RESEARCH COMMUNICATION

Evaluation of a 3 mℓ heartwater (cowdriosis) infective blood vaccine dose

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ABSTRACT

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Three milliliters of blood from the present commercially produced heartwater infective blood vaccine (Ball3 stock) was experimentally tested in sheep and cattle for infectivity and efficacy. Results obtained for this vaccine dose were statistically not different from results for the prescribed 5 mℓ vaccine dose.

Keywords: Blood, cattle, cowdriosis, efficacy, heartwater, infectivity, sheep, vaccine

INTRODUCTION

Heartwater is an acute infectious disease responsible for severe losses among susceptible cattle, sheep and goats (Neitz 1968; Uilenberg 1983). Its causal organism, *Cowdria ruminantium*, is transmitted by certain *Amblyomma* ticks. In nature the disease is transmitted only by the nymphal and adult stages and occurs only in those areas where the tick is present (Petney, Horak & Rechav 1987; Uilenberg 1983). The disease can be controlled by vaccination of susceptible animals (Anonymous 1984; Bezuidenhout 1989; Neitz & Alexander 1941, 1945); treatment of sick animals infected by ticks (Van Amstel & Oberem 1987); chemoprophylaxis (Gruss 1981; Purnell 1987) and judicious tick control (Henning 1956; Stampa 1969; Norval & Lawrence 1979).

The current commercial vaccine produced at the Onderstepoort Veterinary Institute (OVI), consists of blood collected from sheep infected with live *C. ruminantium* organisms. Since the vaccine contains live organisms, vaccinated, susceptible animals will develop variable degrees of heartwater, which may or may not require appropriate treatment. Production cost of the vaccine is very high owing to the fact that sheep are used as vaccine donor animals. A reduction in the volume of the vaccine dose will result in fewer sheep being needed to produce the same number of vaccine doses.

The purpose of this experiment was to determine the infectivity and efficacy of a 3 mℓ vaccine dose as compared with the prescribed 5 mℓ vaccine dose.

MATERIALS AND METHODS

C. ruminantium strain and experimental procedures

The Ball3 isolate of *C. ruminantium* was used to produce frozen heartwater blood vaccine (Bezuidenhout 1989).

Infectivity was tested in sheep by injecting 3 mℓ (n = 19) and 5 mℓ (n = 7) doses of a 1/20 dilution of the vaccine, and in cattle by injecting them with 3 mℓ (n = 37) undiluted vaccine doses.

Infectivity of the vaccine, after long-term storage in liquid nitrogen gas phase for 805 d, was also evaluated. Sheep were injected with 3 mℓ (n = 20) doses

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of a half vaccine dilution after exposure for 8 h on melting ice. Five sheep served as an unvaccinated control group.

The efficacy of the vaccine was evaluated by homologous challenge of vaccinated sheep (only the groups that received 3 mℓ and 5 mℓ of the 1/20 dilution of the vaccine) and cattle.

Monitoring of heartwater reactions

Temperatures of all vaccinated and challenged animals were taken daily between 08:00 and 09:00 for 21 or 30 d post vaccination for sheep and cattle, respectively.

Temperatures ≥40 °C (sheep) or ≥39,5 °C (cattle) were regarded as indicative of a reaction to *Cowdria* organisms (Van der Merwe 1987).

Antibodies against *C. ruminantium* were determined in sera from all cattle by means of both the ELISA (Soldan, Norman, Masaka, Paxton, Edelsten & Sumption 1993) and IFA (Du Plessis & Malan 1987a) techniques, prior to vaccination and again at 42 d post vaccination, but before challenge.

Criteria for treatment

Sheep with temperature reactions ≥40 °C for 3 d were treated intravenously with oxytetracycline (Curamycin 123, Rumevite) for at least two consecutive days. Cattle with temperature reactions ≥40 °C for 2 d or the occurrence of a double reaction (e.g. 39,5 °C; 39,3 °C; 41 °C—J.L. du Plessis, personal communication 1994) were treated intravenously with oxytetracycline [Liquamycin 100, Pfizer Laboratories SA (Pty) Ltd] for two consecutive days.

