Effect of Heterologous Challenge on the Survival of Sheep Immunzed with Inactivated Elementary Bodies and Recombinant Antigens of *Cowdria ruminantium*

Kibor, A. C.[1], Sumption, J. K.[2], and Paxton, E. A.[2]
[1]Egerton University, P. O. Box 536, Egerton, Kenya
[2] CTVM, University of Edinburgh, UK.

Abstract

Immunization of sheep with inactivated elementary bodies (IEB's) confers protection against homologous and heterologous challenge among certain stocks of C. *ruminantium*. Cros-protection between different stocks of C. *ruminantium* using recombinant antigens is being reported in this study. Immunization of sheep with 250µg IEB's of the Gardel stock protected 5 sheep out of 8 (64.5%) against a virulent Kenyan isolate the Kathiani. There was no advantage in terms of protection against virulent challenge when 35µg of recombinant Major Antigen Protein 1 (MAP1=32 kilodalton protein) or MAP2 (21 kilodalton protein) of C. *ruminantium* were combined with 100µg of IEB's. Immunization with recombinant MAP1 antigen did not protect sheep against heterologus challenge, however immunization with 35µg of recombinant MAP2 antigen protected 7 out of 8 (87.5%) sheep immunized with this antigen. This result was surprising and further immunization experiments are required to determine the potential of this recombinant for vaccine production in the future.

Key words: Heterologous, sheep, inactivated elementrary bodies, recombinant antigens, *Cowdria ruminantium*

Introduction

Heartwater/Cowdriosis is an economically important disease of domesticated and wild ruminants cattle, sheep and goats caused by the intracellurar rictettsial organims *Cowdria ruminantium*. It is transmitted by ticks in the genus Amblyomma and occurs in Africa South of the Sahara, islands along the east and west coast of Africa and some islands in the Caribbean region (Uilenberg, 1983, Camus et. al 1996). In Africa this disease occurs in an estimated area of 13 million square kilometres and puts at risk a ruminant population of about 175 million. Heartwatre is a fatal disease particularly in

pure-bred-exotic beef, dairy and their crosses. Indigenous cattle, sheep and goats from vector free areas are susceptible.

The economic losses due to heartwater fall into two main categories namely direct and indirect losses. Direct losses are those, which arise due to mortality while indirect losses, arise from loss of production. Heartwater is controlled by three main methods namely: 1) Tick control by regular application of effective tick acaricides to kill the vector; 2) Treatment of infected animals with chemotherapueutic compounds such as oxytetracycline; and 3) Vaccination by the infection and treatment method using live virulent vaccines.

The three methods listed above have managed to reduce losses due to heartwater. Their effectiveness have however been limited by factors such as: Resistance of ticks to acaricides and their high cost, high cost of chemotherapeutic compounds used for treatment, death arising from late treatment of peracute cases and failure of immunity to develop. Vaccination using live virulent organisms is risky since animals may develop peracute disease and die before treatment, mustering of animals for treatment is cumbersome and expensive while live vaccines are thermolabile and require a working cold chain of preservation.

The limitations given above have necessitated looking for better control alternatives against heartwater. In the 1990s a number of Scientists have carried out vaccine trials and inactivated organisms attenuated, organisms and recombinant antigens (Jonghan, 1991, Martinez, et. al, 1993, Mahan et al, 1994). These trials indicated that there might be a future for inactivated vaccines since there is some protection against homologous and heterologous organisms to some degree. The objectives of the current study were two-fold: 1) Determine the level of cross-protection between a Kenyan isolate (Kathiani) and a Caribbean isolates (Gardel), and 2) Detemine whether immunization of animals with recombinant antigens form *C. ruminatium* would confer protection of sheep after virulent challenge.

