ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

EPIDEMIOLOGICAL AND CLINICO-PATHOLOGICAL STUDY OF MAEDI-VISNA VIRUS INFECTION IN SHEEP IN SELECTED DISTRICTS OF THE AMHARA NATIONAL REGIONAL STATE, ETHIOPIA

By

TSEGAW FENTIE KASSA

June 2004
Debre Zeit, Ethiopia

ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

EPIDEMIOLOGICAL AND CLINICO-PATHOLOGICAL STUDY OF MAEDI-VISNA VIRUS INFECTION IN SHEEP IN SELECTED DISTRICTS OF THE AMHARA NATIONAL REGIONAL STATE, ETHIOPIA

A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in the partial fulfillment of the requirements to the Degree of Master of Science in Tropical Veterinary Epidemiology

By

TSEGAW FENTIE KASSA

June 2004
Debre Zeit, Ethiopia

EPIDEMIOLOGICAL AND CLINICO-PATHOLOGICAL STUDY OF MAEDI-VISNA VIRUS INFECTION IN SHEEP IN SELECTED DISTRICTS OF THE AMHARA NATIONAL REGIONAL STATE, ETHIOPIA

By
TSEGAW FENTIE KASSA

Board of Examiners: Signature

1. Prof. Ph. Dorchies _____
2. Prof. Feseha Gebreab _____
3. Dr. Wondwossen Abebe _____
4. Dr. Giles Innocent _____
5. Dr. Andy Catley _____
6. Dr. David Barrett _____

Academic Advisors:

1. Dr. Ademe Zerihun _____
2. Dr. Francois Roger _____

DEDICATION

This paper is dedicated to Lord Jesus Christ for his everlasting mercy and grace upon me.

To my beloved wife Abeba Afework, to my beloved sons; Jordan, Natnael and Eyassu for their love, patience, prayer, support and encouragement.

To my parents Fentie Kassa and Kebebush Alemie for their encouragement and assuring my education.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXES

LIST OF ABBREVIATIONS

AGID	=	Agar Gel Immunodiffussion Test
AIDS	=	Acquired Immunodeficiency Syndrome
ANRS	=	Amhara National Regional State
BoA	=	Bureau of Agriculture
CACC	=	Central Agricultural Census Commission
CAEV	=	Caprine Arthritis Encephalitis Virus
CI	=	Confidence Interval
CPE	=	Cytopathic Effect
DNA	=	Deoxyribonucleic Acid
ENV	=	Envelop protein
HIV	=	Human Immunodeficiency Virus
i-ELISA	=	indirect-Enzyme Linked Immunosorbent Assay
ILRI	=	International Livestock Research Institute
LTR	=	Long Terminal Repeats
MA	=	Matrix protein
MV	=	Maedi-Visna
MVV	=	Maedi-Visna Virus
NC	=	Nucleocapsid protein
OD	=	Optical Density
OIE	=	Office International des Epizooties
OR	=	Odds Ratio
PCR	=	Polymerase Chain Reaction
RNA	=	Ribonucleic Acid
RT	=	Reverse Transcriptase
ShARC	=	Sheno Agricultural Research Centre

ACKNOWLEDGEMENTS

I express my deep appreciation to the management of Bureau of Agriculture, ANRS for allowing me to join this graduate program and sponsorship, and Amhara Region Agricultural Research Institute for their financial support, the Addis Ababa University, Faculty of Veterinary Medicine for admitting me to the course, financing the serological test materials and field research work and allowing me to use the laboratory and administrative facilities.

I am greatly indebted to my academic advisors Dr. Ademe Zerihun of the AAU, FVM and Francois Roger of the CIRAD-EMVT for their technical and professional advice, encouragement and time devotion to review and correct this manuscript.

My appreciation is to the Dean of the FVM, the Associate Dean for Research and Graduate studies; in particular to Dr. Bayleyegn Molla for his unreserved assistance and an overall coordination of the course program successfully.

My sincere gratitude is to the National Animal Health Research Center, in particular to Dr. Asegedech Sirak for her assistance in undertaking the histopathology work.

Many thanks to the staff of the Kombolcha Regional Veterinary Laboratory for their assistance in providing me materials and vehicle for sample and data collection.

My thanks also go to the staff of the Amed Guya, Debre Berhan and Sheno Agricultural Research centers for their assistance in obtaining data and serum sample.

Finally, I would like to express my heart felt thanks to all my family members for their unreserved encouragement and support.

ABSTRACT

An epidemiological, clinical and pathological study of Maedi-Visna virus (MVV) infection in sheep was carried out in highland areas of the Amhara National Regional State (ANRS). The purpose of the study was to estimate the prevalence and distribution of the disease and identify associated risk factors with the occurrence of the disease. A total of 2417 serum samples were collected from smallholder farms (n = 1491) and sheep breeding ranches (n = 926) from November 2003 to February 2004. Indirect-Enzyme Linked Immunosorbent Assay (i-ELISA) and Agar Gel Immunodiffusion (AGID) tests were employed to determine the presence of antibodies against MVV infection. All sheep sampled were clinically investigated where as few seropositive sheep were slaughtered and examined for gross lesions and histopathological pictures.

The apparent seroprevalence of MVV infection was found to be different between the management types; 6.6% (95% CI = 5.4 - 7.9) in smallholder farms and 30% (95% CI = 27.1 - 33.1) in sheep ranches. The difference in prevalence between the two sheep management types, among ranches and *weredas* was statistically significant ($P < 0.001$). The prevalence of MVV infection increased with increasing flock size and found to be higher in ranches (82.6%) than in the smallholder farms (18.8%). The breed related seroprevalence varied from 16% in indigenous sheep to 77.6% in Awassi sheep. The age-specific seroprevalence increased with the age of sheep from 2.8% in yearlings to 76.5% at 7 years old. Flock size, breed, age and contact with ranch sheep were significant ($p < 0.0001$) risk factors associated with the occurrence and transmission of MVV infection.

In clinical disease investigation, 31 out of 348 (8.3%) MVV seropositive sheep were found to express the clinical disease. Among the seropositives with overt clinical disease, 96.8% were from sheep ranches while 3.2% from smallholder farms. There was statistically significant ($\chi^2 = 88.3$; d.f = 1; $P < 0.001$) association between the clinical disease and the MVV seropositivity. The clinical disease increased with age of animals. The large proportion of the clinical disease in ranches and older animals was associated with the flock management and high level of MVV infection.

The clinical disease was significantly different among sheep breeds ($P<0.05$), higher in Indigenous Menz breeds than the Awassi and their crosses. Clinical manifestations with respiratory symptoms (45.2%) were followed by progressive emaciation (16%). Necropsy

examination revealed focal and diffused consolidation of lungs, broncho-interestitial pneumonia. The histopathological investigations with peribronchiolar and perivascular mononuclear cell infiltration, smooth muscle and lymphoid hyperplasia and thickening of the alveolar septa indicated the progressive interstitial pneumonia characteristic of MVV infection.

The serological, clinical and pathological findings suggested that MVV infection is a major health problem in highland sheep of the ANRS. The ranch management and husbandry practice consisting of large flocks and high proportion of older animals has contributed for the occurrence and spread of MVV in a higher rate in ranches than in smallholder farms. The sheep ranches are considered major sources for the spread of MVV infection to the smallholder farms. The findings of MVV antibodies in areas hypothetically considered free is suggestive for poor knowledge and misdiagnosing of the disease. The serological test agreement between ELISA and AGID found to be almost perfect and this would encourage the use of the two tests in combination for accurate serological diagnostics in the control of MVV infection.

Keywords: Lentivirus, Maedi-Visna Virus, Prevalence, Smallholder Farms, Sheep Breeding Ranches, ELISA, AGID, Pathology, Amhara National Regional State.

1. INTRODUCTION

The *Lentivirinae* subfamily of the *Retroviridae* family comprises taxonomically related viruses that cause fatal disease in human and different species of animals: sheep, goats, horses, cattle, monkeys and cats (Narayan and Clements, 1989; Pepin *et al.*, 1998). The lentiviruses are non-oncogenic, lytic in cell cultures and cause cell fusion resulting in multinucleated giant cells or syncytia. They are exogenic and cause life-long systemic infections, usually with slow development of disease, characterized by chronic inflammation and leading in a progressive manner to severe disability and death in most cases. Although the host responds with development of serological and cell-mediated immune responses to the virus antigens, it is unable to clear itself of the infection.

The name lentivirus is derived from Sigurdsson's (1954) ideas of a special group of infectious diseases which he called "slow" infections. The basic genomic structure of lentiviruses is similar to that of oncoviruses but more complex with a number of regulatory genes, which influence the replication and the expression of the virus genome which is also influenced by the differentiation and the physiological state of the host cells (Petursson *et al.,* 1992).

The earliest recognition of the respiratory form of ovine lentivirus dates back to 1915, when a progressive interstitial pneumonia was reported in South African sheep and later described by Marsh in the United States in 1923 in Montana, and called "Ovine Progressive Pneumonia"(Petursson *et al.,* 1992). In Iceland in 1947, a pulmonary form of similar disease was identified and the disease was called "Maedi", Icelandic word for dyspnea or labored breathing, which describes the disease associated with a progressive interstitial pneumonitis (Sigurdsson *et al.*, 1952). Later in 1957, another form of the disease that caused paralysis was also identified in Iceland. This form was called "Visna", which means wasting or shrinkage in Icelandic, which describes the signs associated with a paralyzing meningio-encephalaitis (Sigurdsson *et al.*, 1957). Since then ovine lentiviruses have been isolated from sheep flocks in many countries. Although there are considerable genetic differences between the various strains of ovine lentiviruses they are referred collectively as "Maedi-Visna"(MV), which is used in many countries to describe the same disease syndrome. Morphogenesis, molecular hybridization, serology and animal experiments have shown these to be caused by the same virus (Kimberling, 1988). The pulmonary form of Maedi-Visna virus (MVV infection has been known under different names in various countries: Graff-Reinet disease in South Africa,

progressive pneumonia in Montana, USA, *zwoegerziekte* in Holland and *la bouhite* in France (Petursson *et al.,* 1992).

The lentiviruses, Maedi-Visna and Caprine Arthritis-Encephalitis virus (CAEV) causes multi-systemic disease processes in sheep and goats. Pathological changes are mainly found in the lungs causing progressive interstitial pneumonia, and other syndromes such as meningeal arthritis with encephalitis, indurative mastitis and non-suppurative arthritis. The organs targeted by the virus in descending order of importance are the lungs, mammary glands, joints and the brain.

The MVV can cause disease with any of the following signs: severe and progressive weight loss, labored breathing or pneumonia, paralysis, swollen joints associated with lameness, and palpably hard, unproductive udders. However, most infected sheep never show clinical signs of disease. Once a sheep is infected with the virus, that animal stays infected for its lifetime and serves as a carrier even in the presence of high levels of circulating antibodies. Unlike some viral diseases, the presence of antibodies is not indicative of immunity (Brodie *et al.,* 1998). Maedi-Visna virus persists and replicates in the presence of active immune responses and cause immune-mediated lesions in several organs and this unusual relationship with the host makes the diagnosis and control difficult and expensive (Cutlip *et al.*, 1988).

Although the animal lentiviruses of veterinary importance have been studied for several decades, the realization that the causative agents of Human Immunodeficiency virus (HIV) belong to the lentivirus group whose biology in humans closely resembles that of MVV of sheep, has greatly intensified the research effort in lentiviruses (Gonda *et al.,* 1986). Although the research effort is by far most concentrated on the human lentiviruses, interest in animal lentivirus research is growing because of the potential comparative value of animal models for Acquire Immunodeficiency Syndrome (AIDS) (Petursson *et al.,* 1992; Simon *et al.*, 1994).

Because of distribution to most major sheep producing countries and a high morbidity, MV is a major disease of the sheep industry and annually results in financial waste. Because it is a disease where there are no immediate clinical signs upon infection, the economical impact on sheep producers may be hard to assess especially if the incidence of clinical disease is low. The economic importance of the disease rests with losses associated with decreased longevity, mortality with clinical disease, decreased value of culled animals and possible effects of subclinical infection on productivity, the cost of treatment of secondary infections and

expensive research and control programs, and loss of marketing opportunities as a result of restrictions that countries impose on the importation of sheep from places where the infection exists (Dohoo *et al.*, 1987; Kimberling, 1988).

The occurrence of MVV in Ethiopia was serologically detected in imported sheep breed (Merino) in 1986 at Agarfa sheep ranch, Bale province. Laboratory test was conducted using AGID both in the country and abroad (UK) and has provided 30% seropositivity for MVV. Due to the detection of the disease, the flocks were destroyed with final closure of the ranch (BoA, 2000).

Maedi-Visna virus was also reported in the year 1996 around Debre Berhan on village sheep and International Livestock Research Institute (ILRI) research station with a prevalence rate of 3.7% (Ayelet *et al.*, 2001). In 1999, a respiratory disease outbreak was investigated in Amed Guya sheep ranch, North Shewa. Histopathological examination of lung tissues had indicated typical MV lesions with a high degree of mononuclear cell infiltration (Tibbo *et al.*, 2001). In the same year a number of serum samples collected from culled and clinical diseased animals from Debre Berhan and Amed Guya sheep ranches were tested serologically and the result has indicated 74% seropositivity for MVV with a significant breed difference varying from 48% in Awassi sheep to 92% in the indigenous Menz sheep (Woldemeskel *et al.*, 2002). Because of the emergence of MVV in both ranches with the exception of some pedigree Awassi sheep breed for replacement through repeated tests, all local, crossbred and exotic sheep from both ranches were slaughtered in the year 2002 and 2003.

In the past two decades, more than 5000 crossbred rams had been distributed from the two sheep ranches to the sheep producing areas of the country to the farmers with high proportion in the Amhara region. The distributed rams could have been possible sources to spread the disease. However, there was no information available about the distribution of the disease in the smallholders' flocks. Therefore, epidemiological, clinical and pathological study was required to be undertaken in major sheep producing areas of the country and this study was conducted particularly in Amhara region to determine the magnitude and impact of the disease in the highland sheep and for designing prompt control measures.

Finally the outputs of this study would provide ground for further research and designing control options to minimize the morbidity and mortality of small ruminants and prevent further spread of the disease. Therefore, the objectives of the study were in general to assess

the magnitude and distribution of MVV infection in the highland sheep of Amhara National Regional State and the specific objectives were:

- To estimate the prevalence and distribution of MV in the highland sheep in selected districts of the Amhara National Regional State,
- To identify the potential risk factors associated with the disease.

2. LITERATURE REVIEW

2.1. The Virus

2.1.1. Viral structure

Lentiviruses are spherical and have a unique three-layered structure. The ultra-structure of lentiviruses is similar to that of C-type retroviruses and the virions are assembled at cellular membranes in a similar way. They can be distinguished from C-type virions by the presence of cone-shaped core in the fully formed virion and the core shell is formed closer to the cell membrane during the budding process and appears thicker than seen in C-type viruses (Gelderblom *et al.,* 1990).

