

***PESTE DES PETITS RUMINANTS* IN SHEEP IN THE SUDAN: A STUDY ON SERO-
PREVALENCE AND RISK FACTORS**

**A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS
ABABA UNIVERSITY, TO THE DEPARTMENT OF PUBLIC HEALTH, FACULTY
OF VETERINARY MEDICINE, FREIE UNIVERSITÄT BERLIN AND TO THE
COLLEGE OF GRADUATE STUDIES, SUDAN UNIVERSITY OF SCIENCE AND
TECHNOLOGY IN THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR
THE ATTAINMENT OF MASTER IN TRANSBOUNDARY ANIMAL DISEASE
MANAGEMENT (MTADM)**

**BY
YASSIR ADAM SHUAIB MOHAMED**

**ADDIS ABABA UNIVERSITY
SCHOOL OF VETERINARY MEDICINE
JOINT MASTER PROGRAM IN TRANSBOUNDARY ANIMAL DISEASE
MANAGEMENT**

DECEMBER, 2011

DEBRE ZEIT, ETHIOPIA

***PESTE DES PETITS RUMINANTS* IN SHEEP IN THE SUDAN: A STUDY ON SERO-
PREVALENCE AND RISK FACTORS**

ADVISORS

Prof. Abd-Alhamid A. M. El-Fadil (SUST, the Sudan)

Prof. Karl-Hans Zessin (FUB, Germany)

Dr. Haileleul Negussie (AAU, Ethiopia)

Prof. Mahasin Elnur Abd-Alrahman (SVRI, the Sudan)

Dr. Gelagay Ayelet (NVI, Ethiopia)

DECEMBER, 2011

DEBRE ZEIT, ETHIOPIA

Board of Examiners

Name	Signature	Date
Abd Al-Hamid M. El-Fadil (BVSc, MSc, PhD) Professor and Head Department of PM and PH, College of Veterinary Medicine, Sudan Univesity of Science and Technology Khartoum North, the Sudan Mobile Phone: +249 9 156 896 30 Email: aeelfadil@yahoo.com	21 st December2011
Karl-Hans Zessin (DVM, MSc, PhD) Professor and Head International Animal Health Faculty of Veterinary Medicine Freie Universität Berlin Berlin, Germany Telephone: +49 30 838 62540 Email: zessin.karl@vetmed.fu-berlin.de	21 st December2011
Berhanu Adamasu (DVM, MSc, PhD) Associate Professor Tufts Univesity Addis Ababa, Ethiopia Mobile Phone: (617) 627 3423 Email: berhanu.admassu@tufts.edu	21 st December2011
Khitma Hassan Almalik (BVSc, MSc, PhD) Associate Professor and Head Department of Veterinay Epidemiology, College of Veterinary Medicine, Univesity of Khartoum Khartoum North, the Sudan Mobile Phone: +249 9 123 525 45 Email: kelmalik@hotmail.com	21 st December2011

ACKNOWLEDGEMENTS

Above all, praise is to my almighty Allah for giving me a good health, wisdom, ability, and strength to carry out this work and for all other graces.

I would like to express my deep and sincere gratitude and appreciation to my supervisors Prof. Abd-Alhamid A. M. El-Fadil, Sudan College of Veterinary Medicine, University of Science and Technology, the Sudan; Prof. Karl-Hans Zessin, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany; Dr. Haileluel Negussie, School of Veterinary Medicine, Addis Ababa University, Ethiopia, for their excellent guidance, support, and constant encouragement throughout this project and also for their invaluable assistance and instructions, without which, it would not have been possible to accomplish this project and for reading and correcting the manuscript.

I am also indebted to Prof. Mahasin Elnur Abd-Alrahman, the head of the Department of Virology, Soba Veterinary Research Institute, the Sudan and Dr. Gelagay Ayelet, National Veterinary Institute, Ethiopia for their kind assistance and guidance and would like to thank them very much. I would also like to thank the people of the Department of Virology, Soba Veterinary Research Institute for their endless and kind help during carrying out the cELISA test.

I would like to express my sincerest appreciation and deepest thank to the German Academic Exchange Service (Deutscher Akademischer Austausch Dienst "DAAD") and the European Union; the African, Caribbean and Pacific Group of States; the ACP-EU Cooperation Programme in Higher Education (EduLink) for their significant contribution and providing scholarship, without which it would not have been possible to accomplish this project.

