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# SEROLOGICAL TESTS FOR DETECTION AND TITRATION OF ANTIBODIES TO RIFT VALLEY FEVER IN SERA OF SHEEP AND GOATS EXPERIMENTALLY VACCINATED WITH LIVE ATTENUATED VACCINE OF SMITHBURN STRAIN (A COMPARATIVE STUDY)

(With 3 Tables and One Figure)

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## الاختبارات المصليه في الكشف عن ومعايرة الأجسام المناعية لحمى الوادي المتصدع في أمصال الأغنام والماعز المحصنة تجريبيا باللقاح الحي المستضعف لعتره سميث بيرن (دراسة مقارنة)

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تـم تقيـيم كفاءه اختبار الاليزا الساندوتشي التجاري واختباري تعادل المصل والفلوروسنت المناعي الغير مباشر في الكشف عن ومعايره الأجسام المناعية IgG النوعية لفيروس حمى الـوادي المتصـدع في أمصال الأغنام والماعز المحصنة تجريبيا باللقاح الحي المستضعف لعتره سميث بيرن، وتمت مقارنه نتائج الاختبارات الثلاثة ومناقشه مزاياها وعيوبها.

## SUMMARY

One commercial IgG-sandwich ELISA (S-ELISA) kit and two local serological tests; serum neutralization test (SNT) and indirect fluorescent antibody (IFA) test were evaluated for detection and titration of Rift Valley Fever (RVF) virus antibodies in sera of sheep and goats experimentally vaccinated with live attenuated vaccine of Smithburn strain. The results of S-ELISA test were compared with those obtained from SNT and IFA test, and the advantages and disadvantages of the three assays were discussed.

***Key words:*** *Rift valley fever, serology, vaccination, antibodies, sheep, goats.*

# INTRODUCTION

Between mid September and the end of November 2000, the 1st epidemic of Rift Valley Fever (RVF) in both humans and livestock in the Kingdom of Saudi Arabia was recorded in Jizan region, Asser and Al-Quenfadah (CDC, 2000 a, b and Jupp *et al.*, 2002). One of the major regulations which had been taken by the veterinary authorities to control the disease was the vaccination of the livestock inside and on the borders of the infected area with live attenuated vaccine of RVF virus, Smithburn strain RVF post-vaccination herd immunity in sheep and goats was checked throughout sero-monitoring of several thousands serum samples collected during the 2nd half of year 2002 and 1st half of year 2003 using commercial kit of RVF virus IgG sandwich ELISA. The obtained results revealed that the protection percentages ranged between (15) to (70) with mean value of (40), (unpublished data). This questionable low achieved herd immunity was the guidance of this work to declare two points of interest, (1) efficacy of the currently used batch of RVF virus live attenuated vaccine of Smithburn strain to immunize native sheep and goats and (2) the benefit of using the serum neutralization test and indirect fluorescent antibody technique as a traditional serological tests for detection and titration of RVF virus-IgG in sera of vaccinated animals in comparison with the validated commercial IgG-sandwich ELISA.

# MATERIALS and METHODS

**Experimental sheep and goats:**

Fifteen clinically healthy, RVF seronegative adult native sheep and 10 goats were vaccinated with live attenuated vaccine of RVF virus, Smithburn strain. One ml containing 4.5 log10 $TCID_{50}$ of virus was inoculated subcutaneously in each animal. Five additional sheep and 3 goats were kept without vaccination. The animals were housed in a local farm at Jizan City. Blood sample for serological tests was collected once from each animal by the 24th day after inoculation.

**Vaccine:**

Commercial live attenuated vaccine of RVF virus, Smithburn strain, Batch No. 100 manufactured by Onderstepoort Laboratory, Pretoria, South Africa was titrated in cell culture and used for vaccination.

**Cell culture:**

Baby Hamster Kidney (BHK) cell line culture was grown and maintained on minimal essential medium (MEM) based on Earl's salt solution and supplemented with 5-10% fetal calf serum. 100 ug of penicillin sodium and 100 ug of streptomycin sulphate were added per one ml of medium.