The size of the virion is about 110-130 nm and the surface is studded with projections or knobs about 9-10 nm in length, composed of the envelop glycoprotein. The central part of the virus is the genome-nucleoprotein complex, associated with the reverse transcriptase. This structure is enclosed within an icosahedral capsid surrounded by an envelope derived from the plasma membrane of the host cell (Gelderblom *et al.,* 1990; Pepin *et al.*, 1998).

Small ruminant lentiviruses, MV and Caprine-Arthritis-Encephalitis viruses (CAEV) are structurally and morphologically similar, cause comparable disease syndromes, and can reciprocally infect sheep and goats, although they are distinguishable at the molecular level and by differences in immunologic reactivity (Narayan and Clements, 1989).

The core contains two copies of the RNA genome associated with a few molecules of the enzyme reverse transcriptase (RT), a small nucleocapsid protein (NC) and is surrounded by a shell composed of the capsid protein. Together with the so called lateral bodies, the core is in turn surrounded by a layer of matrix protein (MA). The outer envelop is composed of a lipid bilayer containing the transmembrane protein (TM) (Gelderblom *et al.,* 1990; Petursson *et al.*, 1992).

2.1.2. Organization of the viral genome

The MVV has a genome organization similar to other retroviruses. It comprises three structural and various regulatory genes (Narayan and Clements, 1989; Pepin *et al.*, 1998). The basic order of structural genes arranged from the 5' end to 3' end of the genome are *gag* (group-specific antigen), *pol* (polymerase) and *env* (envelope) (DeMartini *et al.*, 1991).

The *gag* gene of MVV is highly conserved and encodes information for one major and two minor core proteins (Kwang *et al.*, 1996). The major core protein called the capsid protein (p24-p27) elicits a strong antibody response. For this reason, this protein has been the target to develop sensitive diagnostic assays (Marcom *et al.*, 1991; Juste *et al.*, 1995). Conversely, the two small core proteins, the matrix protein (p16) and the nucleocapsid protein (p14), are poorly immunogenic (Naryan and Clements, 1989).

The *pol* gene encodes for several enzymes important in viral nucleic acid transcription and protein synthesis during replication, including reverse transcriptase, protease, endonuclease, and the dUTPase. Drugs that disrupt the expression of these enzymes have been the focus for HIV chemotherapy, but due to the persistence nature of lentivirus infections and high cost of treatment, these approaches have no practical application for the treatment and control of MVV (Brodie *et al.*, 1998).

The *env* gene encodes the envelope glycoprotein also called the surface protein referred to as gp135 and the transmembrane protein. Gp135 has been identified as an antigenic target during immune responses to MVV infection. The surface protein carries the neutralization and fusion epitopes. Antigen variation within this protein confers the biological and serological properties of different isolates (Leroux *et al.*, 1996). For this reason, recombinant ELISA tests that rely on the detection of antibodies against the surface protein may have low sensitivity (Juste *et al.*, 1995). Conversely, the transmembrane protein, which is much more conserved, has been used successfully in some recombinant ELISA tests (Kwang and Cutlip, 1992).

In contrast to most oncoviruses, the lentiviruses produce several regulatory proteins located between the *pol* and *env* genes and at the 3' terminus, which control the rate of viral genome expression and virus replication. The three major auxiliary genes in MVV, which are essential for virus replication, are *tat*, *vif* (viral infectivity factor), and *rev* (regulator of virion protein expression) (Clements and Zink, 1996). In addition to its regulatory role, the product of the

tat gene may contribute to the pathogenesis of multi-organ proliferative disorders and possibly lesions in the central nervous system (Philipon *et al.*, 1994).

As in the other retroviruses the DNA genomes of lentiviruses are flanked on both sides by regulatory sequences, the so called long terminal repeats (LTRs) that contain enhancer-promoter elements for the initiation of DNA transcription and plays a role in tissue tropism. When the viral RNA enters a permissive cell it is transcribed into double stranded DNA (DeMartini *et al.*, 1991).

2.1.3. Virus replication

Small ruminant lentiviruses generally replicate to high titers in permissive cell cultures of the fibroblastic type, such as choroid plexus and synovial cell cultures. Ovine and caprine cells of the monocyte/macrophage lineage also support productive infection in cultures. Some ovine field strains replicate poorly in fibroblastic cell cultures. Isolates of Small ruminant lentiviruses are divided into two types: 1. lytic isolates such as the Icelandic MVV and 2. non-lytic, persistent isolates typical for CAEV and some sheep isolates of the American ovine progressive pneumonia (Querat *et al.*, 1984).

The general feature of the cytopathic effect of these viruses is the production of multi-nucleated syncytia or giant cells by cell fusion and formation of star-shaped or spider-like cells with a rounded refractile cell body and many long filiform cytoplasmic extensions. As the virus attached to the cell the virion envelope fuses with the cell membrane. Despite the principal receptor molecule for HIV is known to be the CD4 surface molecule of the so called helper-inducer lymphocyte, much less is known about virus receptors for MVV. The attached virion is decoated and the RNA genome is released and transcribed into double-stranded DNA with the help of the reverse transcriptase contained in the virion core (Petursson *et al.*, 1992).

In contrast to the oncogenic retroviruses, where viral DNA synthesis takes place in the cytoplasm, lentiviral DNA is replicated in the nucleus of the cell (Haase *et al.*, 1982). It has been proposed that this explains why lentiviruses can replicate in non-dividing cells. One to two hundred copies of free linear-double stranded DNA molecules are found in lentivirus infected cells. However, very few viral DNA molecules integrate into the host cell DNA. It has been suggested that this high number of free viral DNA molecules in infected cells may

be associated with the cytolytic effects of lentiviruses (Clements *et al.*, 1979; Petursson *et al.*, 1992).

In contrast to the highly productive viral infection in permissive cells in culture, the replication of lentiviruses in the tissues of the animal is highly focal and unproductive (Haase *et al.*, 1986). Very few cells are found to contain the viral DNA and many of those produce little or no viral RNA and even fewer detectable viral antigens. This restriction seems to depend on physiological and developmental state of the cells. When cells from tissues of lentivirus infected sheep are grown in tissue culture, the restriction is relieved with resulting productive infection (Petursson *et al.*, 1976). It has also been shown that when infected monocytes with low production of virus differentiate into macrophages the host cell restriction is lifted leading to increase viral production (Geldelman *et al.*, 1986). Thus it appears that the expression of viral genes and the replication of the lentiviruses is strictly controlled by a complex interaction of viral and host cell genes. The restriction of viral replication in the host is considered a major factor in the slowness of lentiviral diseases (Petursson *et al.*, 1992).

2.1.4. Variability

Genetically, lentiviruses are quite heterogeneous. This manifests itself in their antigenic diversity, differences in virulence and growth characteristics *in vitro*. This genetic plasticity is believed to contribute to viral persistence in the host animal by permitting evasion of the immune response. Moreover, antigenic diversity may also present a problem in the diagnosis of lentviral infections and remains a formidable obstacle to vaccine development. Antigenically distinct viruses have been isolated from sheep persistently infected with MVV; these variants arise by point mutations in the *env* gene (Braun *et al.*, 1987).

Genetic variation in MVV has been determined by polymerase chain reaction (PCR) amplification of portion of the viral genome (Sargan *et al.*, 1995). These studies have allowed the extent of variability in different regions of the genome (LTR, *gag, pol, env*) to be compared and the heterogeneity of MVV strains to be determined. Moreover, these techniques permitted the phylogeny of lentiviruses to be established (Pepin *et al.*, 1998). The high mutation rate must be of importance for the evolution and the epidemiology of lentiviruses and may pose problems in attempts to develop vaccines and drugs to control this disease (Petursson *et al.*, 1992).

When strains of ovine lentivirus from different parts of the world are compared, considerable biological differences are revealed. Some strains are more lytic in tissue culture and grow to higher titres than others and differ in their ability to induce neutralization antibodies (McGuire *et al.*, 1990).

The MVV consists of group of strains, K1514 from Iceland, SA-OMVV from South Africa, and EVI from UK (Pasick, 1998). Sequence analysis studies in the *env* gene of ovine isolates in North America showed more similar to CAEV, which suggests that the ovine and caprine lentiviruses may have descended from a common ancestral genotype (Valas *et al.*, 1997).

2.2. The Disease

2.2.1. Epidemiology

With the exceptions of Australia and New Zealand, MVV is present in all major sheep producing countries of the world; North and South America, Europe, Asia and Africa (Clements and Zink, 1996). Antibodies to MVV are not protective against the disease; therefore if an animal has antibodies to MVV after six months of age then they are considered infected with the MVV and are carriers of the virus. The incidence of infection increases with ewe/ram age, probably due to sheep-to-sheep spread of the virus. The percentage of infected sheep that develop clinical disease is variable and is possibly dependent on flock management, strain and dose of virus, and genetics of sheep.

2.2.1.1. Prevalence

Prevalence of infection and of disease varies greatly. In USA the infection varied from 1% to 67% in culled sheep from different states and from 51-90% in range sheep (Cutlip *et al.*, 1988). The prevalence of seropositive animals increased with age from 4% in less than 1 year to 34% in 4 years period (Cutlip *et al.*, 1992). In range conditions, where lambing occurs in pasture, seropositivity was only 0.5%. Moreover, the differences in seroprevalence suggested the close contact during confinement for lambing and cold weather may favor the transmission of the virus (de la Concha-Bermejillo *et al.*, 1994; Brodie *et al.*, 1998).

A serological survey in Canadian sheep has also indicated a seroprevalence of 19% and a mean flock prevalence of 12% an increased prevalence with increased age and flock size was noted but prevalence in sheep older than 8 years was decreased, probably due to losses through culling and deaths of infected sheep earlier in their life (Simard and Morley, 1991).

2.2.1.2. Susceptibility

Sheep and goats are the only species that have proven to be naturally susceptible to the MVV. According to Cutlip *et al.* (1991) only goats and rabbits could be experimentally infected with the MVV. The infection in rabbits was acute and virus did not persist or induce antibody production as it does in sheep and goats. Goats developed characteristic lesions of MV as of sheep. All breeds and ages of sheep appear to be susceptible to infection. In contrast to susceptibility to infection breeds of sheep appear to differ with respect to susceptibility to development of disease after infection. These findings may also relate to differences in the pathogenicity of the strains of MVV and not solely a function of genotype of the sheep (Cutlip *et al.*, 1991).

Natural disease is more common in 2-3 years old sheep but may occur at any age of adult sheep. The percentage of infected sheep that eventually become clinically affected and the age of onset appear to be related to the level of infection in the flock (DeBoer *et al.*, 1979; Cutlip *et al.*, 1988).

2.2.1.3. Modes of transmission

Secretions of the udder and lungs are thought to be the main source of virus transmission. European and Icelandic studies have shown that lactogenic transmission commonly occurs between ewe and lamb via ingestion of colostrum and milk (DeBoer *et al.*, 1979). In an endemically infected flock, virus infected mononuclear cells and free virus in the colostrum and milk are passed through the intestinal epithelium of the neonate to establish infection (Cutlip *et al.*, 1988). Naturally, lambing is a time of high lentivirus expression, which facilitates the spread of infection. Since mastitis is frequent in affected animals, vertical transmission may be facilitated by the recruitment of mononuclear infected cells to the mammary gland (Zink and Johnsson, 1994; Pepin *et al.*, 1998). When lambs are separated from their dams immediately after birth and reared in isolation they remain with few exceptions free of the infection (Houwers *et al.*, 1983).

A low incidence of ewe to lamb transmission occurs in the early stages of MVV infection whereas chronically infected ewes readily infect their lambs (Sihvonen *et al.*, 1980). There was also a high incidence of transmission with increasing periods of contact, rising to 81% of lambs showing evidence of MV infection when the flock was maintained intact for one year (DeBoer *et al.*, 1979). In infected flocks the progeny of seropositive ewes are much more likely to show evidence of infection than lambs of seronegative dams (Houwers *et al.*, 1990).

There is ample evidence of horizontal transmission of MVV infection in sheep. When virus-carrying sheep are introduced into a virgin population the infection spreads gradually between adult sheep (Houwers *et al.*, 1990; Petursson *et al.*, 1992). Free virus or virus-infected cells are horizontally transmitted by inhalation of respiratory secretions (Zink and Johnsson, 1994). Droplet transmission is facilitated by housing and close confinement and was important in Iceland, where sheep were housed for 6 months during the winter (Carey and Dalziel, 1993). Evidence of respiratory transmission of the disease between adult sheep was also provided by Houwers and Van der Molen (1987) who introduced two infected ewes into a flock of 22 seronegative ewes and found that 80% of the flock became infected within five years. Asymptomatic sheep are rarely a source of virus for infection of other sheep, with the possible exception of ewe to lamb transmission via milk (Carey and Dalziel, 1993).

Experimental transmission of MVV by intra-tracheal inoculation of lung tissue suspensions or cell culture derived virus into newborn lambs has been shown not only to reproduce but also to accelerate the disease process (DeMartini *et al.*, 1987).

The spread of MV infection is frequently very rapid in flocks concurrently infected with the retrovirus causing pulmonary adenomatosis. The copious lung fluid produced by sheep with pulmonary adenomatosis contains MVV in dual infected sheep, and the copious secretion with coughing is believed to increase the risk for horizontal transmission. The increased number of alveolar macrophages in pulmonary adenomatosis lungs has contributed to an increase in MVV replication (Dawson *et al.*, 1990).

Arthropod transmission of MVV and other lentiviruses are limited, and not a primary route of infection (Gurovich, 1989). Dawson (1987) has investigated the potential role of iatrogenic transmission, and he was unable to transmit MVV by inoculating naive sheep with small amounts of blood taken from infected animals.

There is little evidence for in-utero transmission of MV virus. In a study where peripheral blood was taken from lambs immediately following birth and mononuclear cell DNA used for

PCR, it was shown that approximately 10% of lambs were infected in utero (Brodie *et al.*, 1994). No direct evidence for transplacental transmission could be demonstrated in the experiments of Sihvonen (1980), 50 lambs snatched from their infected dams at birth remained uninfected (DeBoer *et al.*, 1979). It is generally accepted that in-utero transmission is of minor significance. However, as fetuses can be experimentally infected in-utero (Narayan *et al.*, 1974) by direct injection of the virus into the fetus, the lack of natural vertical transmission may be due to the cell-associated nature of the virus in the blood rather than a lack of fetal susceptibility (Carey and Dalziel, 1993). There is no evidence in the role of other excretory products, such as saliva and urine in the transmission of MVV (Brodie *et al.*, 1998).

