectively). The results demonstrate a strong positive association between detection of IgG antibodies in the sera of vaccinated animals and vaccination (OR = 208, p-value = 0.0002).

Both sheep and goats in the vaccination group responded to the vaccine. No significant difference was observed between vaccinated sheep and goats regarding detection of IgG antibodies in the second, third or fourth week following inoculation. Regarding detection of IgM antibodies, significant differences were observed between vaccinated sheep and goats (p-value = or < 0.005). The percent of IgM antibodies positive sheep was more than the percent of IgM positive goats in the three examinations.
INTRODUCTION

Vaccination of animals is an effective control method widely used to protect animals against infectious diseases and Rift Valley fever (RVF) is no exception. Two types of RVF vaccine have been used to control the disease in endemic areas and during the outbreaks\(^1\). These are the inactivated and the live attenuated RVF vaccine (Smithburn strain). The inactivated vaccine, inoculated subcutaneously in one-ml dose and another booster dose after two weeks usually induces immunity for 6-12 months. The live attenuated vaccine, inoculated subcutaneously in a single one-ml dose, usually induces a life long immunity\(^1\). However, in both types of vaccine not all vaccinated animals will be protected against the disease.

The live attenuated RVF vaccine (Smithburn strain) has been used to control the 2000/2001 RVF outbreaks in Southwestern Saudi Arabia. In the control campaign, most of the animals in the affected regions have been vaccinated\(^2\). However, a serological survey carried out in the February /March 2003 to measure the level of herd immunity in the affected regions, revealed a low level of herd immunity [Elfadil, unpublished]. This field trial was performed to evaluate the immunogenicity of the live attenuated RVF vaccine in local breeds of sheep and goats in Southwestern Saudi Arabia.

MATERIALS AND METHODS

Sixty-three local breed animals (43 goats and 20 sheep) in a governmental farm in Jazan district were included in the field trial after they have tested negative for the presence of IgM and IgG antibodies to RVF virus. These 63 animals were divided into two groups. Group A (vaccination group) consisted of 30 females (22 goats and 8 sheep). Group B (control group) consisted of 33 females (21 goats and 12 sheep). Female animals were chosen in the vaccination group to allow evaluating the effect of the live attenuated vaccine on pregnancy.

The live attenuated RVF vaccine (Smithburn strain) used in the filed trial was manufactured by Onderstepoort Biological Products (Ltd.), South Africa. The expiry date is the first of November 2003. The batch of the vaccine was transported to Saudi Arabia in early 2002 and it was stored in cold rooms since then.

All animals included in the field trial were clinically examined prior to inoculating group a animals with the vaccine and then clinically observed for five days. Temperature was measured for all animals just before inoculation and then daily for five days. All mature females in group A were examined by palpation for pregnancy and observed for abortion for eight weeks following inoculation.

Blood specimens were obtained from all animals included in the field trial in a schedule of one, two, three, and four week following inoculation. The tubes containing the blood were kept in a tilted position in a shad for at least 15 minutes. The blood specimens were then transported to the veterinary diagnostic laboratory in Jizan city. The enzyme linked immunosorbent assay (ELISA) diagnostic test was performed throughout the filed trial to detect IgM and/or IgG antibodies to RVF virus. The result of the test was considered positive when the difference between the specimen and control optical density readings was 0.4 or higher and negative when it was lower than 0.4.

Statistical analysis was performed using the odds ratio (OR) statistic to investigate the association between the development of IgM or IgG antibodies and vaccination. The F-test was used to determine the presence of significant differences between vaccinated and control animals regarding detection of IgM or IgG antibodies, and between vaccinated sheep and goats regarding strength of response to the vaccine.
RESULTS

(Group A) animals were inoculated with the live attenuated vaccine on 5/7/2003. Blood specimens were obtained from all animals included in the field trial and examined for the presence of IgM and/or IgG antibodies on 13/7, 22/7, 29/7 and 10/8/2003.

