

Copyright is owned by the Author of this thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.



**Comparison of antibody titres between intradermal  
and intramuscular rabies vaccination using inactivated  
vaccine in cattle in Bhutan**

A thesis presented in the partial fulfilment of the requirements for the  
degree of

Master of Veterinary Science

at Massey University, Palmerston North  
New Zealand

**Karma Wangmo**

**2018**



## **Abstract**

In developing countries, the cost of vaccination limits the use of prophylactic rabies vaccination, especially in cattle. Intradermal vaccination delivers antigen directly to an area with higher number of antigen-presenting cells. Therefore, it can produce equivalent or higher antibody titres than conventional intramuscular vaccination even when a lower dose is given.

This study aimed to compare the antibody response in cattle vaccinated intramuscularly with 1mL of inactivated rabies vaccine (Raksharab, Indian Immunologicals) against intradermally vaccinated cattle with 0.2mL of the same vaccine. The study was conducted in Haa province of Bhutan where rabies is not endemic. One hundred cattle from 27 farms were selected for the study. Virus neutralising antibody (VNA) response was measured using the fluorescent antibody virus neutralisation test on the day of vaccination (day 0) and 14, 30, 60 and 90 days later.

Overall, 71% of intradermally vaccinated cattle and 89% of the intramuscularly vaccinated cattle produced a protective response ( $\geq 0.5$  IU/mL). This difference was significant ( $P < 0.02$ ) on days 14 and 30 post vaccination with 36 and 56% in the intradermal group having titres  $\geq 0.5$  IU/mL respectively compared to the equivalent figures of 78 and 76% in the intramuscular group. The mean VNA titres were lower for intradermal group than intramuscular group ( $p < 0.001$ ) with the mean difference being greater than 0.6 IU/mL. Although low dose intradermal vaccination did produce a detectable antibody response, it was inferior to intramuscular vaccination. Thus, although, intradermal vaccination has the potential to reduce the cost of vaccination by reducing the dose required, this study showed that a single dose of 0.2mL intradermally was inferior to an intramuscular dose of 1mL. Further research evaluating dose and dose regimen is needed before intradermal vaccination using the Raksharab rabies vaccine can be recommended in cattle.

## **Acknowledgements**

My sincere gratitude goes to a lot of people for their support and guidance throughout my Masters project.

I am really thankful to my supervisor, Dr. Richard Laven for his excellent supervision. I am lucky to have got an opportunity to work with an experienced professional like him. I am really grateful for his timely feedbacks and support in spite of his busy schedule.

I am grateful to Dr. Florence Cliquet, Marine Wasniewski, Jonathan Rieder, Alexandre Servat and the entire team at OIE/EU/WHO reference laboratory on Rabies in France for analysing my samples and sending the results on time. I am indebted to Dr. Florence Cliquet, who offered to process my samples for free without which this study would not have been possible. I am forever grateful for this generosity. I would also like to thank Marine and the team for timely correspondence and for sharing information and materials related to this project.

I am ever grateful to my dear friend Dr. Sonam Peldon and her entire team at Haa, Bhutan for agreeing to help me collect and process samples at odd hours of the day and making this project possible.

I am also thankful to all the cattle owners who agreed to participate in this study and gave me permission to collect blood samples from their cattle.

My acknowledgment would be incomplete without thanking New Zealand Development Aid for providing this scholarship to pursue Masters of Veterinary Science without whose financial support; I won't have been able to carry out this study.

I also thank the Royal Government of Bhutan and Department of Livestock for giving me permission to conduct this study in the country.

I thank Kencho Sum (Triple Gem) and almighty Buddha for blessing me and giving determination to complete this study.

I would also like to thank Dr. Mary Gaddam (my landlord) and Dr. Linda Laven (my Supervisor's wife) for their guidance and encouragement to complete this study.

Lastly I thank my family members, friend (Xue Qi Soon) and for giving me the moral support and inspiration to pursue and complete this study.