Intra-peritoneal inoculation of withers with semen from MVV infected rams failed to infect them (Dawson, 1987). The result indicates that if venereal infection does occur, it does not appear to be a significant route of transmission. In a recent study (de la Concha-Bermejillo *et al.*, 1996), MVV was detected in the ejaculate of rams with concurrent *Brucella ovis*-induced epididymitis. Large numbers of white blood cells, including macrophages, were present in these fluids suggesting that these cells harbor the virus. There are no reports of successful detection of MVV genome in ova embryos, or purified spermatocytes. In addition, there is no evidence to suggest that wild animal species act as a reservoir for MVV (Cutlip *et al.*, 1991).

2.2.2. Clinical and pathological features

The extraordinary long incubation period for MVV noted during the initial outbreaks as of one to three years or more is followed by the onset of disease symptoms with a generally progressive clinical course. Because of the long silent preclinical phase of the infection, clinical signs are rarely seen in animals less than 3-4 years of age. Sheep may exhibit signs of disease in the second year of infection and die after a protracted and progressive illness (Haase, 1986). Progressive emaciation, in spite of a normal appetite and temperature, is usually the earliest symptom recognized (Cutlip *et al.*, 1988).

Maedi-Visna virus causes multi-systemic disease: the lungs and regional lymph nodes, joints, mammary glands, spleen and blood vessels are affected by the disease, and any or all of these organs may be involved in a single sheep (Cutlip *et al.*, 1988; Desrosiers, 2001). Histopathologically the lesions are characterized by their inflammatory nature, mononuclear cell infiltration and lympho-proliferation. Persistent production of viral antigen by infected

macrophages may then cause chronic stimulation of the immune system, resulting in lymphocytic hyperplasia (Cutlip *et al.*, 1988).

Previously, the disease has been known by pulmonary and neurologic disease process. Indurative mastitis and non-suppurative arthritis are now recognized as components of the MV disease complex. Vasculitis some times affects the small arteries and arterioles of the kidneys and may also be associated with lesions of the lungs, joints, and central nervous system (Cutlip *et al.*, 1988; Brodie *et al.*, 1998).

Lymphoproliferative lesions in the testicles of rams seropositive for MVV have been described although there was no attempt to isolate virus (Palfi *et al.*, 1989). Recently, it was shown that rams with B*rucella ovis*-induced epididymitis shed MVV in their semen associated with infected white blood cells (de la Concha-Bermejillo *et al.*, 1996). Ram epidedimitis is relatively common and thus may contribute to the venereal spread of MVV (Brodie *et al.*, 1998).

The clinical and pathological effects of MVV on other organs have not been reported. No studies investigated MVV as an agent of abortion or have definitively shown the virus to be a cause of a reproductive failure, although it is known that the virus can be transmitted in utero but at a frequency of less than 10% (Brodie *et al.*, 1994).

2.2.2.1. Respiratory tract involvement

The most common clinical manifestation of MVV infection of sheep is a chronic pneumonia with signs of progressive respiratory failure. When sheep are driven, the affected animals lag behind, they exhibit labored breathing and after exertion, the respiration becomes rapid and shallow. These sheep are often referred to as "lungers". As the severity of respiratory disease progresses, the respiration at rest becomes gradually more difficult with dilated nostril and flank breathing aided by accessory respiratory muscles accompanied by characteristic rhythmic jerks of the head. In advanced cases the sheep loose condition and become emaciated and often recumbent (Cutlip *et al.*, 1986). Coughing is not a prominent sign and not productive when it occurs. Death is inevitable once symptoms are recognized and may occur within 6 months to one year. Secondary bacterial pneumonia is a common sequela to the respiratory form of MVV and the cause of death (Cutlip *et al.*, 1986; Brodie *et al.*, 1998).

At necropsy, the lungs of sheep with chronic MVV infection are consolidated, 2-3 times heavier than the normal weight; they have a firm rubbery consistency, lack normal crepitations. The lungs fail to collapse when the thoracic cavity is opened, and show multiple gray areas on the pleural surface. In cases with secondary bacterial infection, coalescent cranioventral areas of consolidation are observed. The tracheobronchial and mediastinal lymph nodes are found enlarged (Georgsson and Palsson, 1971).

Histologically there is hyperplasia of the fibrous tissue and smooth muscle of the alveolar septae along with inflammatory mononuclear cell infiltration and accumulation of plasma cells, lymphocytes and macrophages. Lymphoid aggregates frequently with germinal centers often surround airways and blood vessels and are prominent in sheep with MVV associated interstitial pneumonia (Georgsson and Palsson, 1971).

In certain flocks, MVV is found as a co-infection with Jaagsiekte retrovirus, the causative agent of sheep pulmonary adenomatosis (Payne *et al.*, 1986). There have been tentative suggestions of *in vivo* synergism between the two retroviruses based on accelerated development of lesions when both viruses are present (DeMartini *et al.*, 1987).

2.2.2.2. Udder involvement

Maedi-Visna virus can also infect the udder resulting in a chronic, indurative mastitis. This involvement becomes apparent as an enlarged firm udder that has reduced milk flow to no milk flow. This is often referred to as "hard bag". Milk production is decreased because of infiltration of lymphocytes and macrophages into the surrounding milk ducts where milk is produced. The changes found with udder involvement during the course of MVV infection are different from changes associated with mastitis. The udder is not hot or painful, nor are there abnormal milk secretions as are found in the case of mastitis with bacterial infection. The main difference is that the changes associated with udder involvement in MVV are generally irreversible, while the other causes may be reversible (Van der Molen *et al.*, 1985).

Large numbers of MVV-infected macrophages can be found in association with lymphocyte infiltrates. Multiple breaks occur in the acinar and ductal epithelium that may be the source of infected cells, which transmit virus to lambs (Cutlip *et al.*, 1988). Blockage of teat ducts during lactation may be associated with failure to properly nurse lambs. Poor lamb growth

prior to weaning was found to occur in flocks with a high incidence of antibody to MVV and concurrent indurative mastitis (Van der Molen *et al.*, 1985).

2.2.2.3. Arthritis

Arthritis has been observed in MV infected sheep, involving swelling and calcification of soft tissue, fibrosis of the joint capsule and synovium and perivascular lymphocytic infiltration (Oliver *et al.*, 1981), which is similar to arthritis observed in CAEV infected goats (Crowford *et al.*, 1980). Infection can result in lameness and painful swelling of one or several joints especially apparent with the "knee" joint of the forelimb and the hock joints of the rear limbs, the carpus and tarsus. Maedi-Visna virus was isolated from joints in a group of naturally infected sheep and reproduced disease when introduced into naive animals by intra-articular injection (Oliver *et al.*, 1981).

Affected joints are non-suppurative with fibrin deposition and the proliferated membranes are hyperemic and infiltrated with lymphocytes and macrophages. Severe erosion of articular cartilage and subchondral bone and periarticular tissues can also occur in chronic lesions. Synovial fluids from animals with clinical arthritis often contain infectious virus, viral antigens, and or high titer anti-viral antibody to MVV surface proteins: particularly the membrane proteins that may further exacerbate joint lesions (Brodie *et al.*, 1995b).

2.2.2.4. Central nervous system involvement

Central nervous system involvement is the least frequent form of MVV in sheep. In animals with neurological infection, gait is affected and early signs are muscle quivering and imbalance seen in the rear legs, with paresis of the hindquarters progressing until paraplegia develops (Cutlip *et al.*, 1988).

At necropsy, the brain and spinal cord usually appear normal. Histologically, central nervous system lesions consist of varying degrees of infiltration of the meninges, choroid plexus, white matter of the brain and spinal cord with lymphocytes (Oliver *et al.*, 1981; Brodie *et al.*, 1995b). Lymphocytes and macrophages are accumulated around blood vessels, and degeneration of nervous tissue is seen too. Infectious virus, viral antigens, and anti-viral antibody to MVV are relatively common in cerebrospinal fluid of sheep in association with subclinical MVV-associated encephalomyelitis (Brodie *et al.*, 1995b). In contrast to European

strains, central nervous system disorders associated with infection by North American strains of ovine lentiviruses are infrequent and almost always subclinical (Brodie *et al.*, 1998).

2.3. Virus-Host Interactions

2.3.1. Pathogenesis

Cells of monocytes /macrophage lineage have been shown to be infected in all lentivirus infections of animals and man (Narayan and Clements, 1989). In addition other cell types have been found to express virus antigens by immunohistochemical methods. They include lymphocytes, plasma cells, endothelial cells, fibroblasts, pericytes choroidal epithelial cells and meningeal cells in MVV infection of sheep (Georgsson *et al.*, 1989).

Strains of MVV vary in their pathogenecity *in vivo* and *in vitro* (Carey and Dalziel, 1993). Both highly lytic and persistent (*in vitro)* strains were isolated from naturally infected sheep (Querat *et al.,* 1984). Intra-tracheal injection of low passage (ten passes) or high passage virus into lambs, with killing at various time points up to 28 weeks, resulted in the development of histological and clinical lesions only on those animals infected with low passage virus (Lairmore *et al.*, 1986).

The predominantly inflammatory nature of the lesions in MVV-induced disease suggested that the disease mechanism could be immunopathological in nature. Inflammatory lesions result in part from interactions between viral antigens and mechanisms of the host immune response. Since few cells in the lesions express viral antigens as revealed by immunostaining (Georgsson *et al.*, 1989), it appears that tissue damage may be in large part due to amplification of the immune response to viral antigens with a great influx of macrophages and lymphocytes and secretions of cytokines and other soluble factors resulting in non-specific tissue damage (Petursson *et al.*, 1992).

A number of studies have attempted to characterize the cellular filtrates, which are a feature of MVV infection in the lung. The basic lesions are characterized by dense infiltrates of lymphocytes, plasma cells and macrophages. Interferons have been identified and may be involved in inducing lymphocyte infiltration and proliferation (Narayan *et al.*, 1985; Lairmore *et al.*, 1988). Infected macrophages synthesize interferon and other cytokines to which T and B-lyphocytes respond by accumulating and/or proliferating in target tissues.

The severe immunodeficiency with associated opportunistic infections and tumors characteristic of human AIDS is not seen in small ruminant lentivirus infections. However, there are disturbances of immune functions associated with MVV infection attributed to viral infection of macrophages rather than to a depletion of CD4 positive lymphocytes (Bird *et al.*, 1990)

Persistence of infection within the host is fundamental to the pathogenesis of MVV-induced diseases and is accomplished by infection of a small percentage of cells of monocytes-macrophage lineage and the subsequent restriction of virus replication within these cells (Clements and Gabuzda, 1990). The proviral DNA genome of the virus may integrate into the host DNA of the macrophage stem cells in the bone marrow and the virus gene expression occurs only during maturational processes of the cells. This results a constant supply of virus infected cells from a niche safe from defense mechanism of the host but also provides a mechanism for macrophages to leave the bone marrow carrying latent viral genomes to various tissues (Narayan, 1990).

The mechanism of lentivirus persistence is through the formation of antigenic variants of the virus during the course of infection (Clements *et al.*, 1988). Mutations in the *env* gene are compatible with survival of the virus but they cause structural and conformational changes in the envelope of the particle. Frequently, these changes affect neutralization sites on the virus and allow the agent to escape from antibodies directed against the parental virus. These antibody-escape mutants are the basis of antigenic drift by lentiviruses (Clements *et al.*, 1988).

The occurrence of antigenic variation on MVV has been well documented (Clements *et al.*, 1988). Studies of genomic changes associated with antigenic variation indicate that variants are a consequence of genomic instability resulting from high rates of mutations, insertions and deletions, and recombination between different viral strains. Although the significance of antigenic variation and its contribution to virus persistence and pathogenesis remains obscure and it is likely to be a major constraint in developing MVV vaccines (Brodie *et al.*, 1998).

Experimental evidence indicates that although there is a long delay between initial infection and the development of disease, the majority of lentivirus-infected individuals go through an acute phase of virus replication during the first few weeks after primary infection. This phase

of acute viraemia is followed by a vigorous immune response that restricts virus replication to low levels but fails to eliminate the virus completely (Clements and Zink, 1996).

In sheep experimentally infected with MVV cell associated viraemia peaks between 2 and 8 weeks following infection and gradually declines to a low level by 16 weeks. However, MVV persists in cells of macrophage lineage of infected animals despite the presence of neutralizing antibodies and cell mediated immune responses (de la Concha-Bermejillo *et al.*, 1995b).

2.3.2. Immune responses

In experimental and natural infections with MVV of sheep, antibodies specific for the major viral antigen develops. The lentivirus persists in an infected animal despite an active immune response to the virus. Both humoral and cell mediated immune responses occurring in MVV infected animal appear to be incapable of clearing the virus in the early stage of infection and may contribute to the disease process and there by permitting establishment of infection in the host tissues (Carey and Dalziel, 1993). The majority of infected sheep respond to infection by the production of viral specific antibodies (Brodie, *et al.*, 1998)

The length of time from infection to seroconversion is unpredictable, and may take several months to years before detectable antibody can be assayed (Petursson and Hoff-Jorgensen, 1990), this reflects the low level of antigen typically presented to the immune system in lentivirus infections (Brodie *et al.*, 1998).

It is not clear whether antibodies to gp135 are protective, or may have a deleterious immunopathological effect. In CAEV, the titres of anti-gp135 in the synovia correlated directly with disease severity (Knowles *et al.*, 1990). In studies of lambs experimentally infected with MVV strains of different *in vitro* replication characteristics, the mean titre to gp135 was three times higher in lambs, which developed lymphoid interstitial pneumonia (Kajikawa *et al.*, 1990).

Fewer data are available on cell-mediated response to MVV infection. An early cell- mediated immune response to MVV has been demonstrated one week following experimental intracerebral inoculation (Griffin *et al.*, 1978) and 4-6 weeks following respiratory infection (Sihvonen, 1981). A proliferative response to virions and involving $CD4^+$ and $CD8^+$ cells, has been consistently demonstrated 1-4 years post-infection (Reyburn *et al.*, 1992).

2.4. Detection of infected animals

Although clinical symptoms may be highly suggestive and histopathological lesions practically diagnostic in typical or advanced cases of lentivirus disease in sheep and goats, serological and virological methods are necessary to confirm the diagnosis, especially in early phases of the infection and in animals which are infected but show little or no pathological changes.

2.4.1. Serology

Several serological assays have been used to identify sheep exposed to MVV, although currently there is no single recommended test. Early detection of MVV infection in sheep may be challenging. The accuracy of diagnosis largely depends on sensitivity and specificity of the assay used. It can also be influenced by the duration of infection, levels of viraemia, integrity of the host immune system and virus phenotype (virulence factors and propensity for antigenic variation) (Lairemore *et al.*, 1988; Brodie *et al.*, 1993)

Of the serological tests, the AGID and the ELISA tests are most suitable for practical application (Dawson *et al.*, 1982). The AGID test was one of the first successful serological assays developed for the detection of specific antibody to MVV (Marcom *et al.*, 1991). The AGID test is relatively inexpensive, commercially available, easy to perform, and commonly used. However, recent studies suggest that the AGID test is of limited value in detecting infected animals at early stages of infection when plasma antibody concentrations are low (Simard and Briscoe, 1990b).