I offer my sincere gratitude also to Dr. Yilkal Asfaw, the dean of the School of Veterinary Medicine, Addis Ababa University, Ethiopia, Dr. Maxmillian Baumann, Freie Universität Berlin, Faculty of Veterinary Medicine, all the representatives and academic staff of the partner universities, and Dr. Kelay Belihu, Food and Agriculture Organization of the United Nations (FAO), Ethiopia, for their very useful assistance and instructions from the beginning

of the course up to the end. The input of the late Dr. Moses Kyule during the course is acknowledged.

I would like to thank Dr. Zerihun Tadesse, International Livestock Research Institute (ILRI), Ethiopia, for his significant assistance during the workshop of the statistical analysis.

I am grateful to the Unit of Field Investigation, Directorate of Animal Health and Epizootic Diseases Control (DAH and ED), Ministry of Animal Resources and Fisheries (MARF), Khartoum, the Sudan and to Soba Veterinary Research Institute (SVRI), Khartoum, the Sudan for their kind help during sampling and field missions. I am also grateful to the Unit of Statistics and Geographical Information System (GIS), Directorate of Animal Health and Epizootic Diseases Control, Ministry of Animal Resources and Fisheries for providing the Chloropleth maps. I would also like to thank the Federal Ministry of Animal Resources and Fisheries and the Ministries of Animal Resources of North Kordofan and Kassala States for their support, help, and facilitating the field missions and sampling.

I am really grateful to Dr. Mohamed Abd-Alraazig the director general of MARF, Prof. Amel Omer Bakhiet the dean of CVM, SUST, Dr. Wilfried Hartwig the technical consultant of the Livestock Epidemio-Surveillance Project (LESP), Prof. Abdel Rahim Mohamed El-Hussein the head of Animal Resources Research Corporation (ARRC), Prof. Mohamed Abd-Alsalam Abdalla the deputy dean of CVM, SUST, Dr. Khedir Mohamed Al-Faky the head of DAH and ED, Dr. Al-Fatih Ahmed Abd Alrahman, Dr. Nawal Sarsar, Dr. Haitham Fadlallah Al-Tayeb, Dr. Yahia Hassan Ali, Dr. Burhan Nasar Mahjoob, Dr. Idris Ahmed Yagoub, Dr. Ibtihal Hammad, Dr. Ahmed Younis Al-Souffi, Dr. Leila Mohamed Ibrahim, and Dr. Asma Ibnouf Abdalla for their kind cooperation and support. I am thankful to all my colleagues and sheep owners and herders for answering the questionnaires and also thankful to all people who helped me in way or another to accomplish this work.

Many thanks to my class mates of the first joint Master Program in Transboundary Animal Disease Management (MTADM) for their everlasting cooperation and encouragement.

Finally, I am thankful to my parents, my beloved sisters and brother, and to all my friends for their everlasting support.

DEDICATION

*THIS WORK IS DEDICATED TO MY
FAMILY, ESPECIALLY MY PARENTS, WHO
BELIEVED IN THE IMPORTANCE OF
EDUCATION. IT IS ALSO DEDICATED TO
MY GRANDFATHERS AND
GRANDMOTHERS, TO MY FRIENDS,
ASSOCIATES AND TO THE SOUL OF THE
LATE PROFESSOR MOHAMED MUSA
MOHAMED-AHMED.*

TABLE OF CONTENTS

LIST OF TABLES

Page No.

Table 1: Number of detected outbreaks of PPRV in different parts of the Sudan and number of herds at risk (2000 to 2007).....	12
Table 2: Rank of the most common and important diseases for which animals were barred by veterinary inspectors from selling at the primary and secondary markets in the three states of Sudan.....	13
Table 3: Crude case fatality rates for diseases reported by household respondents in Sudan as important (2003 to 2005).....	14
Table 4: Detection of PPRV in wildlife species	22
Table 5: Number of vaccinated small ruminants against PPRV in the Sudan in the period (2005 to 2008).....	39
Table 6: PPRV (genus <i>Morbillivirus</i>) strains and sequences retrieved from GenBank, Africa (2000 to 2009).....	45
Table 7: Frequencies and distributions of tested serum samples by state, locality, breed, age, and sex for PPR in North Kordofan and Kassala States (April to July 2011).....	58
Table 8: Estimated sero-prevalence rates of PPR by state, locality, breed, age and sex in North Kordofan and Kassala States (April to July 2011).....	66
Table 9: Univariate associations of risk factors with cELISA PPR-sero-positivity in sheep in North Kordofan and Kassala States (April to July 2011).....	67
Table 10: Results of univariate associations of herd size, number of males, females, and young animals in herds with cELISA PPR-sero-positivity in sheep in North Kordofan and Kassala States (April to July 2011).....	68
Table 11: Results of univariate associations of herd management risk factors with cELISA PPR-sero-positivity in sheep in North Kordofan and Kassala States (April to July 2011).....	69
Table 12: Results of multivariate analyses of associations of risk factors with cELISA PPR-sero-positivity in sheep in North Kordofan and Kassala States (April to July 2011).....	70
Table 13: Frequencies of owners' and herders' general information in North Kordofan and Kassala States (survey from April to July 2011).....	72
Table 14: Mentioning and ranking of economically important animal diseases by owners and herders in North Kordofan and Kassala States (survey from April to	