Table (1): Distribution of Vaccinated Animals Tested Positive to IgM and/or IgG Antibodies, and Control Animals Tested for the Presence of These Antibodies by Date of Examination

<table>
<thead>
<tr>
<th>Examination (Date)</th>
<th>Vaccinated animals (n=30)</th>
<th>Control animals (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM+ve (%)</td>
<td>IgG+ve (%)</td>
</tr>
<tr>
<td>First examination (13/7/2003)</td>
<td>3(10%)</td>
<td>None</td>
</tr>
<tr>
<td>Second examination (22/7/2003)</td>
<td>1(3%)</td>
<td>11(37%)</td>
</tr>
<tr>
<td>Third examination (29/7/2003)</td>
<td>3(10%)</td>
<td>24(80%)</td>
</tr>
<tr>
<td>Fourth examination (10/8/2003)</td>
<td>None*</td>
<td>26(87%)</td>
</tr>
</tbody>
</table>

Tested on 1/10/2003

The results are summarized in (Table 1). Immunoglobulin M (IgM) antibodies were detected in the sera of vaccinated animals in a low percentage as early as the first week following inoculation. However, IgG antibodies were detected in the second week with increasing percentage from 37% in the first week to 87% in the fourth week (Table 1). Except for one animals (3%) tested positive to IgG antibodies in the fourth week, control animals were negative to IgM and IgG antibodies throughout the field trial (Table 1). There was a highly significant difference between vaccinated and control animals regarding detection of IgG antibodies in the fourth week (P-value = 0.0002). The results indicate a strong positive association between the development of IgG antibodies in the sera of vaccinated animals and vaccination (OR = 208, P-value = 0.0002). Comparison between sheep and goats in response to the vaccine is summarized in (Table 2). In IgG antibodies no significant difference was observed between sheep and goats in the second, third or fourth week following inoculation. Immunoglobulin M (IgM) antibodies were detected in vaccinated sheep and goats in the first, second and third week following vaccination (Table 2). There were significant differences between sheep and goats in developing IgM antibodies (P-value = or <0.005). In the second and third week, IgM antibodies were detected only in sheep. No IgM antibodies were detected in the fourth week.

Table (2): Comparison between Sheep and Goats in Response to Vaccination with RVF Live Attenuated Vaccine by Date of Examination

<table>
<thead>
<tr>
<th>Examination (Date)</th>
<th>Vaccinated Animals (n=30)</th>
<th>Control Animals (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM+ve (%)</td>
<td>IgG+ve (%)</td>
</tr>
<tr>
<td>First examination (13/7/2003)</td>
<td>1(5%)</td>
<td>None</td>
</tr>
<tr>
<td>Second examination (22/7/2003)</td>
<td>None</td>
<td>9(41%)</td>
</tr>
<tr>
<td>Third examination (29/7/2003)</td>
<td>None</td>
<td>18(82%)</td>
</tr>
</tbody>
</table>
Although a slight rise in body temperature was measured in most vaccinated animals, no significant was observed between vaccinated and control animals.

Either one or combination of the following symptoms: eye mucous membrane congestion, nasal mucous fluids, diarrhea and swelling of one or more of the lymph nodes were observed in 10 out of 30 (33%) vaccinated animals, and in 11 out of 33 (33%) control animals. No significant difference was observed between vaccinated and control animals. Regarding the occurrence of these symptoms.

Neither abortion nor fetus malformation was reported among vaccinated females during the eight weeks of observation. There was no even normal birth during that period.