The Western blot and ELISA (Kajikawa *et al.*, 1990; Simard and Briscoe, 1990a) have more recently been used to diagnose MVV infection. The Western blot is helpful in confirming doubtful results in the other tests (Zanoni *et al.*, 1990).

Simard and Briscoe, (1990b) compared indirect-ELISA with AGID for the detection of the antibodies to MVV, and the ELISA detected antibody at 2.6 weeks prior to the AGID. The ELISA had a specificity of at least 98.8% and an increased relative sensitivity of 15.5% compared to the AGID (the relative sensitivity of the ELISA was 94.7% compared to 79.2% for AGID). Hence, it was concluded that the AGID test might not be adequate to monitor serum samples for an eradication scheme (Simard and Briscoe, 1990b).

Brodie *et al.*, (1993) has also demonstrated ELISA to be significantly more sensitive in detecting antibody to MVV when compared with AGID, but less sensitive than immunoblotting. However, most animals in their study were older and had pathologic evidence of chronic infection (Brodie *et al.*, 1993).

In contrast, de la Concha-Bermejillo, (1995a) has shown that when comparing AGID with two recombinant ELISAs, the specificity of AGID was always 100%, with the sensitivity ranging from 11% two weeks following infection, and 100% five weeks after infection and remained constant until the end of the experiment. In this study, the specificity and sensitivity of the recombinant ELISA tests varied depending on the recombinant MVV protein used. The sensitivity and specificity of a recombinant ELISA based on a MVV major core protein varied from 22% to 100% (average 88%) and 50% to 100% (average 95%), respectively. It was concluded that because most ELISA tests only detect IgG antibody, animals recently infected and tested by ELISA might have false negative results and IgM responses would be missed (Juste *et al.*, 1995). Therefore, a combination of serological tests should perhaps be used for evaluating antibody production to MVV and when these tests give conflicting results, the serological status of individual animals can be confirmed by immunoblot (Brodie *et al.*, 1998). In addition to ELISA, AGID and immunoblot, the indirect immunoflourescence, haemaglutination, complement fixation and neutralization assays have been used for the diagnosis of MVV and other lentiviruses (Desrosiers, 2001).

Some MVV infected sheep are less likely to be detected with the ELISA because of fluctuating antibody levels (Simard and Briscoe, 1990b). Houwers and Nauta, (1989) have also reported a decline of antibody activity over time using immunoblot. In such cases, the MVV infected seronegative animals especially ewes might continue to propagate the infection since the detection of the antibody in their progeny is often quite long and the percentage of infectivity within the flock might continue to increase (Houwers and DeBoer, 1984).

2.4.2. Virology

The animals seropositive for MVV remain infected for life but infection of individual animals may be corroborated by virus isolation. Virus isolation from autopsy material (spleen, lymph nodes, CNS, joints, lungs) can be used to confirm the diagnosis. Isolation could be done by culturing clinical specimens like peripheral blood cells, lung lavage cells and other tissues collected from a suspect animal with an indicator cell line. In the living animal virus may be

isolated from buffy coat cells by cocultivation with permissive cells such as choroid plexus cells. Attempts to isolate virus from the buffy coat are not always positive in infected animals so negative results are not reliable (Petursson *et al.*, 1992).

In permissive cells *in vitro*, the MVV cause extensive cytopathic effect (CPE) with formation of multinucleated giant cells and release free infectious virus into the culture medium. Virus isolation may take several weeks, making the technique time-consuming and expensive (Carey and Dalziel, 1993; Brodie *et al.*, 1998).

New analytical methods based on immunological and molecular techniques have been developed. These techniques are based on the detection of viral nucleic acid or viral proteins and can circumvent the need for virus isolation in culture (Brodie *et al.*, 1995b). Amplification of viral DNA sequences using the Polymerase chain reaction (PCR) has been used as a research and diagnostic tool for detection of lentivirus infection (Brodie *et al.*, 1995b). Using PCR, MVV DNA could be detected at one-day post infection in cultured sheep choroid plexus cells. This was 4 days before viral proteins could be demonstrated by immunoblot (Zanoni *et al.*, 1990). PCR has recently been used as a diagnostic tool to demonstrate specific MVV DNA in clinical specimens from naturally infected sheep and experimentally infected cell culture. In these studies, viral DNA was best demonstrated in cell culture lysates with which MVV-infected cells or tissues had been cultivated for at least several days (Brodie *et al.*, 1998).

2.5. Prevention and Control

2.5.1. Test and removal

Disease could be controlled through drug use, changes in animal management, and vaccination. In the case of MVV infection several compounds, including phosphonoformate, 2', 3'-dideoxynucleosides, and interferon alpha have been shown to inhibit MVV replication *in vitro* (Beaussoleil *et al.*, 1989). In addition, recombinant ovine interferon tau, a non-toxic Type I interferon has been shown to have *in vitro* and *in vivo* antiviral activity against MVV (Juste *et al.*, 2000). Because of costs and the need for continued therapy, the method is not practical and economical.

One of the practical and effective means of controlling the spread of MVV and associated diseases has been through test and slaughter or quarantine policies. Eradication of MV was first accomplished successfully in Iceland in about 20 years (1944-1965) before reliable diagnostic tests were available. Over 650,000 MV infected and exposed sheep were slaughtered, and farms repopulated with unexposed sheep (Cutlip *et al.*, 1988). Today Iceland remains the only country where a lentiviral infection has been totally eradicated.

After serological tests became available, some countries have eradicated MVV from sheep flocks by annual or semi-annual bleeding of all sheep in the farms, testing serologically for antibodies and culling of all seropositive animals and their progeny of less than one year of age (Cutlip *et al.*, 1988). Such practices require extensive, repeated serological testing and restrictions of movement of animals between flocks (Houwers *et al.*, 1990). Infection was eradicated in 6 years from a closed flock and was controlled in an open flock by annual AGID testing and culling of seropositive sheep (Cutlip and Lehmkuhl, 1986). On the basis of this method, Voluntary control programs have been established in some European countries and have achieved considerable success (Houwers *et al.*, 1987).

2.5.2. Isolation and artificial rearing of progeny

Attempts to clean up infected flock by removal of lambs at birth and raising them artificially in isolation from the flock either on pasteurized milk or milk substitute have been successful in some countries (Houwers *et al.*, 1987; Cutlip *et al.*, 1988). Wiliams-Fulton and Simard (1989) had evaluated two management procedures for eradicating MVV from a single Canadian sheep flock. In the first management program, all seropositive ewes and lambs were removed from the flock. The prevalence of infected sheep decreased gradually and a seronegative flock was obtained after 30 months of monitoring. In the second program, lambs were removed from seropositive ewes before taking colostrum. Antibodies to MVV were not demonstrated in this flock. The results indicated that MVV could be successfully eliminated from a flock by either of the methods. The second program is more effective because of the early establishment of a MVV seronegative flock (Wiliams-Fulton and Simard, 1989).

Methods of elimination of infection from infected flocks are mostly difficult, expensive and time consuming. It is therefore of great importance to prevent introduction of infection into lentivirus-free populations. There are numerous examples of introduction of lentiviruses into free countries invariably associated with importation of infected animals. In order to prevent

import controls are necessary. Serological testing of individual animals is not enough; the whole flock of origin must be tested repeatedly; since animals in early stages of infection may be serologically negative (Petursson *et al.*, 1992).

2.5.3. Vaccines

The unusual character of lentiviruses makes the development of conventional vaccines for MVV and other lentiviruses a major challenge (Pearson *et al.*, 1989). Vaccines have not been successful in controlling the infection, although the vaccinated sheep produced precipitating antibodies against the virus, but were not protected against infection when challenged with live MVV (*Cutlip et al.*, 1988). Attempts to prevent infection by using formalin-inactivated lentivirus vaccines have failed (Cutlip *et al.,* 1987), and in some cases have resulted in exacerbated signs of disease in immunized animals (McGuire *et al.*, 1986).

Since the discovery that the agent causing AIDS in humans is a lentivirus, intensive research has been directed towards the identification of protective immunogens and new approaches for vaccine development, which could improve prospects for a MVV vaccine (Perk *et al.*, 1996). Commercial availability of a subunit vaccine for feline leukemia and development of genetically engineered virus-vectored vaccines for bovine leukemia, both retrovirus-induced diseases, give some cause for optimism (Brodie *et al.*, 1998). A preliminary work in using genetically engineered ovine lentivirus that contains the green fluorescent protein gene (GFP) attenuated for pathogenecity indicated effective protection against MVV (Zhang *et al.*, 2000) .

The only lentivirus for which vaccine has been so far used in the field is equine infectious anemia virus. The vaccine is a live attenuated strain developed by researchers in China by repeated passage in donkey cells. The vaccine has been extensively used in China and Cuba for horses with apparent safety and efficiency (Desrosiers, 2001).

2.6. Economic Importance of Maedi-Visna

The economic importance of MV and whether control efforts are financially justifiable are much debated issues. The economic consequences of MVV infection for sheep breeding vary depending on several factors, the virus strain, the animal breed, husbandry and management (Petursson *et al.*, 1992). The most dramatic example of direct losses due to MVV infection

comes from Iceland where the importation of silent carrier sheep in 1933 resulted in severe economic losses (Carey and Dalziel, 1993). The economic importance of the MV disease rests with losses associated with decreased longevity, mortality with clinical disease, decreased value of cull animals and possible effects of subclinical infection on productivity (Radostits *et al.*, 2000).

The major economic loss associated with infection rests with the effects of subclinical infection of breeding ewes in some flocks associated with a reduction in conception rates, and lowered birth weights and reduced growth rates in lambs associated with changes in the udder of the ewe and probably from lowered milk intake (Dohoo *et al.*, 1987). It was estimated that MVV infection of Dutch sheep caused 10-20% animal losses (De Boer *et al.*, 1979) and Dohoo *et al.* (1987) estimated 33% reduction in conception rate and 3-6% reduction in birth weight of lambs due to MVV infection of sheep.

Maedi-Visna has been considered as a major cause of ovine pneumonia and able to predispose to other respiratory infections. A Canadian study over a 4-year period identified MVV as the primary causative agent in 26% of sheep with pneumonia (Dohoo *et al.*, 1985). The incidence and importance of MVV in mixed or concurrent infections is unknown. Loss of trade due to international export restrictions of breeding sheep from a MVV infected country has got considerable effects (Houwers *et al.*, 1987).

The economic consequences of lentiviral infection of sheep are difficult to evaluate in exact financial terms. They may be predicted to become of increasing concern with the apparent tendency towards intensification of the sheep industry and further information from well controlled studies of the effects of MVV infection on sheep productivity (Petursson *et al.*, 1992).

2.7. Importance of Maedi-Visna in Ethiopian Sheep

The occurrence of MVV in Ethiopia was serologically detected for the first time in 1986 in imported sheep breed, Merino (BoA, 2000). In Bale province, there had been a serious respiratory disease problem with high mortality on the Merino sheep at Agarfa sheep ranch in 1983 and animals tested for MVV using AGID both in the country and abroad (UK) resulted 30% seropositivity. Due to the disease and other management problems, the flocks were de-stocked, which resulted in total closure of the ranch. Even if the cause was not confirmed,

similar clinical symptoms with high mortality were observed at Debre Berhan sheep ranch in that same year (BoA, 2000).

Maedi-Visna virus was also reported in indigenous Menz and Horro sheep breeds of the village farms and International Livestock Research Institute (ILRI) research station at Debre Berhan in 1996 (Ayelet *et al.*, 2001). Serological test using AGID indicated a prevalence rate of 3.7%. High prevalence was observed in sheep flock of ILRI Debre Berhan (5.4%) and 1.4% on village sheep farms (Ayelet *et al.*, 2001).

In a recent investigation of respiratory disease outbreak, alarmingly high mortality rate was observed in Amed Guya sheep ranch, North Shewa. A histopathological examination of lung tissues from morbid sheep revealed a characteristic MV lesion with a high degree of mononuclear cell infiltrations (Tibbo *et al.*, 2001). In similar year, serum samples collected from culled and diseased animals from both Debre Berhan and Amed Guya sheep ranches were serologically tested and 74% were seropositive for MVV. The infection was detected in all breeds of sheep examined (Awassi, Hampshire, Corriedale, indigenous Menz breeds and their crosses) with a significant breed difference varying from 48% in Awassi sheep to 92% in the indigenous Menz sheep (Woldemeskel *et al.*, 2001).

Because of the emergence of MVV and continuous build up of respiratory disease cases in sheep at ranches and to minimize the risk of contaminating the smallholder farm flocks, a decision has been made for a complete de-stocking of sheep from both ranches and to repopulate with MV free sheep through repeated serological tests (BoA, 2001). Based on the decision, with the exception of little pedigree Awassi sheep, all local and crossbred sheep (about 6000) from both ranches were slaughtered in the year 2002/3.

There is a strong assumption that the MVV might have been introduced to Ethiopia when exotic sheep breeds were imported from abroad (Table 1) with out testing for the disease. All sheep source countries were known with MV disease (Brodie *et al.*, 1998).

Table 1. Exotic sheep breeds introduced to Ethiopia

Sheep breed	Source country	Year of introduction
Merino	Italy	1944
Romney	Kenya	1968
Corriediale	Kenya	1968
Hampshire	Kenya	1968
Ramboyle	USA	1968
Awassi	Israel	1980, 1985,1994

Source: BoA (2001)

For the last 20 years, more than 5000 crossbred rams had been distributed from sheep breeding ranches to sheep producing areas of the country. Those distributed rams might have been possible carriers to spread the disease as well. However, the extent and distribution of the disease in the Ethiopian indigenous sheep in smallholders in different parts of the country is not established and demands further investigation.

3. MATERIALS AND METHODS

3.1. Description of the Study Area and Livestock Population

The study was conducted in selected sheep rearing highland *weredas* and sheep breeding and research centers in the ANRS (Fig.1). MVV was diagnosed in the Amhara region recently during respiratory disease outbreak investigations in sheep ranches and has been considered as a major sheep health concern in the region. Six administrative *weredas* (districts); Meket and Wadila (North Wollo), Dessie Zuria and Legambo (South Wollo), Gera Keya and Basona-worana (North Shewa) were selected for the study. The sheep breeding and research centers available in the region; Debre Berhan, Amed Guya, Sheno (North Shewa) and Guguftu private sheep farm (North Wollo) were also included in the study.