Materials and Methods

Experimental animals: Sixty-four (64) Corriedale wethers and hoggets 6-25 months old were used. They were obtained from Ngongogeri Farm of Egerton University. Sheep were divided into 8 treatment groups of 8 animals each as shown in Table 1. The sheep were raised on a heartwater free farm, with no record of heartwater in the 20 years in the farm records. Ambylomma ticks are not seen on the farm, and there were wild antelopes/bufallo on the farm.

Antigens: Three antigen preparations were used in the trial namely: Inactivated elementary bodies (IEB's) of the Gardel isolate of C. *ruminantium* used in a three dose regiment and recombinant antigens (Rags) encoded by genes isolated from genomic-DNA library of *C. ruminantium* MAP1 (31kDa Protein) and MAP2 (21kDa Protein). The Rags were expressed in E. Coli and purified using a standard procedure. The recombinant antigens were used in combination with inactivated elementary bodies or in without (Table 1).

Table 1: Treatment groups

Group	Inoculation	Dose IEB/Recomb Ag	No. of animals
1	IEB	250 µg	8
2	IEB	150 µg	8
3	IEB	50 µg	8
4	EB/MAP1	100/35µg	8
5	MAP1	35 µg	8
6	IEB/MAP2	100/35µg	8
7	MAP2	0/35 µg	8
8	Control	0	8

Key: IEB- Inactivated elementary bodies; MAP1- Major antigenic protein 1 (32 kilodalton protein of C. *ruminantium*); MAP2- Major antigenic protein 2 (21 kilodalton protein of C. *ruminantium*)

Immunization: Animals in respective experimental groups received 2 doses of antigen administered 14 days apart in the neck region. The antigens were prepared for inoculation by mixing equal volumes (1 ml) of each antigen with the adjuvant Mantonide ISA50 (Seppadec' Quay d'Orsay, Paris France) before inoculation.

Sample collection: Blood samples for serum preparation were collected using vacutainer tubes and needles from all animals on days), 14, 21, 30 and 48 days post immunization (P.I.). Further samplin was carried out 21 days after challenge from surviving animals and the sera were tested for sero-conversation by ELISA.

Challenge of immunized sheep: The Kathiani isolate of *C. ruminantium* was used to challenge all the sheep. The challenge material was a blood stabilate obtained from experimentally infected sheep (courtesy of Dr. Rumberia of KARI Muguga). Each animal receive 5ml of inoculum intravenous though the jugular vein.

Monitoring of Animal after Challenge

The sheep were transferred into an isolation unit one-week before challenge. Their body temperatures were taken daily and recorded. Temperatures taken throughout the challenge period up-to 30 days post challenge. The animals were checked daily for signs of heartwater and any cases that died were autopsied and the pathological findings were recorded.

Results

A summary of challenging the immunized sheep is given in Tables 2 and 3.

Table 2: Outcome of challenging immunized and control sheep with live virulent *C. ruminantium* (Kathiani isolate)

Group	Antigens	Dose in ug	Total animals	Animals survived	Animals died	Survival % group
1	IEB	250 µg	8	5	3	62.5
2	IEB	150 µg	8	4	4	50
3	IEB	50 µg	8	3	5	37.5
4	IEB/MAP1	100/35µg	8	5	3	62.5
5	MAP1	35µg	8	3	5	37.5
6	IEB/MAP2	100/35µg	8	6	2	75
7	MAP2	0/35 µg	8	7	1	87.5
8	Controls	0	8	3	5	37.5

Key: IEB= Inactivated elementary bodies; MAP1= Major antigenic protein 1 (31 kilodalton protein); MAP2 =Major antigenic protein 2 (21 kilodalton protein)

Table 3: Challenge of immunized and control sheep with live virulent *C. ruminantium* (Kathiani isolate)