July 2011).....	73
Table 15: Frequencies of responses of sheep owners and herders on clinical symptoms of PPRV infection in their herds in North Kordofan and Kassala States (survey from April to July 2011).....	74
Table 16: Frequencies of responses of sheep owners and herders on vaccination against PPRV and number of vaccinated animals in North Kordofan and Kassala States (survey April to July 2011).....	75
Table 17: Frequencies of responses of sheep owners and herders on the susceptibility of different age groups and sexes to PPRV infection in North Kordofan and Kassala States (survey from April to July 2011).....	76
Table 18: Frequencies of responses of sheep owners and herders on sources of PPRV infections and their economic impacts in North Kordofan and Kassala States (survey from April to July 2011).....	77
Table 19: Frequencies of responses of sheep owners and herders on seasonality and frequency of occurrence of PPRV infections in North Kordofan and Kassala States (survey from April to July 2011).....	77
Table 20: Frequencies of responses of sheep owners and herders on seasonality and frequency of occurrence of PPRV infections in North Kordofan and Kassala States, (survey from April to July 2011).....	78
Table 21: Frequencies of responses of sheep owners and herders on experience with PPRV outbreaks in North Kordofan and Kassala States (survey from April to July 2011).....	78
Table 22: Frequencies of veterinarians' general information in North Kordofan and Kassala States (interviews from April to July 2011).....	79
Table 23: Ranking of economically important animal diseases by veterinarians in North Kordofan and Kassala States (interviews from April to July 2011).....	80
Table 24: Frequencies of responses of veterinarians on routine diagnosis, control practices and control measures taken for the ranked diseases in North Kordofan and Kassala States (interviews from April to July 2011).....	81
Table 25: Frequencies of responses of veterinarians on the last occurrence of PPR and its seasonality in North Kordofan and Kassala States (survey from April to July 2011).....	82
Table 26: Frequencies of responses of veterinarians on diagnosis of PPRV and control measures taken against PPRV in North Kordofan and Kassala States (survey from April to July 2011).....	83
Table 27: Frequencies of responses of veterinarians on the most susceptible species and breed to PPRV infection in North Kordofan and Kassala States (survey from April to July 2011).....	84

Table 28: Frequencies of responses of veterinarians on the most susceptible age group and sex to PPRV infection in North Kordofan and Kassala States (survey from April to July 2011).....	84
Table 29: Frequencies of responses of veterinarians on the major clinical signs of PPRV infection seen frequently in North Kordofan and Kassala States (survey: April to July 2011).....	85
Table 30: Frequencies of responses of veterinarians on last vaccination against PPRV and the number of vaccinated animals in North Kordofan and Kassala States (survey from April to July 2011).....	86
Table 31: Frequencies of responses of veterinarians on farming systems, migratory routes of nomads, and the sources of PPRV outbreaks in North Kordofan and Kassala States (survey from April to July 2011).....	87
Table 32: Responses of veterinarians on problems they face when implementing disease control programs in North Kordofan and Kassala States (survey: April to July 2011).....	88
Table 33: Comments, advises, and additional information of veterinarians they want to give to the MARF/public/policy makers on PPR control and management of PPR in North Kordofan and Kassala States (survey: April to July 2011).....	89