The altitude of the study area ranges from 2500 to 3100 meters above sea level with annual rainfall ranging between 800-1000mm with bimodal rainfall in pattern: a long rainy season that extends from June to September, and a short rainy season from February to May. Average monthly minimum air temperature ranges 2.4^{0}C in November to 8.5^{0}C in August where as the average monthly maximum air temperature ranges from 18.3^{0}C to 23.3^{0}C in June. Mean relative humidity is 68^{0}C. Frost usually occurs between the months of October and January.

In the ANRS, the livestock comprises of cattle, sheep, goats, equines and camels raised as integrated part of the crop agriculture. Backyard poultry and apiculture are also practiced and have significant contribution in supplementing the smallholders' subsistence economy. Sheep and equines are dominant species in the highlands, while cattle, goats and camels are mainly reared in the mid to lowland areas of the region. The livestock and poultry population in the region is estimated at 10.5 million cattle, 5.3 million sheep, 3.8 million goat, 1.88 million equines, 18,186 camels and 13.4 million chickens (CACC, 2003). The major livestock production system in the region is mixed crop-livestock farming.

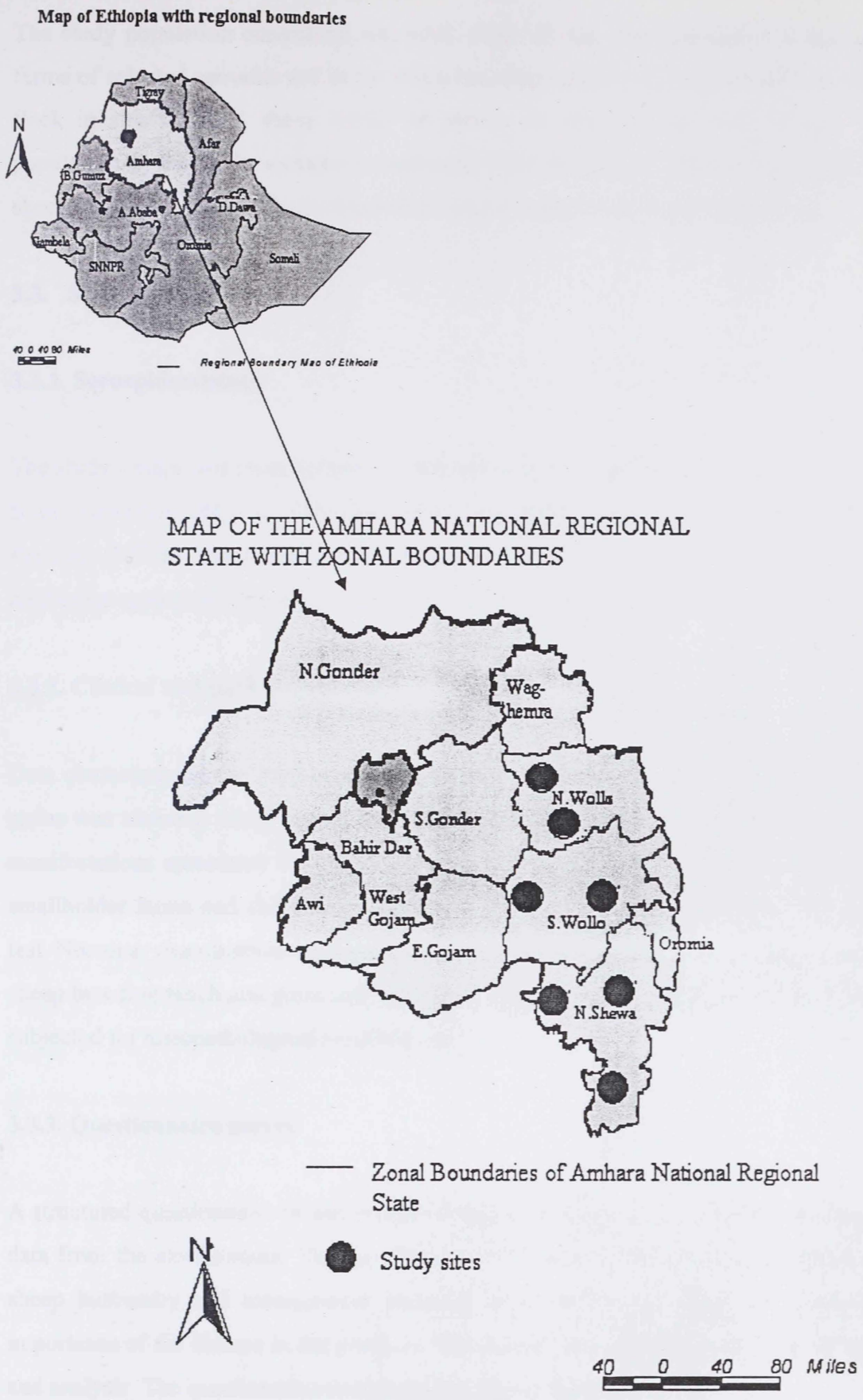


Fig.1. Map of the Amhara National Regional State and specific Maedi-Visna study sites

3.2. Study Population

The study population considered was adult sheep of one year and above in the smallholder farms of selected *weredas* and in the sheep breeding ranches. It was assumed that 65% of the flock in smallholders' sheep would be greater or equal to one year of age. The sheep population in the study *weredas* is estimated 976,049 (CACC, 2003). The number of adult sheep in ranches was determined based on animal records and found to be 4630.

3.3. Study Design

3.3.1. Seroepidemiology

The study design was cross-sectional. Data and sera were collected from the study population from November 2003 to February 2004. Serological tests; indirect-ELISA (Simard and Briscoe, (1990b) and AGID (OIE, 1996) were used to determine the presence of specific antibodies against MVV and to estimate the seroprevalence in the study population.

3.3.2. Clinical and pathological study

Data characterizing the host; age, sex, and breed description of the animal and the clinical status was recorded during serum collection. Clinical assessment was done based on clinical manifestations associated with MVV infection. The assessment was made in sheep both in smallholder farms and sheep breeding ranches during sera sample collection for serological test. Necropsy examinations were made on seropositive adult sheep culled from Debre Berhan sheep breeding ranch and gross pathological findings were recorded and tissue collected were subjected for histopathological examination.

3.3.3. Questionnaire survey

A structured questionnaire format was developed and administered with the aim of collecting data from the stockowners. The questionnaire was designed to generate information on the sheep husbandry and management practices, their knowledge about the disease and the importance of the disease in the producer. The format was coded for computer database entry and analysis. The questionnaire format used is shown in Annex 4.

3.4. Sample Size Determination

The sample size was determined using 95% levels of confidence and 7% estimated prevalence of MVV antibodies and with a desired absolute precision of 1%. The estimated prevalence was determined based on a previous report of MV (Ayelet *et al.*, 2001). Sampling size was calculated using the formula for random sampling (Thrusfield, 1995).

$$n = \left[\frac{tx\sqrt{p(1-p)}}{L}\right]^2$$

(Where, n = sample size, t_x = student t-value (1.96 at 95%), P = estimated prevalence of MV, L = desired absolute precision)

Then the required sample size (n = 2501) for prevalence estimate was obtained using the Win Episcope software 2.0 (2000).

3.5. Sampling Strategy

The study population was stratified into two strata based on the management types; sheep flocks in ranches and smallholder farms. The stratification was required because the study population is kept under different management types, which could influence the prevalence of MV antibodies to be estimated. Stratification with a variable sampling fraction was used and the proportional allocation was 20% for ranch sheep and 0.25% for smallholder farms.

Stratum 1. ***Sheep flocks on-station:*** the adult sheep population in ranches was 4630. The sampling frame was comprised of individual animals from farm records and animals were sampled by taking 20% random sample. Individual animals (n = 930) were sampled using systematic random sampling.

Stratum 2. ***Sheep flocks on smallholder farms:*** the adult sheep population in selected districts/weredas was estimated 634,432 and then, 0.25% of the adult population (n = 1580) was considered as sample size. The sample was proportionally distributed to randomly selected weredas (n = 6) and kebeles/localities. Adult animals in each kebele were sampled using systematic random sampling.

3.6. Sample Collection

3.6.1. Sera

Whole blood was collected aseptically from jugular vein of each sample animal using 10ml non-heparinized vacutainer tubes. The blood containing tubes were provided identification number. After centrifugation, the serum was decanted into another 5ml vial similarly identified. The collected sera were held under refrigeration until submission to the laboratory. Data including the owner's name, address, flock location, name of sheep/ear tag number, age of animal, sex, and breed description and clinical status of animal and vial number was recorded at the time of blood sampling.

3.6.2. Tissue specimens

Tissue samples were collected form slaughtered seropositive adult sheep (n = 12) during necropsy examination. The post mortem examination was conducted on seropositive animals that were culled from the ranch flocks and slaughtered in Debre Berhan. Tissue specimens having relevance for the detection of MV associated lesions were collected from lungs, mammary glands and brains, and preserved in 10% buffered formalin solution until submitted and processed in the laboratory.

3.7. Serological Tests

All serum samples were processed and tested by i-ELISA and AGID techniques using readily available kits. Both kits used were products of Pourquier Institute, France. The test procedures recommended by the manufacturer (Annex 1 and 2) were strictly followed in both tests for MVV antibody verification. Sera sample were processed and tested within 2 months period.

3.7.1. Indirect-ELISA

The ELISA method used for the detection of MV antibodies in this kit was an indirect ELISA based on the use of an immunogenic peptide of a trans-membrane protein (TM, ENV gene) and on the other hand, on the use of the recombinant P28 protein which enters into the composition of the viral capsid (*gag* gene). The appearance of anti-P28 antibodies can occur slightly later than that of the anti-viral envelope protein antibodies. The use of the much conserved viral capsid protein p28 allows the serological detection of a very wide spectrum of

serological variants; the use of the trans-membrane protein allows a premature detection of infection and improves the sensibility of the kit.

3.7.1.1. Enzyme Linked Immunosorbent Assay test protocol

- The wells of the polystyrene microplates were precoated with viral antigen (only the wells of even-numbered columns were coated with MV antigen).
- Sera samples to be tested were diluted and incubated in the wells for an hour. Antibodies specific to the antigen present in the serum form an antigen-antibodies immune-complex and remains bound in the wells.
- After a washing steps (three washes), a monoclonal anti-ovine IgG antibody coupled to peroxydase was added to incubate for half an hour. This conjugate binds to the immune complex.
- After a washing steps (three washes), the enzyme substrate (TMB) was added to the conjugate, forming a blue compound becoming yellow after blocking. The intensity of the color was a measure of the level of antibodies present in the serum sample.
- The cut-off/limit of positivity was set by using a positive control serum supplied with the kit, which must be added to each microplate.

3.7.1.2. Enzyme Linked Immunosorbent Assay plate lay out

The ELISA plate lay out for the distribution of the sample and control sera was used according to the instruction of the manufacturer (Fig.2). The position of controls in A1, A2 and B1, B2 was not important, they were dispensed any where on the plate and replicates of controls (one negative and one positive) were added in the middle of the same plate in order to establish an average OD (Optical Density) value.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**N**	**N**										
B	**P**	**P**										
C	1	1										
D	2	2										
E	3	3										
F	4	4										
G	5	5										
H	..	..										

Key: N = Negative control, P = Positive control, 1 = Sample no. 1, 2 = Sample no. 2, 3 = …

Fig.2. Enzyme Linked Immunosorbent Assay plate lay out for sera distribution

3.7.1.3. Enzyme Linked Immunosorbent Assay test validation and interpretation

The samples were tested in duplicate on antigen pre-coated polystyrene microplates with a total of 44 samples per plate. Each serum samples and control sera were diluted 1:20 in the ELISA buffer. Negative and positive reference sera were tested on each plate in replicate.

The optical density for each sample was read at 450nm after the photometer was blanked on air. The corrected OD 450 was calculated for each serum by subtracting the OD 450 value obtained from the uncoated well from the OD 450 value of the coated well. The test result was considered reliable when the positive control serum has a minimum uncorrected OD 450 value of 0.350 and a ratio between the corrected OD 450 value of the positive control and uncorrected OD 450 value of the negative control is greater than or equal to 3.5.

Formula for calculation of the ratio S/P for each sample;

$$S/P = \frac{\text{Corrected OD 450 value of the sample}}{\text{Corrected OD 450 value of the positive control}} \times 100$$

Each sample was considered seronegative with (S/P % less than or equal to 110), doubtful (S/P % between 110 and 120) or seropositive (S/P % greater than or equal to 120). Suspicious reactions were confirmed by a second test.

3.7.2. Agar Gel Immunodiffusion test

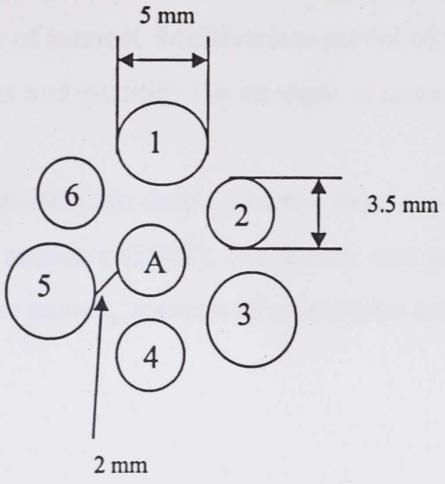
The AGID kit was used to detect antibodies to the MV viral envelop glycoprotein (gp 135) using the agar gel immuno-diffusion technique. The kit uses Ouchterlony method (also called double immuno-diffusin method) of precipitation on agar-gel.

3.7.2.1. Agar Gel Immunodiffusion test protocol

- The antigen was placed in the central well and the sera to be tested were placed in the wells around the antigen.
- Antigens and antibodies of the sera diffuse in the agar-gel. Their concentration decreases proportionally to the distance from the deposit.
- A zone of equivalence between gp135 antigens and antibodies in the serum tested was demonstrated by a continuous line between the control serum and the wells containing the serum tested.
- The reading was standardized by adding, from time to time, a positive limit and negative control sera in each series of test analysis.

3.7.2.2. Agar Gel Immunodiffusion test lay out

Ready-to-use agar-gel supplied with the kit was heated and poured (33ml) in 90x90mm Petri dishes at most one day earlier. A metal gel cutter of seven cylinders was used to cut seven wells of 5mm diameter for sample sera and 3.5mm diameter for control sera and antigen deposition (Fig 2).



Key: A = Antigen
1, 3 and 5 = sample sera
2, 4, and 6 = positive control sera

Fig.3. Agar Gel Immunodiffusion test lay out for serum and antigen distribution

The negative serum shows spontaneous inflection of the control precipitation line. Sera displaying a precipitin arc inflection greater or equal to that of obtained with the positive limit control serum was considered positive.