## Table of Contents

Abstract .....	i
Acknowledgements .....	ii
List of tables .....	v
List of figures .....	vi
Introduction .....	1
Literature review .....	6
1.0 Types of rabies vaccine .....	6
1.1 First generation rabies vaccines .....	6
1.2 Second generation rabies vaccines.....	7
1.2.1 Attenuated or live vaccines .....	7
1.2.2 Inactivated or killed vaccines.....	8
1.3 Third generation rabies vaccines.....	10
2.0 Rabies vaccination of reservoir hosts and vectors .....	10
2.1 Rabies control of free ranging wildlife .....	11
2.2 Rabies control in domestic dog reservoirs .....	12
2.2.1 Canine rabies free regions of the world .....	12
2.2.2 Canine rabies control in Asia and Africa .....	13
3.0 Rabies vaccination in cattle.....	14
4.0 Immune response to rabies virus and rabies vaccination .....	15
4.1 Innate immunity .....	16
4.2 Adaptive immune response.....	17
4.2.1 Cell mediated immune response .....	17
4.2.2 Humoral immune response .....	18
5.0 Tests for detection and quantification of rabies virus antibody titres .....	19
5.1 Sensitivity and specificity .....	19
5.1.1 Sample quality .....	21
5.2 Reproducibility .....	21
5.3 Rapidity.....	22
5.4 Cost .....	22
5.5 Summary .....	22
5.6 Limitations of virus neutralisation tests.....	23
6.0 Efficacy of rabies vaccination in cattle .....	24
6.1 Indication of vaccine efficacy .....	24
6.2 Factors influencing the efficacy of rabies vaccination.....	25
6.2.1 Effect of vaccine potency.....	26
6.2.2 Effect of vaccine storage.....	27
6.2.3 Effect of different types of vaccine.....	28
6.2.4 Efficacy based on virus strains.....	29

6.2.5 Effect of booster vaccination .....	29
6.2.6 Effect of age and colostral antibodies .....	30
7.0 Effect of route on rabies vaccination response in cattle.....	30
7.1 Methods of intradermal vaccination .....	31
7.2 Efficacy of vaccination via intradermal and intramuscular routes in cattle.....	31
7.3 Intradermal rabies vaccination in species other than cattle.....	32
7.4 Comparison of rabies vaccination via intradermal and intramuscular routes in cattle .....	33
8.0 Cattle rabies in Bhutan .....	37
8.1 Cattle farming in Bhutan.....	37
8.2 Rabies in cattle.....	38
8.2.1 Risk factors .....	39
8.2.2 Prevention .....	39
9.0 Research objectives.....	40
10.0 Materials and Methods.....	41
10.1 Study design.....	41
10.2 Study area.....	41
10.3 Sample size calculation.....	41
10.4 Farm selection.....	41
10.5 Animal selection .....	42
10.6 Vaccination .....	42
10.7 Animal management .....	43
10.8 Sample collection and shipment .....	43
10.9 Sample analysis.....	44
10.10 Calculation of the rabies virus neutralising antibody titres.....	45
10.12 Test validation.....	45
10.13 Data analysis .....	45
10.14 Potency testing of rabies vaccine (Raksharab).....	46
11.0 Results.....	46
11.1 Descriptive data .....	46
11.2 Potency of rabies vaccine.....	47
11.3 Proportion of animals that responded to the vaccination.....	47
11.4 Proportion of animals with protective VNA titres .....	47
11.5 Effect of vaccination route and time on VNA titres .....	49
12.0 Discussion .....	50
13.0 Conclusion .....	54
14.0 References .....	55



## List of tables

1. Table 1: Summary of different tests used to measure rabies antibody titres.
2. Table 2: Effect of dose, route and injection site on rabies vaccine response.
3. Table 3: Effect of routes of vaccination on antibody response.
4. Table 4: Effect of routes of vaccination on antibody response.
5. Table 5: Dose and route of rabies vaccine and number of cattle in each treatment group.
6. Table 6: Distribution of age, sex, breed and BCS in each treatment groups.
7. Table 7: Proportion of cattle that responded to rabies vaccination (VNA titre  $\geq 0.1$  IU/mL).
8. Table 8: Proportion of cattle in each vaccination group with at least VNA titre  $\geq 0.24$  IU/mL at any time point.
9. Table 9: Proportion of cattle with VNA titre  $\geq 0.5$  IU/mL at 0,14,30,60 and 90 days.
10. Table 10. Effect of vaccine routes on mean (SEM) VNA titres in cattle.
11. Table 11. Effect in cattle of vaccine routes on mean (SD) VNA titres.

## List of figures

1. Figure 1: Pairwise comparisons of the effect of time since vaccination on proportion of vaccinated cattle with rabies VNA titres  $\geq 0.24$ .
2. Figure 2: Geometric mean VNA titres of intramuscularly (im) and intradermally (id) vaccinated cattle on 0, 14, 30, 60 and 90 days post vaccination.