Group	Immunization dose & antigen	Median days to death	Survived	Died	Survival % group
1	250 µg IEB	18	5	3	62.5
2	100 µg IEB	19	4	4	50
3	50 µg IEB	15.5	4	4	50
4	100 µg IEB/MAP1	19.5	5	3	62.5
5	100 µg MAP1	16	3	5	37.5
6	100 µg IEB/35 ug MAP1	16.5	6	2	75
7	100 µg MAP2	14	7	1	87.5
8	Controls	14	3	5	37.5

Key: IEB= Inactivated elementary bodies, MAP1=Major antigenic protein 1 (31 kilodalton protein); MAP2=Major antigenic protein 2 (21 kilodalton protein)

Discussion

There was some level of cross-protection between the Gardel and Kathiani stocks of C. *ruminantium*. This is because 62.5% of the goats immunized with 250 ug inactivated elementary bodies of the Gardel stock were protected against virulent challenge of the Kathiani stock used for challenge. There was no advantage in 50 ug or 100 ug of IEBs since both doses protected 50% of the immunized sheep. Combination of 100 ug of IEBs and 35 ug of the recombinant MAP1 antigen had a survival rate of 62.5% the same as that confeered by using 250 ug of IEBs. Immunization with recombinant antigen MAP at the rate of 100 ug did not protect sheep at all.

The most important findings of this study were achieved by immunization with 100 ug of the recombinant MAP2 antigen. The survival rate of sheep immunized with this antigen was 87.5%. Combination of 100 ug of IEBs and 35 ug of MAP lead to a survival rate of 75% of sheep after virulent challenge. These results are quite encouraging since we have shown that there is a recombinant antigen that confers protection to sheep by itself or in combination with IEBs. Inactivated elementary bodies alone protected 50% of the immunized sheep indicating that there is a potential for a future IEB vaccine. Since vaccination prolonged the survival time of sheep.

Three non-immunized controls survived challenge, which was unexpected. All the immunized sheep sero-converted by day 14 post inoculation which indicated that there was an active immunity response to the antigens used. The length of survival of those, which succumbed to the disease, was longer (18 days vs. 14 days, median) for immunized versus control animals.

Higher proportion of animals, which survive longer after developing heartwater, may assist in controlling heartwater in the field where immunization is chosen. A longer period of survival may give a better "window of an opportunity" for treatment of heartwater affected animals after field infection therefore saving them from death since this disease is curable with tetracylines.

Acknowledgements

This project was supported by funds provided by the Department for International Development of the United Kingdom (DfiD). We also wish to thank the Vice Chancellor Egerton University for allowing us to use the institution's facilities to carry out the experiments and availing support staff to the project. Our appreciation also to go Ms. Elaine Chirnside, Edith Paxton and College Kelly for providing technical support, Dr. Rumberia of KARI for kindly donating the Cowdria *ruminantium* challenge stabilate (the

Kathiani) and last but not least we to appreciate the support given by staff of Animal Health Egerton University.

References

Barre, C. E., N. Martinez, D. an Uilenberg G. (1996). Heartwater a review OIE Paris.

Jongejan F. & Thielemans, M. J. C. (1989). The identification of an immunodominan antigenically conserved 32 kilodalton protein antigen from Cowdrian *ruminantium*. Infection and Immunity 57:3243-3246.

Mahan S. M., Andrew, H. R. Tebele, N. Burridge, M. J. and Barbet A. F. (1994). Immunozation of sheep against heartwater with inactivated *Cowdira ruminantium*. Research in Veterinary Science 58:46-49.

Martinez, D., Shekboudou, Courad, P. O. and Bensaid, A. (1993). Protection of goats against heartwater is acquired with inactivated elementary bodies of *Cowdria ruminantium*. Revue Elevue Medicine Veterinaire Pays Tropicaux 46. 229.

Norval, R. A. I. (1991). Heartwater disease. Developing World Agriuclture, Grosvenor Press International Ltd. Pp. 180-185.

Uilenberg. (1983). Heartwater (*Cowdria ruminantium* infection). Current Status Advances in Veterinary Science Comparative Medicine 227:427-480.