3.8. Data Storage and Management

Data collected during sampling, laboratory results and questionnaire data were entered and stored in a separate database in both MS-Access database and MS-Excel spread sheet. Data were screened for proper coding and errors were corrected prior to statistical analysis.

3.9. Data Analysis

Descriptive statistics such as mean, median, standard errors and confidence intervals of means were used to approximate the seroprevalence of MVV antibodies. Chi-square tests of independence and multiple logistic regressions were used to investigate differences and associations between serological test results and different locations, flock sizes, breed, age and sex categories of test animals. STATA computer software (Stata Corp. 2001) was used for analysis. Kappa statistics using Win Episcope software (2000) software was used for the analysis of test agreement and evaluation between the ELISA and AGID tests.

Factors of epidemiological relevance such as breed, flock sizes, age, crossbred ram distribution, contact between sheep in ranches and smallholders were considered as risk factors for comparison of test results (out come variables). Animals having the same risk

factor were measured to have some degree of dependency in contrast to those with out the factor of interest. Multivariate model of logistic regression was used to identify the actual risk factors and quantify the strength of associations with the individual serostatus.

A questionnaire data collected from stockowners was analyzed using descriptive statistics of Stata software (2001). Frequency and proportions were used for disease ranking, identifying disease causes, seasons of occurrence and syndromes.

4. RESULT

4.1. Seroprevalence of Maedi-Visna Virus Infection in Sheep Using ELISA

A total of 2510 serum samples were collected from selected study *weredas* and sheep breeding ranches. One hundred serum samples were not tested for limited test materials. A total of 2417 sera samples (1491 from smallholder farms and 926 from sheep ranches) were tested for the presence of serum antibodies against MVV infection. The sera were tested using established ELISA and AGID tests for the detection of antibodies to MVV. All the seroprevalence estimates presented in the paper are with reference to ELISA result.

The mean seroprevalence of MVV infection in the smallholder farms was 6.6% (95% CI = 5.4 - 7.9) and 30% (95% CI = 27.1 - 33.1) in the sheep breeding ranches as given in Table 2 and 3, respectively. There was a significant difference ($p < 0.001$) in seroprevalence between the two sheep management types.

The distribution of Maedi-Visna seroprevalence was given in Fig.5. Among different *weredas*, the seroprevalence for MV antibodies was higher in Gera Keya (15.4%) followed by 6.9% and 4.5% in Basona-Worana and Wadilla *weredas*, respectively. In sheep breeding ranches, the seroprevalence was much higher (87.4%) in Sheno Agricultural Research Centre than Amed Guya (20.5%) and Debre Berhan (20%) ($P < 0.001$).

Table 2. Seroprevalence of Maedi-Visna in smallholder farms (*weredas*) using Enzyme Linked Immunosorbent Assay

Zones	***Weredas***	**Sera**		**Sero-**	**95% Conf.**
		Tested	**Positive**	**prevalence (%)**	**Interval**
North Shewa	Gera Keya	364	56	15.4	11.8 - 19.5
	Basona-Worana	202	14	6.9	3.8 - 11.4
South Wollo	Dessie Zuria	240	6	2.5	0.9 - 5.4
	Legambo	181	4	2.2	0.6 - 5.6
North Wollo	Meket	259	7	2.7	1.1 - 5.5
	Wadilla	245	11	4.5	2.3 - 7.9
Total		**1491**	**98**	**6.6**	**5.4 - 7.9**

Table 3: Seroprevalence of Maedi-Visna in sheep ranches using Enzyme Linked Immunosorbent Assay

Sheep Breeding Ranches	Sera		Seroprevalence (%)	95% Conf. Interval
	No. Tested	No. Positive		
Debre Berhan	335	67	20.0	15.9 - 24.7
Amed Guya	375	77	20.5	16.6 - 24.9
Sheno ARC	151	132	87.4	81.1 - 92.3
Guguftu	65	2	3.1	0.4 - 10.7
Total	**926**	**278**	**30.0**	**27.08 - 33.09**

The seroprevalence distribution in smallholder farms varied among the three zones (Fig. 4). The prevalence was as high as 12.4% in North Shewa followed by North Wollo (3.6%) and South Wollo (2.4%). The seroprevalence difference, North Wollo and South Wollo compared to North Shewa was significant ($p < 0.001$), whereas the difference between North Wollo and South Wollo was not significant ($p > 0.05$).

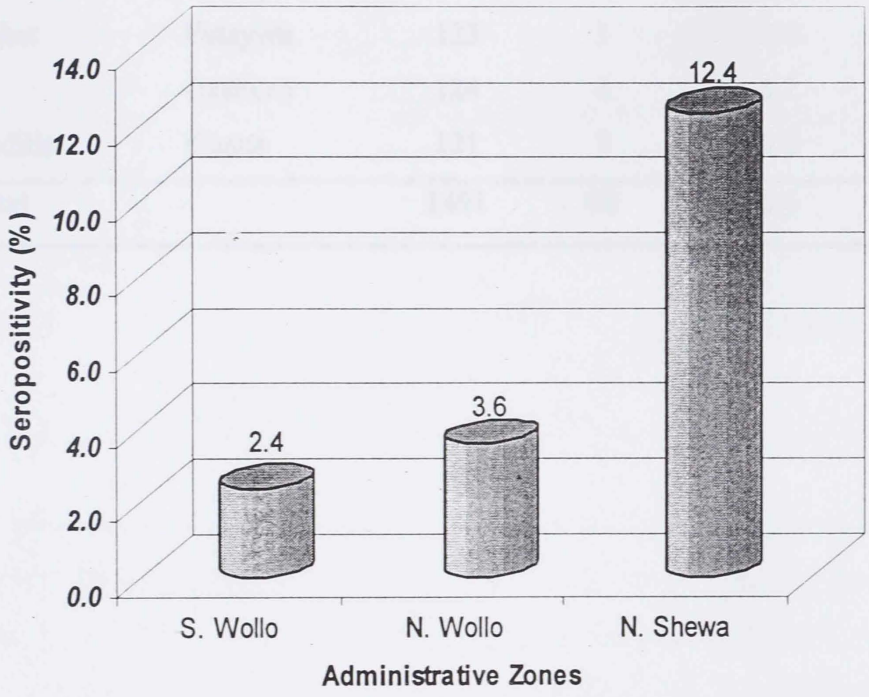


Fig.4. Zonal distribution of Maedi-Visna seroprevalence in smallholder farms

The seroprevalence of MV in sheep also varied among the different localities (*Kebeles*) ranging from 2.2% to 37.9%. Seroprevalence was higher in two localities of North Shewa namely Tsehaysina (37.9%), which borders the Amed Guya sheep ranch and Faji (12.1%), which surrounds the previous ILRI sheep research centre (Table 4).

Table 4. Seroprevalence of Maedi-Visna in smallholder farms at *kebele* level based on Enzyme-Linked Immunosorbent Assay result

Weredas	*Kebeles*	Sera		Seropreval-ence (%)	95% Confidence Interval
		No. Tested	No. Positive		
Basona-	Angolela	103	2	1.9	0.2 - 6.8
Worana	Faji	99	12	12.1	6.4 - 20.2
	Sarmidir	121	3	2.5	0.5 - 7.1
Gera- Keya	Sinamba	127	9	7.1	3.3 - 13.0
	Tsehaysina	116	44	37.9	29.1 - 47.4
Dessie- Zuria	Gelsha	119	2	1.7	0.2 - 5.9
	Gerado	121	4	3.3	0.9 - 8.3
Legambo	Chiro	91	2	2.2	0.3 - 7.7
	Segnogebiya	90	2	2.2	0.3 - 7.8
	Akat	136	4	2.9	0.8 - 7.4
Meket	Estayish	123	3	2.4	0.5 - 6.9
	Gashena	124	4	3.2	0.9 - 8.1
Wadilla	Kuana	121	7	5.8	2.4 - 11.6
Total		**1491**	**98**	**6.6**	**5.4 - 7.9**

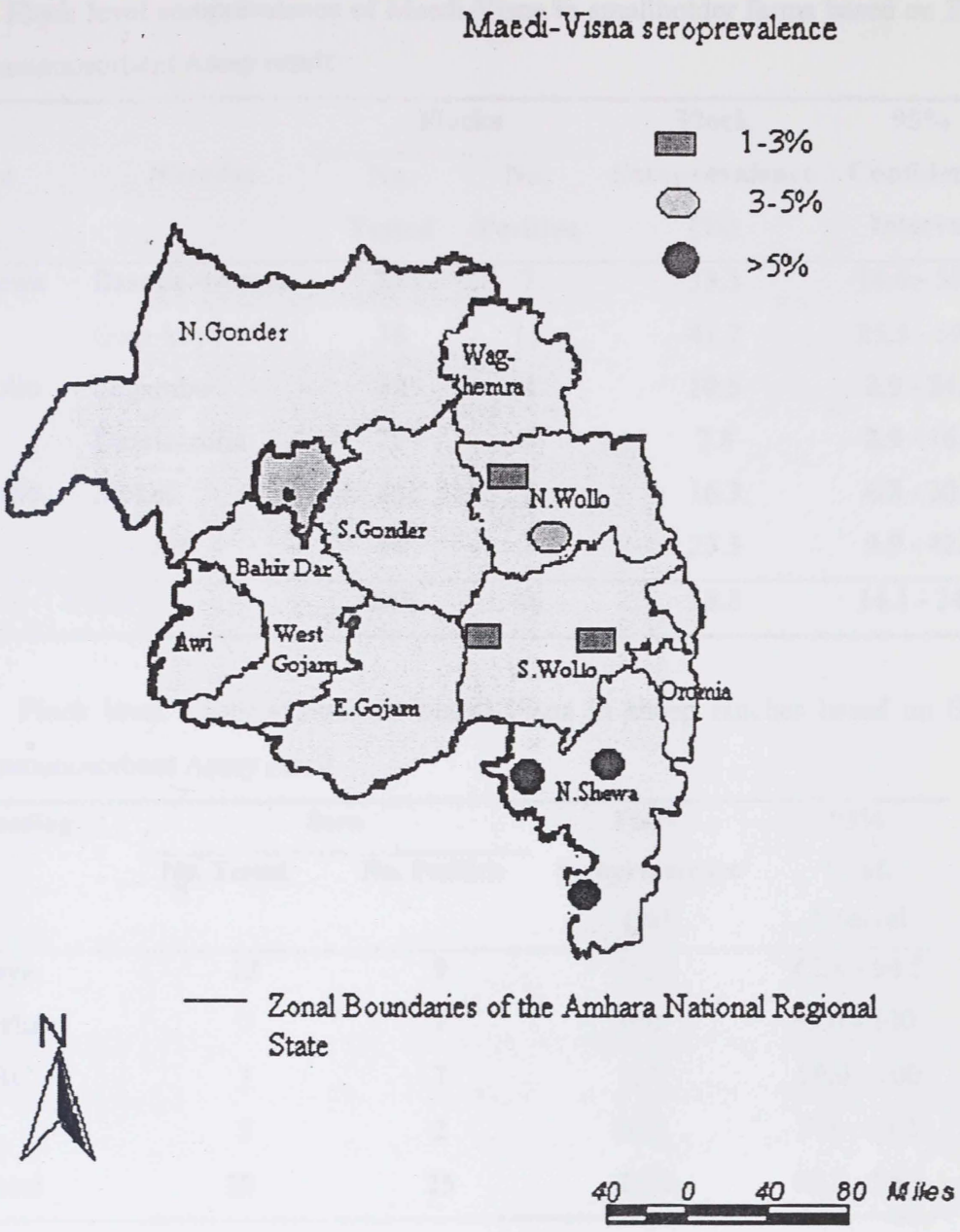


Fig.5. A map showing seroprevalence of Maedi-Visna in the Amhara National Regional State

The mean flock seroprevalence with at least one seropositive sheep in smallholder farms was 18.8% (95% CI = 14.1 – 24.2) and 86.2% (95% CI = 68.3 – 96.1) in sheep ranches as given in Table 5 and 6, respectively. In smallholder farms, the flock seropositivity varied considerably among the *weredas* and it was higher in Gera Keya (41.7%) followed by Basona-Worana (33.3%) while in ranches all flocks (100%) in Sheno and Debre Berhan had at least one sheep seropositive.

Table 5. Flock level seroprevalence of Maedi-Visna in smallholder farms based on Enzyme Linked Immunosorbent Assay result

Zones	*Weredas*	Flocks		Flock Seroprevalence (%)	95% Confidence Interval
		No. Tested	No. Positive		
North Shewa	Basona -worana	21	7	33.3	14.6 - 56.9
	Gera-keya	36	15	41.7	25.5 - 59.2
North Wollo	Legambo	38	4	10.5	2.9 - 24.8
	Dessie-zuria	77	6	7.8	2.9 - 16.2
North Wollo	Meket	43	7	16.3	6.8 - 30.7
	Wadilla	30	7	23.3	9.9 - 42.3
Total		**245**	**46**	**18.8**	**14.1 - 24.2**

Table 6. Flock level seroprevalence of Maedi-Visna in sheep ranches based on Enzyme Linked Immunosorbent Assay result

Sheep Breeding Ranches	Sera		Flock Seroprevalence (%)	95% Conf. Interval
	No. Tested	No. Positive		
Amed Guya	12	9	75.0	42.8 - 94.5
Debre Berhan	7	7	100	59.0 - 100
Sheno ARC	7	7	100	59.0 - 100
Guguftu	3	2	66.7	9.4 - 99.2
Total	**29**	**25**	**86.2**	**68.3 - 96.1**

4.2. Univariate Analysis of Risk Factors for Maedi-Visna Infection in sheep

Univariate analysis using logistic regression was conducted for assumed risk factors which have epidemiological importance. The effect of Breed, age, sex flock size, contact of sheep between smallholders and ranches and Awassi crossbred rams distribution to smallholder farms on the out come (seropositivity of Maedi-Visna) was analyzed and presented in Table 7.

Table 7: Univariate logistic regression estimates for risk factors of Maedi-Visna in sheep ranches and smallholder farms

Risk Factors	Odds Ratio	P-value	95% CI
Awassi breed Vs Indigenous	18.3	0.000	9.9-33.6
Awassi cross Vs Indigenous	7.9	0.000	5.6-11.3
Awassi cross Vs Awassi breed	0.4	0.000	0.2-0.8
Age category 3<6 Vs 1≤3	2.06	0.000	1.6-2.6
Age category ≥6 Vs 1≤3	7.8	0.000	5.3-11.6
Age category 3<6 Vs ≥6	0.3	0.000	0.2-0.4
Sex	1.9	0.000	1.4-2.5
Flock size 11-20 Vs 1-10	4.6	0.000	3.0-6.9
Flock size >20 Vs 1-10	9.6	0.000	6.4-14.3
Flock size 11-20 Vs >20	0.5	0.000	0.4-0.6
Contact with ranches	6.1	0.000	4.0-9.3
Awassi crossbred ram access	1.4	0.135	0.9-2.1

4.2.1. Breed

The seroprevalence among three breeds of sheep varied from 16% in Indigenous sheep to 77.6% in Awassi sheep (Table 8). The difference was significant ($p < 0.001$). Using logistic regression, the OR value for Awassi sheep was 18.3 (95% CI = 9.9 - 33.6) and 7.9 (95% CI = 5.6 - 11.3) for Awassi x Indigenous crosses as compared to the Indigenous sheep while the OR value for Awassi crosses as compared to Awassi breed was 0.4 (95% CI = 0.2 - 0.8).