**Serological tests:**

**Serum neutralization test (SNT):**

SNT was conducted according to a method of Swanepoel *et al.* (1986) as follows:

Two fold dilutions between 1/2 to 1/256 of experimental sera, negative and positive control serum samples were prepared in growth medium in 96-well tissue culture plates, two wells/dilution, 50µl/well. 100 $TCID_{50}$/50µl of RVF virus, Smithburn strain was added for each serum dilution and back titration of the virus was done using 4 well per each 10-fold dilution, 50µl /well. Plates were shaked well, then incubated at 37°C for 60 minutes prior to the addition of 100 µl of BHK cells suspension (15,000-20,000 cells/well) to each well. Tested plates were sealed and incubated at 37°C for 3-5 days with cultures media change until the back titration indicated the working virus contained 100 $TCID_{50}$. The 50% end titre of sera was geometrically calculated.

**Indirect fluorescent antibody (IFA) test:**

IFA test was carried out using the method of Carol *et al.* (1990). BHK cells were grown on a 96-well tissue culture plate at 37°C until cells were confluent. Each well was inoculated with RVF virus, Smithburn strain sufficient to produce scattered foci of cytopathic effect within 45 hours of incubation. Negative control platted cells were also prepared. Plate culture medium was decanted and cells were washed carefully with filling and decanting of PBS, pH 7.2 (5 min/each), then fixed with cold 80% acetone in PBS, pH 7.2 and stored at 4°C until used after washing twice with PBS, pH 7.2. Experimental sera as well as negative and positive control serum samples were diluted two fold between 1/2 to 1/256 in PBS, pH 7.2, then added to each of infected platted cells and normal platted cells, two wells/dilution, 50µl /well. The plates were sealed and incubated at 37°C humid chamber for 60 min, and subsequently washed twice with PBS, pH 7.2. Rabbit-antisheep IgG-FITC conjugate (Eoppel, Organon Teknika, West Chester, Lot II 392264) was diluted 1:80 in PBS, pH 7.2 and added to the wells of plates 50 µl /well. The plates were then sealed and incubated at 37°C in

humid chamber for 45 min. Test plates were washed twice with PBS, pH 7.2. Specificity of immunofluorescence was evaluated by first examining of negative control plated cells for each sample. The 50% end titre of sera was geometrically calculated.

**IgG-sandwich-ELISA:**

Commercial sandwich enzyme linked immunosorbent assay (S-ELISA) kit for detection and titration of anti-RVF virus-IgG antibodies in sera of sheep and goats produced by Special Pathogens Unit, National Institute for Virology, Johannesburg, South Africa, Batch No. 2002/11 was used according to the manufacturer's recommendation. The test is based on sandwich formation in which the plates are coated with mouse anti-RVF virus serum and then reacted with RVF virus antigen. Test sera are applied and specific anti-RVF virus IgG antibody is detected with an anti-species IgG-HRPO conjugate and ABTS substrate as developed and validated by Paweska *et al.* (2003).

## RESULTS

Susceptible native adult sheep and goats inoculated each with 4.5 $\log_{10}$ $TCID_{50}$ of the live attenuated vaccine of RVF virus, Smithburn strain developed a good immune response. RVF virus-neutralizing antibodies were detected in 100% of the inoculated sheep and goats by the $24^{th}$ day after vaccination using the SNT. The IFA test was capable to detect RVF-virus IgG antibodies in 100% and 99% of vaccinated sheep and goats respectively while commercial IgG sandwich ELISA (S-ELISA) scored positive percentages of (93.3) and (72.7) in sera of vaccinated sheep and goats respectively (table 1).

On the other hand, all unvaccinated sheep and goats (control animals) were serologically negative to RVF virus IgG antibodies by SNT, IFA and S-ELISA on the $24^{th}$ terminal day of the experiment.