LIST OF FIGURES

	Page No.
Figure 1: Structure of <i>Morbillivirus</i>	8
Figure 2: Phylogenetic Analysis of the 1232–1560 nt Sequence of the N Protein Gene of Sequenced PPRV Strains.....	10
Figure 3: Distribution of PPRV across West Africa from 2000 to 2010.....	11
Figure 4: Distribution of PPRV across East Africa from 2000 to 2010.....	15
Figure 5: Distribution of PPRV across Central Africa from 2000 to 2010.....	16
Figure 6: Distribution of PPRV across North Africa from 2000 to 2010.....	17
Figure 7: Distribution of PPRV across Asia from 2000 to 2010.....	19
Figure 8: Common Clinical Signs of Acute PPRV infection in Small Ruminants: Ocular and Nasal Discharges.....	24
Figure 9: Common Clinical Signs of Acute PPRV infection in Small Ruminants: Early Oral Lesions and Necrosis of the Gum.....	24
Figure 10: Map of the Study Regions.....	48
Figure 11: Map of the Selected States in the Study Regions.....	51
Figure 12: Map of the Selected Localities in each State in the Study Regions.....	52
Figure 13: Plate Layout of cELISA for PPR.....	55
Figure 14: Estimated state sero-prevalence rates for PPR in sheep in North Kordofan and Kassala States (April to July 2011).....	59
Figure 15: Map of state sero-prevalence rates for PPR in sheep in North Kordofan and Kassala States (April to July 2011).....	60

Figure 16: Estimated locality sero-prevalence rates for PPR in sheep at localities in North Kordofan and Kassala States (April to July 2011).....	61
Figure 17: Map of locality sero-prevalence rates for PPR in sheep in North Kordofan and Kassala States (April to July 2011).....	62
Figure 18: Estimated breed sero-prevalence rates for PPR in sheep in North Kordofan and Kassala States with 95% CI (April to July 2011).....	63
Figure 19: Estimated age group sero-prevalence rates for PPR in sheep in North Kordofan and Kassala States (April to July 2011).....	64
Figure 20: Estimated sex sero-prevalence rates for PPR in sheep in North Kordofan and Kassala States (April to July 2011).....	65
LIST OF ANNEXES	Page No.
Annex 1: Competitive ELISA Reagents and Solutions.....	124
Annex 2: PPR Questionnaire Format for Owners and Herders.....	125
Annex 3: PPR Questionnaire Format for Veterinarians.....	133

LIST OF ACRONYMS

aa	Amino Acid
Abs	Antibodies
Ag	Antigen
AGID	Agar Gel Immunodiffusion
AGPT	Agar Gel Precipitation Test
APS	Animal Production System
ATCC # CCL81	American Type Cell Culture
BB	Blocking Buffer
BDSL	Biological Diagnostic Supplies Limited
BHK-21	Baby Hamster Kidney
bp	Base pair
BPS	Buffered Physiological Saline
CBS	Central Bank of Sudan
CCFR	Crude Case Fatality Rate
CCPP	Contagious Caprine Pleuro-Pneumonia
cDNA	Complementary Deoxyribonucleic Acid
CDV	Canine Distemper Virus
cELISA	Competitive Enzyme Linked Immuno-Sorbent Assay
CFT	Complement Fixation Test
CIEP	Counter immunoelectrophoresis
CIRAD	Centre de Coopération Internationale en Recherché Agronomique pour le Développement
CIRAD	The International Cooperation Centre in Agronomic Research for Development
CPE	Cytopathic Effect
DAAD	Deutscher Akademischer Austausch Dienst
DAAD	The German Academic Exchange Service
DAH & ED	Directorate of Animal Health and Epizootic Diseases Control
DDW	De-ionized Distilled Water
DIVA	Differentiation of Infected from Vaccinated Animals
DNA	Deoxyribonucleic Acid
dNTPs	Deoxonucleoside triphosphate
EDI	ELISA Data Interchanges Software
EduLink	The Connecting Learning Communities
F	The Fusion Gene
FAO	Food and Agriculture Organization of the United Nations
FEE	Foreign Exchange Earnings
GDP	Gross Domestic Product
GHA	The Greater Horn of Africa
GIS	Geographical Information System
GIT	The Gastrointestinal Tract
GREP	Global Rinderpest Eradication Project
H	The Haemagglutinin Gene
H ₂ O ₂	Hydrogen Peroxide
HA	Hemagglutination Test