Table 8. Seroprevalence of Maedi-Visna in different breeds of sheep in ranches in the Amhara National Regional State

Breed Type	Sera		Seroprevalence (%)	95% confidence Interval
	Tested	Positive		
Awassi sheep	67	52	77.6	65.8 - 86.9
Awassi x Indigenous crossbred	201	121	60.2	53.1 - 67.0
Indigenous sheep	658	105	16	13.2 - 19.0
Total	**926**	**278**	**30**	**27.1 - 33.1**

4.2.2. Age

Age-specific seroprevalence increased from 2.8% in yearlings to 76.5% in 7 years old sheep but decreased slightly in older animals (Table 9). The majority of seropositive sheep were in the range of 3-5 years old.

Table 9. Seroprevalence of Maedi-Visna in relation to age groups of sheep in the Amhara National Regional State

Age	Sera		Seroprevalence	95% Confidence
	Tested	Positive	(%)	Interval
1	36	1	2.8	0.1 - 14.5
2	443	49	11.1	8.3 - 14.4
3	868	88	10.1	8.2 - 12.3
4	580	83	14.3	11.6 -17.4
5	367	97	26.4	22.0 - 31.3
6	85	30	35.3	25.2 - 46.4
7	17	13	76.5	50.1 - 93.2
8	17	12	70.6	44.0 - 89.6
9	4	3	75.0	19.4 - 99.4
Total	**2417**	**376**	**15.6**	**14.1 - 17.1**

The age was categorized in 3 groups as given in Fig.6. The seroprevalence difference was significant ($p < 0.001$) among age categories ranging from 10.2% in age category 1≤3yrs to 47.2% in age category ≥6yrs. Logistic regression analysis for age and seropositivity provided an OR value of 2.06 (95% CI = 1.6-2.6) for category 3<6yrs compared to category 1≤3yrs and 7.8 (95% CI = 5.3-11.6) for age category ≥ 6yrs compared to age category 1≤3yrs. The OR value for age category 3<6yrs compared to age category ≥6yrs was 0.3 (95% CI = 0.2 - 0.4).

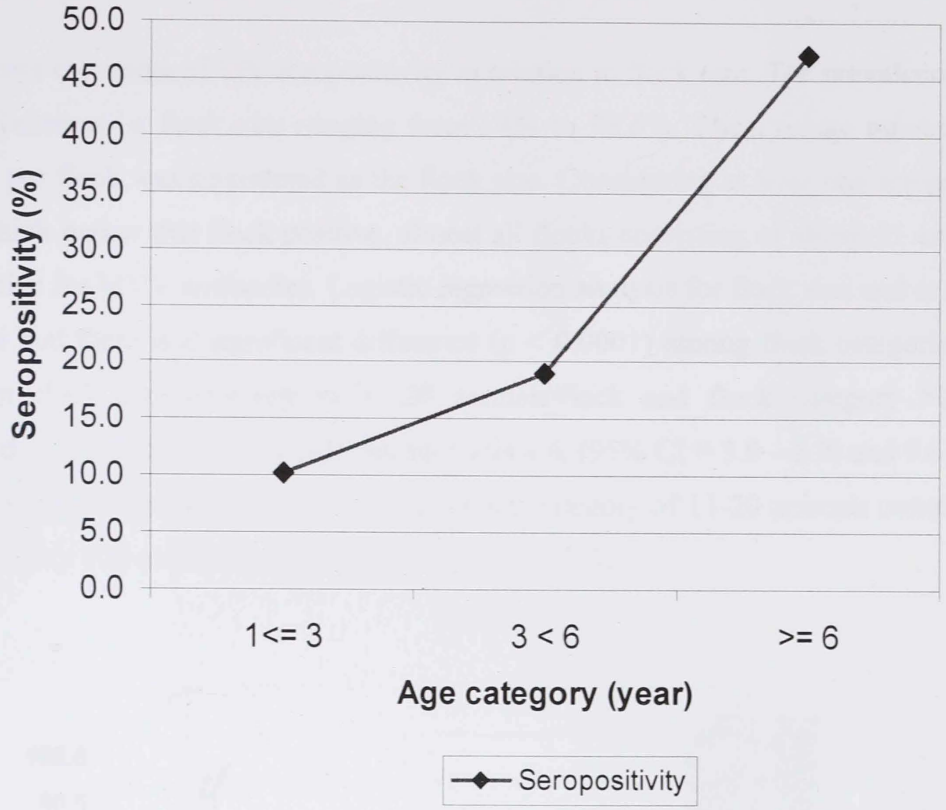


Fig.6. Seroprevalence of Maedi-Visna by age category of sheep in smallholders and ranches

4.2.3. Sex

A considerable difference was also observed between female (14.4%) and male (24.1%) in MV seropositivity (Table 10). However, the ratio of female to male sheep tested was 7:1. The difference was significant ($p < 0.001$) and the OR value was 1.4 (95% CI = 0.9 – 2.1).

Table 10. Sex related seroprevalence of Maedi-Visna in sheep

Sex	Sera Tested	Sera Positive	Seropositivity (%)	95% Confidence Interval
Female	2118	304	14.4	12.9 - 15.9
Male	299	72	24.1	19.3 - 29.3
Total	**2417**	**376**	**15.6**	**14.1 - 17.1**

4.2.4. Flock size

Fig.7 shows the status of MV seropositivity in relation to flock size. The prevalence increased with an increase in flock size ranging from 14% to 98.6%. The average number of sheep sampled per flock was considered as the flock size. Considering at least one seropositive in a flock, which makes that flock positive, almost all flocks consisting of above 21 animals were seropositive for MVV antibodies. Logistic regression analysis for flock size and seropositivity indicated that there is a significant difference ($p < 0.0001$) among flock categories. The OR value for flock size category of 11-20 animals/flock and flock category >21 animals compared to the flock category 1-10 animals was 4.6 (95% CI = 3.0 – 6.9) and 9.6 (95% CI = 6.4 - 14.3), respectively. The OR value for flock category of 11-20 animals compared to the flock category >21 animals was 0.5 (95% CI = 0.4 – 0.6).

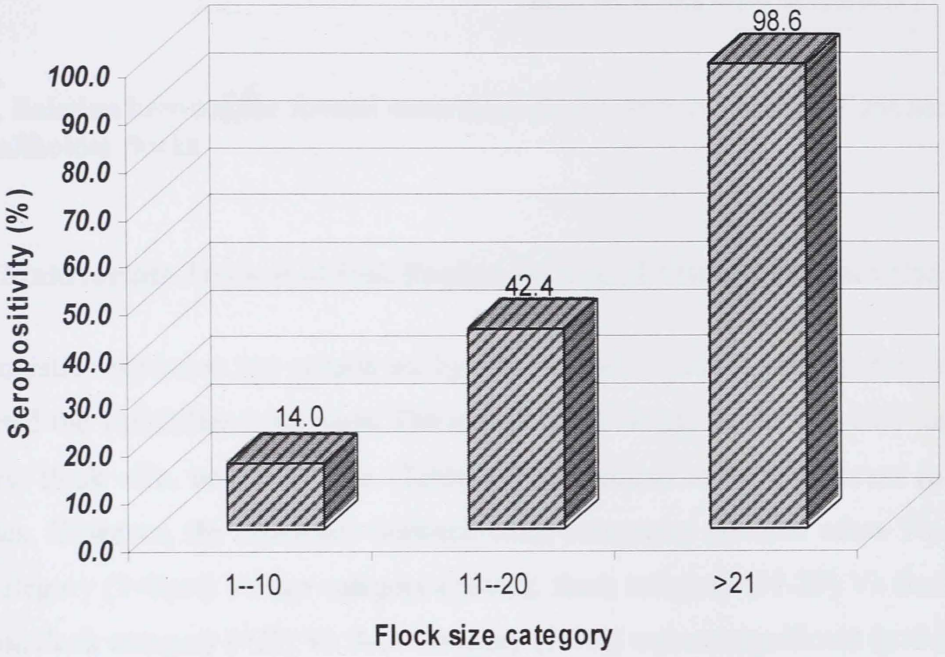


Fig.7. Seroprevalence of Maedi-Visna in relation to flock size

4.2.5. Awassi crossbred rams distribution

The number of Awassi x Menz cross rams distributed since 1990 to1999 from sheep breeding ranches and introduced to village flocks in the study *weredas* was compared along with the serology result in each study *wereda* (Fig 8). However, no statistical significant seroprevalence difference ($p < 0.05$) was observed.

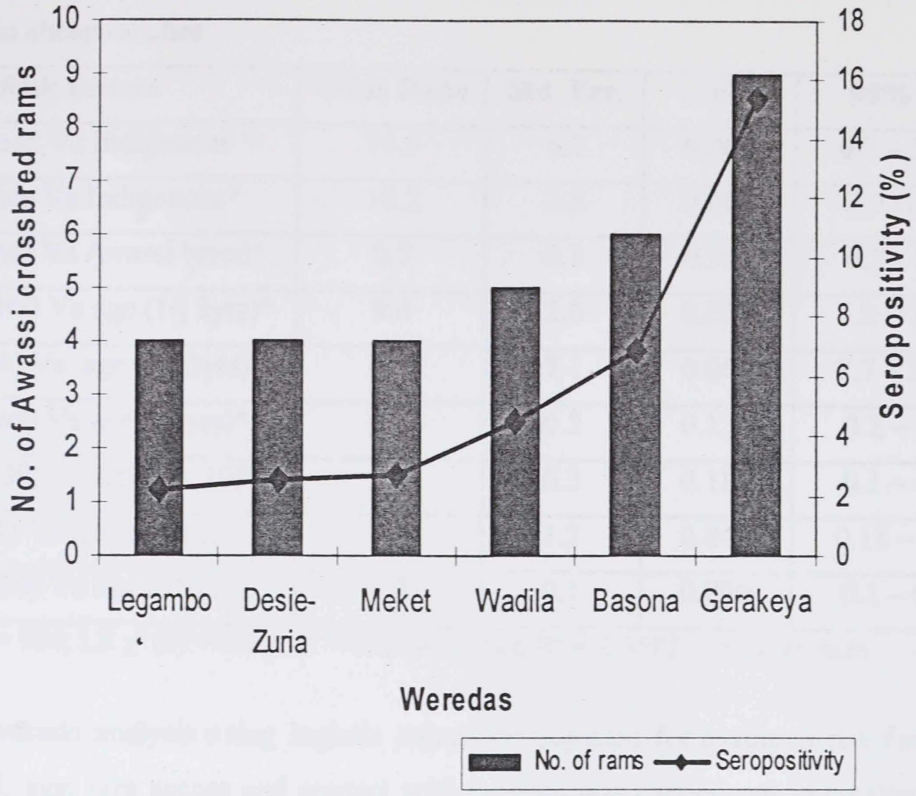


Fig.8. Relation between the Awassi cross rams distribution and Maedi-Visna seroprevalence in smallholder flocks

4.3. Multivariate Analysis of Risk Factors for Maedi-Visna Virus Infection

The logistic regression test criteria set by Stata software (2001) was used to fit a model that reflected the variability in the data. The overall multivariate model adjusted for assumed risk factors; flock size, breed and age (Table 11) was found to be significant ($p < 0.0001$) in ranches. However, the difference between some categories (Awassi cross Vs Awassi breed; age category (3<6yrs) Vs age category (≥6yrs); flock category (11-20) Vs flock category (1-10) and flock category (>21) Vs flock category (1-10)) was no significant ($p>0.05$).

Table 11. Multivariate logistic regression estimates for risk factors of Maedi-Visna virus infection in sheep ranches

Risk factors	Odds Ratio	Std. Err.	p	95% CI
Awassi breed Vs Indigenous *	14.9	6.7	0.000	6.2 – 35.8
Awassi cross Vs Indigenous*	10.2	2.8	0.000	5.9 -17.3
Awassi cross Vs Awassi breed*	0.7	0.3	0.362	0.3 – 1.6
Age (3<6yrs) Vs age (1≤ 3yrs)*	9.0	2.0	0.000	5.8 – 13.9
Age (≥6yrs) Vs age (1≤ 3yrs)*	17.3	7.1	0.000	7.7 – 38.8
Age (3<6yrs) Vs age (≥6yrs)*	0.5	0.2	0.138	0.2 – 1.2
Flock (11-20) Vs flock (1-10)*	0.3	0.3	0.180	0.1 – 1.8
Flock (>21) Vs flock (1-10)*	1.2	1.2	0.842	0.18 – 8.1
Flock (11-20) Vs flock (>21)*	0.2	0.1	0.000	0.1 – 0.4

No. of obs = 926; LR χ^2 (9) = 396.3; P = 0.0000; Pseudo R^2 = 0.3502 * References

The multivariate analysis using logistic regression adjusted for assumed risk factors; flock size, breed, age, ram access and contact with ranches was carried out in smallholder farms (Table 12). The overall model with assumed risk factors; flock size, age and contact was found to be statistically significant ($p < 0.05$) with the exception of some categories (flock category (11-20) Vs flock category (>21), age category (3<6yrs) and age category (≥6yrs) Vs age category (1≤ 3yrs) and could be considered as risk factors for the occurrence of the disease in the smallholder farms.

Table 12. Multivariate logistic regression estimates for risk factors of Maedi-Visna virus infection in smallholder farms

Risk factors	Odds Ratio	Std. Err.	p	95% C I
Flock (11-20) Vs flock (1-10)*	2.2	0.6	0.009	1.2 – 3.9
Flock (>21) Vs flock (1-10)*	2.5	1.2	0.046	1.0 – 6.1
Flock (11-20) Vs flock (>21)*	0.9	0.3	0.717	0.4 – 1.8
Age (3<6yrs) Vs age (1≤ 3yrs)*	1.6	0.4	0.052	1.0 – 2.4
Age (≥6yrs) Vs age (1≤ 3yrs)*	0.5	0.6	0.547	0.1 – 4.1
Age (3<6yrs) Vs age (>6yrs)*	2.9	3.0	0.303	0.4 – 21.7
Ram access	1.2	0.3	0.559	0.7 – 1.8
Contact to ranches	4.4	1.1	0.000	2.7 – 7.1

Number of obs = 1491; LR χ2 (8) = 84.53; Prob = 0.0000; Pseudo R^2 = 0.1169, * References

4.4. Comparison between ELISA and AGID Tests

Maedi-Visna seropositivity comparison between the ELISA and the AGID tests is given in Fig.9 and 10. In the ELISA test, positive results were found in 376(15.6%) samples, while positive results in the AGID test were 353 (14.6%). There were discordant results between the two tests. Fifty two samples which were identified as negative by AGID were positive by ELISA while 29 samples identified as negative by ELISA were positive by AGID (Table 10 and Annex 5). In smallholder farms more animals were positive with ELISA than in ranches. In this study the relative sensitivity and specificity was 91.8% and 97.5% for ELISA while 86.2% and 98.6% for AGID test, respectively.