From the results recorded in tables (2 and 3), it can be summarized that RVF virus IgG antibodies titer ranges detected by SNT, IFA and S-ELISA, (1) in sera of vaccinated sheep on the $24^{th}$ day of vaccination were (8-192), (4-128) and (100-1600) with mean values of (65.06), (54.13) and (607.14) respectively and (2) in sera of vaccinated goats were (2-128), (2-128) and (100-1600) with mean values of (47.09), (52.60) and (612.50) respectively.

It was clear that RVF virus neutralizing antibody titers 8, 24, 32, 48, 64, 92, 128 and 196 in sera collected from sheep and goats by the

24th day of vaccination were dramatically related with RVF virus IgG antibody titres (0-100), (200), (200-400), (400), (400-500), (800), (800-1600), (1600) as detected S-ELISA (Fig. 1).

**Table 1:** Comparison of SNT, IFA test and S-ELISA test results on sera collected from sheep and goats by the 24th day, after vaccination

| Serological test | Sheep | | Goats | |
|---|---|---|---|---|
| | No. of +ve sera / No. of tested sera | Positive % | No. of +ve sera / No. of tested sera | Positive % |
| SNT | 15/15 | 100 | 11/11 | 100 |
| IFA | 15/15 | 100 | 10/11 | 90.9 |
| S-ELISA | 14/15 | 93.3 | 8/11 | 72.7 |

**Table 2:** RVF virus IgG detected and titrated by SNT, IFA test and S-ELISA in sera collected from 15 sheep on the 24th day after vaccination

| Number of animals | RVF virus IgG titer | | |
|---|---|---|---|
| | SNT | IFA | S-ELISA |
| 1 | 8 * | 4 * | - |
| 1 | 8 | 8 | 100 * |
| 2 | 24 | 32 | 200 |
| 2 | 32 | 32 | 400 |
| 2 | 32 | 64 | 400 |
| 1 | 48 | 64 | 400 |
| 1 | 64 | 32 | 400 |
| 1 | 96 | 64 | 800 |
| 2 | 128 | 64 | 800 |
| 1 | 128 | 128 | 1600 |
| 1 | 192 | 128 | 1600 |
| Mean ** | 65.06 | 54.13 | 607.14 |

(*): Reciprocal of serum dilution.

(-): Negative, 100 is the minimal recommended positive titer as detected by commercial IgG S-ELISA test kit

(**) $\frac{\text{Assembled values}}{\text{Number of positive animals}}$

**Table 3:** RVF virus IgG detected and titrated by SNT, IFA test and S-ELISA in sera collected from 11 goats on the 24th day after vaccination.

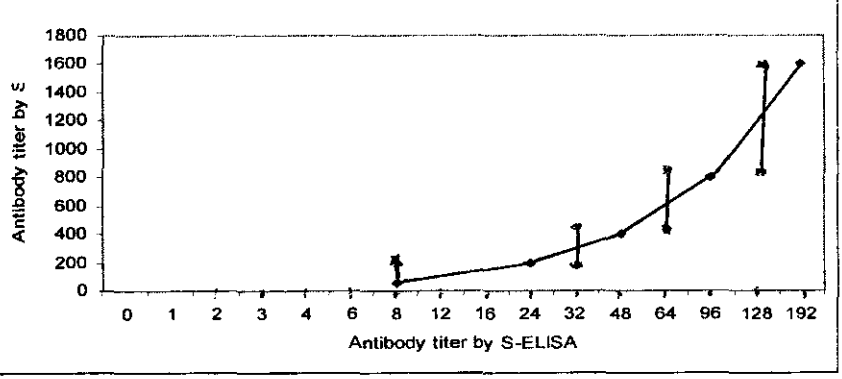
| Number of animals | RVF virus IgG titer | | |
|---|---|---|---|
| | SNT | IFA | S-ELISA |
| 1 | 2 * | < 2 * | - |
| 1 | 4 | 2 | - |
| 1 | 8 | 4 | - |
| 1 | 8 | 8 | 100 * |
| 2 | 32 | 48 | 200 |
| 1 | 48 | 32 | 400 |
| 1 | 64 | 64 | 800 |
| 1 | 64 | 128 | 800 |
| 1 | 128 | 64 | 800 |
| 1 | 128 | 128 | 1600 |
| Mean ** | 47.09 | 52.60 | 612.50 |

(*): Reciprocal of serum dilution.