Evaluation of vaccination and challenge responses

The following criteria were used to evaluate responses:

- The number of animals requiring treatment as opposed to the number of animals with temperature reactions ≥40 °C or ≥39,5 °C for sheep and cattle, respectively.
- Temperatures were taken from each animal for 5 d before inoculation to obtain the animals' mean pre-inoculation normal temperature (m.p.n.t.). The maximum temperature rise above normal for each animal was calculated by subtracting the m.p.n.t. of that specific animal from the highest temperature recorded over the expected reaction period. The total temperature rise above normal for each animal was calculated by subtracting the animals' m.p.n.t. from the daily recorded temperatures in excess of the normal and adding these values to obtain a total.
- The reaction time for each animal was determined by adding the number of days on which the animal had had a rectal temperature of ≥40 °C (sheep) or ≥39,5 °C (cattle).
- In order to obtain equal representation, five sheep and eight cattle were chosen at random from each vaccinated group and the results compared for homogeneity of variances by means of the Bartlett test. The Bonferroni multiple-comparison test was used to determine whether results differed significantly.

RESULTS

All the sheep vaccinated with 3 mℓ and 5 mℓ of 1/20 vaccine dilution showed typical heartwater temperature reactions and all of these animals required treatment. Homologous challenge of the 3 mℓ of 1/20 vaccine dilution group resulted in 7/19 sheep reacting. However, none qualified for treatment. Challenge of the 5 mℓ of 1/20 vaccine dilution group resulted in 3/7 animals reacting with temperatures of ≥40 °C and only one animal had to be treated. No statistically significant differences could be shown between the results obtained for the 3 mℓ and 5 mℓ of 1/20 vaccine dilution groups with regard to mean maximum temperature rise, mean total temperature rise and mean reaction time. However, results obtained in the homologous challenge of these groups differed significantly from the results obtained for the initial vaccination.

Nineteen of the 20 sheep injected with 3 mℓ of half vaccine dilution (vaccine stored for 805 d in the gas phase of liquid nitrogen) reacted and 18 required treatment. Results obtained for the mean maximum temperature rise above normal for this group were significantly different from the results of the unvaccinated control group. However, no significant difference could be shown between the vaccinated and unvaccinated control group for mean total temperature rise, the reason being that one of the animals in the control group not only displayed an inexplicable temperature of 40 °C on day 3 of the experiment, but also had a temperature that was constantly higher than the norm for the group.

Twenty-one of the 37 cattle had temperature reactions ≥39,5 °C but only two animals required treatment. All the cattle seroconverted after initial vaccination. On homologous challenge all were found to be immune and significant differences were recorded for mean maximum temperature rise between vaccination and homologous challenge reactions.

DISCUSSION

No difference was found in the results when sheep were vaccinated with either 3 mℓ or 5 mℓ doses of

heartwater-infective blood vaccine at dilutions of 1/20, with 1/20 being the highest dilution at which the infectivity of the commercially produced batches are being tested. Resulting reactions from the homologous challenge of these animals were insignificant compared with the original vaccination results (only one of the sheep qualified for treatment).

This study has again confirmed that infective blood, after prolonged storage in liquid nitrogen, will retain its infectivity for at least 8 h after having been thawed on melting ice. This is in compliance with the recommendations for the use of the vaccine.

Infectivity testing in cattle is difficult because a large percentage of animals show non-specific resistance to the clinical signs of the disease (Du Plessis & Bezuidenhout 1979; Du Plessis & Malan 1987b). As can be seen in these results, not all the cattle showed temperature reactions after vaccination, and the reactions of only two animals required treatment, compared with 26/26 of the sheep requiring treatment. All the cattle seroconverted after vaccination and there were virtually no detectable reactions in cattle after homologous challenge.

These results therefore indicate that a 3 mℓ instead of a 5 mℓ dose of the heartwater infective blood vaccine can be used effectively for the vaccination of ruminants against heartwater.

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