## DISCUSSION

Few studies on antibody and immune response of animals to RVF inactivated or live attenuated vaccine have been performed and published in the scientific literature\[3,4,5,6\]. However, none of these studies has evaluated the immunogenicity of the vaccine in terms of IgM and IgG antibodies separately. This study is the first to evaluate the IgM and IgG antibody response to the live attenuated RVF vaccine (Smithburn strain) in local breeds of sheep and goats in Southwestern Saudi Arabia. Immunoglobulin M (IgM) antibodies were detected in the blood of vaccinated animals as early as first week following vaccination. However, the percentages of IgM positive animals were very low (3-10%). Since the sensitivity of the ELISA diagnostic test in detecting IgM antibodies has been reported to be 100\[%\], it is possible that the vaccine could not induce IgM antibodies in the blood of most (90-93%) vaccinated animals. These results are in agreement with the results of a previous study reported a poor antibody response to a single dose of a live attenuated vaccine\[3\]. It is also possible, and unlike what has been reported, that the ELISA diagnostic test could not detect IgM antibodies in all responded animals. This speculation is supported by the findings of a recent study indicated that the ELISA diagnostic test detected IgM antibodies in only 51% of RVF confirmed cases\[8\].

No IgM antibodies were detected IgM in the blood of vaccinated animals in the fourth week following inoculation. These results are in agreement with previous findings indicated that IgM antibodies usually disappear from the blood of vaccinated or naturally infected animals within 4 to 7 weeks following vaccination or infection [Swanepoel, unpublished].

However, since the test for IgM antibodies was carried out approximately seven weeks after collection of the blood specimens, disappearance of IgM antibodies could have been due to adverse storage conditions in the laboratory. The significant difference between vaccinated sheep and goats regarding detection of IgM antibodies may indicate that sheep respond to the vaccine much better than goats. This speculation is supported by the findings of a previous study indicated that sheep are highly susceptible to RVF virus\[9\]. Also, a recent study reported a higher prevalence of RVF (diagnosed by detection of IgM antibodies) in sheep compared to goats\[2\].

Although IgG antibodies were detected in the blood of vaccinated animals starting in the second week following inoculation, the percent of IgG positive animals was significantly high compared to IgM antibodies positive animals (Table 1). Since the sensitivity of the ELISA diagnostic test in detecting IgM and IgG antibodies has

<table>
<thead>
<tr>
<th>Fourth examination</th>
<th>None*</th>
<th>19(86%)</th>
<th>None*</th>
<th>7(88%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(10/8/2003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tested on 1/10/2003
been reported to be 100% and from 99% to 100% respectively\textsuperscript{7}, it is possible that the vaccine induces more detectable IgG antibodies in vaccinated animals compared to IgM antibodies. Neither IgM nor IgG antibodies were detected in all vaccinated animals even in the fourth week following inoculation. The highest IgG antibodies positive animals percentage was 87% (Table 1). The results of this study indicate that the vaccine could not.

Induce IgG antibodies in 13% of vaccinated animals. These results are in agreement with pervious finding indicated that the vaccine prepared from a neurotrophic strain of virus propagated by serial intracerebral passage in mice is not sufficiently immunogenic\textsuperscript{10}.

Detection of IgG antibodies in one animal in the control group is not expected. One possibility is that the animals was positive from the beginning and was missed in the first test according to which the animals were chosen to the filed trial and also in the three tests thereafter. It is also possible that the animal acquired the vaccine virus by direct contact via copulation with vaccinated females and consequently developed IgG antibodies. Since the vaccine virus has been reported to invade the uterine tissues\textsuperscript{6,10}, it is possible it could be secreted in the vaginal secretions and hence copulating males could have been exposed to the vaccine virus. This possibility may be strengthened by detection of the vaccine virus in the semen of bulls for three week following vaccination\textsuperscript{11}. It is worth mentioning that animals in the two groups were not separated during the trial.

It has been reported that the live attenuated vaccine may cause abortion in a low percentage of vaccinated animals\textsuperscript{6,10}. Since no pregnant animals were detected in the vaccination group, it was not possible to evaluate the abortigenicity of the live attenuated vaccine in this study.

No significant difference was observed between vaccinated and control animals regarding post-vaccination clinical symptoms. This is in agreement with pervious findings indicated a mild pyrexia following vaccination\textsuperscript{4,9}.

REFERENCES