HRPO	Horseradish Peroxidase Conjugate
IcELISA	Immunocapture Enzyme Linked Immuno-Sorbent Assay
IGAD	Intergovernmental Authority on Development
ILRI	International Livestock Research Institute
M	The Matrix Gene
MAb	Monoclonal Antibody
MAR	Mean Average Rainfall
MARF	Ministry of Animal Resources and Fisheries
MDBK	Madin-Darby Bovine Kidney Cells
MDT	Mean Daily Temperatures
MEPD	Ministry of Environment and Physical Development
mRNA	Messenger Ribonucleic Acid
MT	Metric Tones
MTADM	Master Program in Transboundary Animal Disease Management
MV	Measles Virus of Humans
N	The Nucleocapsid Gene
NES	Nuclear Export Signal
NLS	Nuclear Localization Signal
No.	Number
NP	Nucleoprotein
NPV	Net Present Value
NS	Normal saline
nt	Nucleotide
°C	Degree Centigrade
OD	Optical Density
OIE	The International Organization for Animal Health
OPD	Ortho-Phenylenediamine
Orf	Contagious Ecthyma
ORF	Open Reading Frame
PAGE	Electrophoretic Profile in Polyacrylamide Gel
PANVAC	The Pan African Veterinary Vaccine Centre
PARC	The Pan-African Rinderpest Campaign
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PD	Phosphate diluents
pH	Measure of the Acidity or Basicity
PI	Percentage of Inhibition
PPR	Peste Des Petits Ruminants
PPRV	Peste Des Petits Ruminants Virus
RBCs	Red Blood Cells
RBOK	The Kabete 0 Strain of Rinderpest
RNA	Ribo-nucleic Acid
RNP	Ribonucleo-Protein
RPV	Rinderpest Virus
RT	Reverse Transcriptase Enzyme
RT-PCR	Reverse Transcription Polymerase Chain Reaction
Shoats	Sheep and Goats
SP	Strong Positive

SPS	Sanitary and Phyto-Sanitary
SPSS	The Statistical Package for Social Sciences for Windows®
SVRI	Soba Veterinary Research Institute
T cells CD4+	T helper Lymphocytes
T cells CD8+	Cytotoxic T Lymphocytes
Taq	Thermostable DNA Polymerase
TCID	Tissue Culture Infective Dose
TCID ₅₀	50% Tissue Culture Infective Dose
TCRV	The Tissue Culture Rinderpest Vaccine
UN	The United Nations
US\$	United States Dollar
USAID	United States Agency for International Development
Vero	African Green Monkey Kidney Cell
VNT	Virus Neutralization Test
WP	Weak Positive
µl	Microliter

SUMMARY

The results of the study have increased knowledge on the epidemiology of PPR in sheep in North Kordofan and Kassala States of the Sudan, by using cELISA testing and a questionnaire survey. The estimated overall sero-prevalence rate was found to be 70.2% (576/820) (95% CI: 67.1 - 73.3). The sero-prevalence rates estimated in the two states were statistically not different, while there were differences in the sero-prevalence rates between individual surveyed localities, with both Jebrat Al-Shiekh and Shiekan locality showing significantly higher sero-prevalence rates than the other 5 localities. For breeds, Zaghawa was showing a higher sero-prevalence rate than other breeds. There were no statistically significant differences in the sero-prevalence rates among differentiated age groups. For sexes, females were showing a higher sero-prevalence rate than males. Significant risk factors associated

with a cELISA positive status for PPRV in the univariate analysis using the chi square test were found to be state, locality, breed, sex and number of males with p-value ≤ 0.05 . Age, herd size, number of females in the herd, number of young animals in the herd, buying animals from outside, measures taken before introducing a new animal into the herd, production system practiced, mixing herds at communal points, and where herds get mixed, in contrast, were not identified as significant risk factors. The factors found to be significantly associated with increased odds of being cELISA positive in the multivariate analysis were localities (Jebrat Al-Shiekh, Barra, and Al-Girba) and sex (females), number of males (≤ 10) and number of young animals (>40) in the herd, while the factors found not significantly associated with increased odds of being cELISA positive were breeds, ages, and where herds get mixed. The results of the questionnaire survey showed that sheep owners and herders in North Kordofan and Kassala States have a solid good knowledge of PPR infection, its clinical signs, the effect of animal movements, practicing communal grazing and watering on the spread of the disease and its impact on their animals. At the same time there exist considerable reservations of a sizeable number of herders against vaccination against PPR. Investigation results suggest that PPR has taken an endemic pattern of occurrence in the Sudan as reported from other countries in East Africa. Urgent need therefore exists to initiate a realistic network for surveillance, control and eradication of this important disease in the Sudan and in the region. Such scheme is suggested and supported at high levels and it should immediately be launched as recommended by OIE.

Keywords: Sudan, sheep, PPR, sero-prevalence, risk factors