(-): Negative, 100 is the minimal recommended positive titer as detected by commercial IgG S-ELISA test kit

(**) $\frac{\text{Assembled values}}{\text{Number of positive animals}}$

**Fig. 1:** Correlation between the results of SNT and S-ELISA test for titration of RVFV-IgG antibodies in sera of vaccinated sheep and goats on the 24th day after inoculaton




## DISCUSSION

In spite of extensive works on the immunization of domestic ruminants with the neurotropic attenuated Smithburn strain of RVF immunogenicity of this strain is not sufficiently proved (George, 1986).

Based on the present data, experimental inoculation of RVF-seronegative adult naïve sheep and goats with the recommended dose of the live attenuated vaccine of Smithburn strain (4.5 log10 TCID/dose) was capable to produce RVF virus-IgG sero-positive in (100% both), (100% & 90.09%) and (93.5% & 72.7%) on the 24th day after vaccination as tested by SNT, IFA and S-ELISA respectively. These results proved that the test vaccine was absolutely satisfactory to immunize sheep and goats.

The vaccinated sheep and goats sera provided an unique battery of samples to compare the sensitivity of the three serological tests and to determine the relatedness of each other that could validate each one.

SNT is the current gold standard serological test although it s laborious and requires cell culture facilities. All test sera collected from sheep and goats by the 24th day after vaccination exhibited positive to RVF virus IgG by SNT. RVF Virus neutralizing antibody titre ranges were (5-192) and (2-128) with mean values of (65.06) and (47.09) in sheep and goats respectively. These results declared that sheep reacted immunogenically relatively higher than goats. However, few test sera exhibited toxicity on BHK cell culture at dilutions 1/2 to 1/8 in SNT. This was overcome by inoculating confluent monolayer cell cultures rather than cell suspension with changing their culture media by the 3rd hour after inoculation.

Comparing to the results of SNT, sensitivity of IFA test was relatively similar, the obtained antibody titre ranges were (4-128) and (2-128) with mean values of (54.13) and (52.60) in sheep and goats respectively. Number of test sera of high antibody titres were reacted non-specifically with control BHK cell cultures at dilutions between 1/2 to 1/8. IFA is sensitive and more convenient as a screening test but some sera may give a false positive reaction in low serum dilution possibly due to the animal being recently vaccinated with cell culture materials (Carol *et al.*, 1990).

Again, the sensitivity of commercial S-ELISA for detection of RVF virus-IgG in sera of vaccinated sheep and goats was approximately 10 times higher than SNT and IFA test. It recorded a titre range of (100-1600) with mean values of (607.14) and (612.5) respectively in sheep and goats. However, it was true that one out of 15 sheep and 3 out of 11 goats sera which reacted positive to SNT with titres between 2 and 8 gave a negative result with S-ELISA because the marginal positive dilution of serum samples in S-ELISA was 1/100. the results of commercial S-ELISA test accuracy validation which was carried out by

Paweska *et al.* (2003), S-ELISA and SNT were capable to detect RVF virus-IgG in 100% of test sera of sheep between 10-34 days after vaccination with Smithburn strain vaccine.

In conclusion, the results of the present study demonstrate that: (1) the test vaccine is potent, (2) the SNT is the method of choice to achieve reproducible results for assessment of RVF virus-IgG in sera of experimentally vaccinated animals and IFA test is the alternative one, and (3) the current use of commercial RVF virus IgG S-ELISA test kit under the field conditions in Jizan area for investigation of the immune status of sheep and goats herds on month after vaccination offers an advantage to examine a large number of field samples in a short time compared with SNT and IFA test.

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