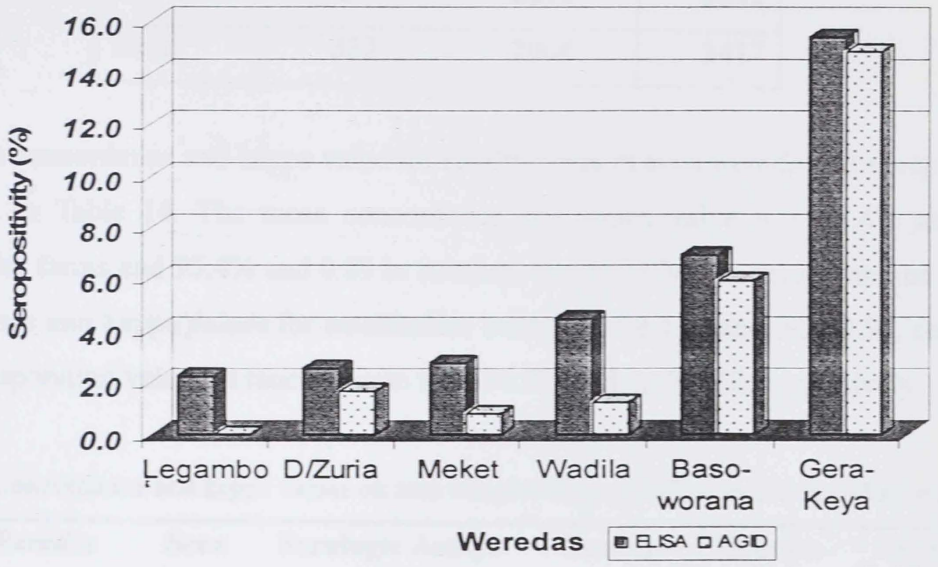


Fig.9. Maedi-Visna seropositivity comparison between ELISA and AGID tests in smallholder farms

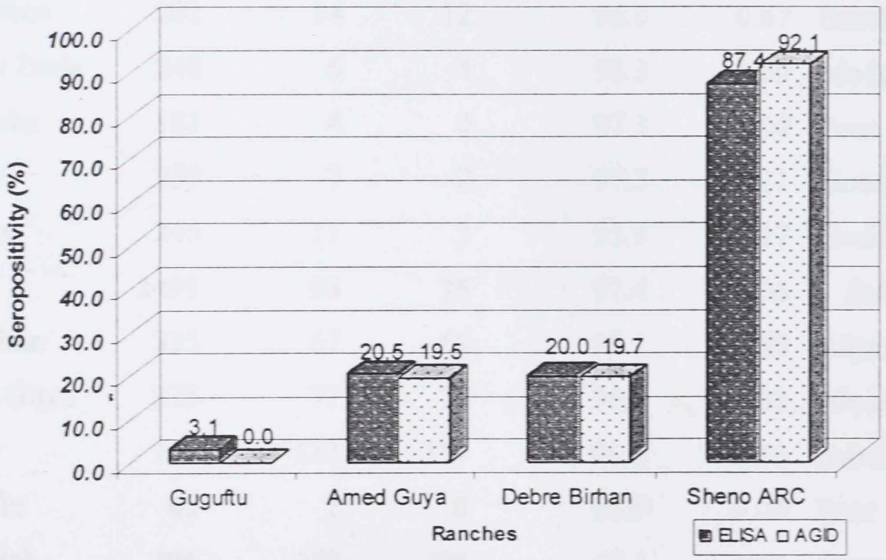


Fig.10. Maedi-Visna seropositivity comparison between ELISA and AGID tests in ranches

Test agreement for ELISA and AGID based on the crude agreement (concordance) and the agreement beyond chance (Kappa value) were performed on sera samples from different localities and ranches (Table 13). The mean concordance and kappa value for overall sera samples regardless of the origin, was found to be 96.6% and 0.87 (95% CI = 0.83 - 0.91), respectively. The statistical agreement between the AGID and ELISA tests was almost perfect.

Table 13. Test agreement between ELISA and AGID in Maedi-Visna diagnosis in sheep

		AGID		
		Positive	Negative	**Total**
ELISA	Positive	324	52	**376**
	Negative	29	2012	**2041**
	Total	**353**	**2064**	**2417**

The mean concordance and kappa value for smallholders in each *wereda* and sheep ranches is presented in Table 14. The mean concordance and kappa value was 97.4% and 0.76 in smallholder farms and 95.4% and 0.89 in ranches, respectively. The minimum and maximum concordance and kappa values for smallholder were 95.9-98.4 % and 0.00-0.94, respectively. The corresponding values in ranches were 91.4-96.9% and 0.00-0.88, respectively.

Table 14. Concordance and kappa values on sera samples from smallholder farms and sheep ranches

Zone	*Weredas*	Sera Tested	Serologic Assays		Concor-dance (%)	Kappa Value	Agreement
			ELISA	AGID			
North	Gera Keya	364	56	54	98.4	0.94	Almost perfect
Shewa	B/Worana	202	14	12	96.0	0.67	Substantial
South	Dessie Zuria	240	6	4	98.3	0.59	Moderate
Wollo	Legambo	181	4	0	97.3	0.00	Poor
North	Meket	259	7	2	97.3	0.21	Good
Wollo	Wadila	245	11	3	95.9	0.27	Good
Total		**1491**	**98**	**75**	**97.4**	**0.76**	**Substantial**
	D/Berhan	335	67	66	96.1	0.88	Almost perfect
Sheep	Amed Guya	375	77	73	96.3	0.88	Almost perfect
Ranches	Sheno	151	132	139	91.4	0.53	Substantial
	Guguftu	65	2	0	96.9	0.00	Poor
	Sub total	**926**	**278**	**278**	**95.4**	**0.89**	Almost perfect
Overall total		**2417**	**376**	**353**	**96.6**	**0.87**	Almost perfect

4.5. Clinical and Pathological Findings

4.5.1. Clinical evaluation

Forty one clinical cases were identified from smallholder flocks and ranches. Of 376 MVV seropositive sheep 8.3% had overt clinical disease and 0.4% of the clinical cases were seronegative (Table 15). The result indicated that there was statistically significant association ($\chi^2 = 88.3$; d.f = 1, $p < 0.001$) between the clinical disease and the ser status.

Table 15. Relationship between the clinical disease and Maedi-Visna seropositivity in sheep

Serological Result	Health status		Total	Percentage
	Apparently Healthy	Clinically Diseased		
Positive	348	31	376	8.3
Negative	2028	10	2041	0.4
Total	2376	41	2417	1.7

Among 31 seropositive animals with overt clinical disease, 30 (96.8%) were from sheep ranches and 3.2% from smallholder farms (Table 16).

Table 16. The proportion of clinically diseased sheep in smallholders and ranches

Management Type	No. of Flocks	No. of Clinical Cases	No. of Seropositive Animals	Proportion Exposed (%)
Smallholder farms	6	7	1	3.2
Sheep ranches	8	34	30	96.8
Total	**14**	**41**	**31**	**73.2**

The percentage of clinically diseased MVV seropositive animals increases with age ranging from 56.3% in animals less or equal to 4 years to 100% in above 6years old (Fig.11). However, the difference among age groups was not significant (P>0.05).

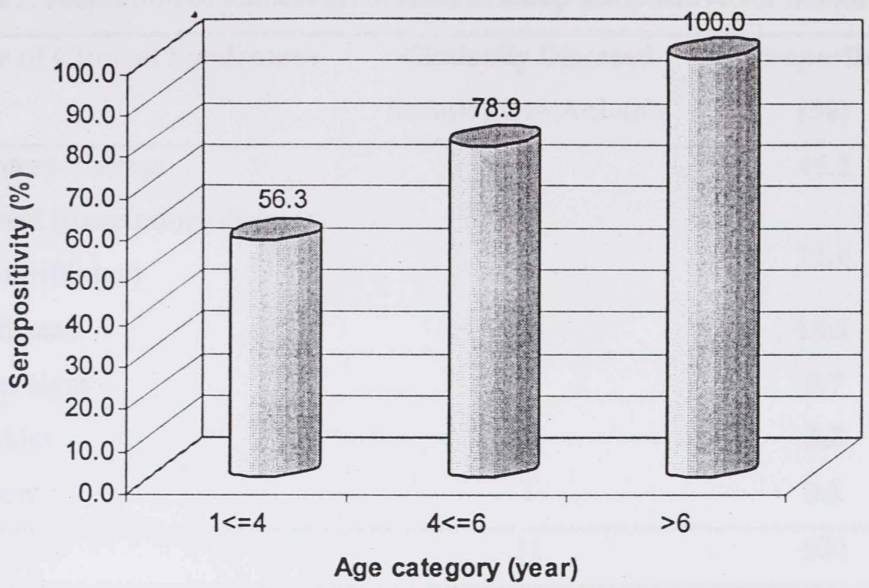


Fig.11. Age distribution of clinically diseased sheep in ranches and smallholder farms.

The percentage of clinical disease varied considerably among breeds (Fig.12), 61% in the Indigenous sheep followed by the Awassi x Indigenous crosses (36%) and Awassi (4%).

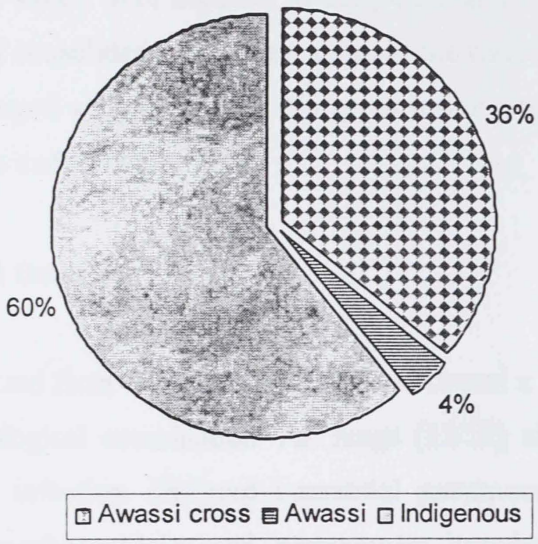


Fig.12. Clinical cases of Maedi-Visna related to breeds of sheep

The distribution of clinical signs in seropositive sheep is given in Table 17. Among the clinical signs observed, respiratory distress was dominant (45.2%) followed by combined signs of ill-thriftiness and respiratory distress (22.6%). Firm udder and lameness were rare events (one each).

Table 17. Proportion of clinical syndromes in sheep seropositive for Maedi-Visna

Type of Clinical Syndromes	Clinically Diseased Seropositive Animals	Proportion (%)
Respiratory distress	14	**45.2**
Combined (Respiratory distress and Ill-thriftiness)	7	**22.6**
Ill-thriftiness	5	**16.1**
Nervous signs	3	**9.7**
Firm udder	1	**3.2**
Lameness	1	**3.2**
Total	**31**	**100**

4.5.2. Gross pathological findings

Necropsy examination was made on seropositive Awassi x Menz crossbred sheep(n = 12) at Debre Berhan sheep ranch and the seroprevalence of source flocks was 82.4% and 84.6%. At postmortem examination, 11/12 animals (91.7%) showed gross lesions including heavy and enlarged lungs some of which were attached to the pleura and ribs and mottled by diffused gray and brown areas of consolidation. In some animals, the tracheobronchial and mediastinal lymph nodes were enlarged and edematous. No gross lesion was detected in the examined mammary glands, brains and joints.

4.5.3 Histopathological findings

Tissue specimens collected from sacrificed seropositive Awassi x Menz crosses (n =12) were subjected to histopathological examination. All lungs (12/12) showed microscopic lesions characteristic to MVV infection. Diffused interstitial pneumonia with multiple lymphoid follicles and marked peribronchiolar and perivascular lymphoid hyperplasia were seen through out the lungs (Fig.13 and 14). There was also hyperplasia of the smooth muscle of the respiratory bronchioles (Fig.14) and diffuse thickening of the alveolar septa by mononuclear cells (macrophages and lymphocytes). In some foci there was a connective tissue proliferation in the interstitium. In severe lymphoid infiltrations the combined peribronchiolar and perivascular changes have reduced and obliterated the alveoli.

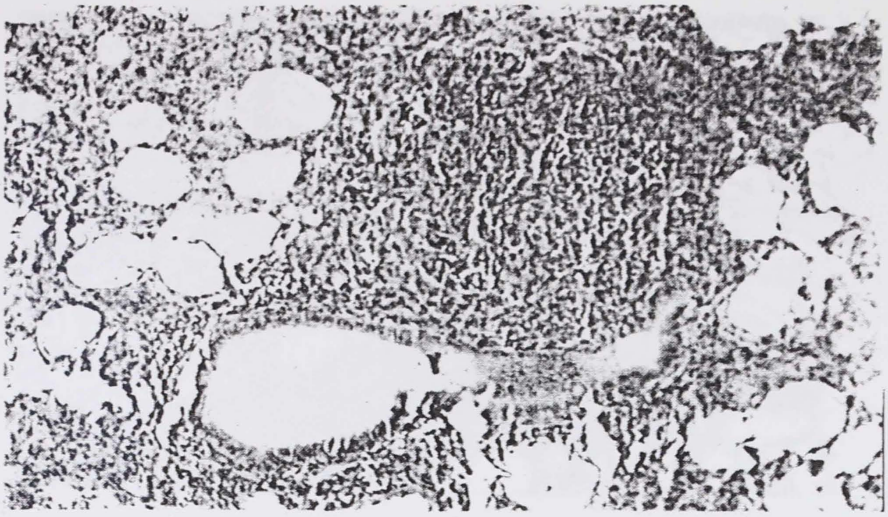


Fig.13. Lymphoid infiltration and hyperplasia in the lung of Maedi-Visna infected sheep

In one of the animals, there was perivascular and peribronchiolar cellular infiltration with some polymorphic nuclear and a high degree of mononuclear cells in the interstitium and in the lumen of the bronchioles and the alveoli (Fig.14) indicating presence of secondary complication forming Maedi-bronchopneumonia complex.

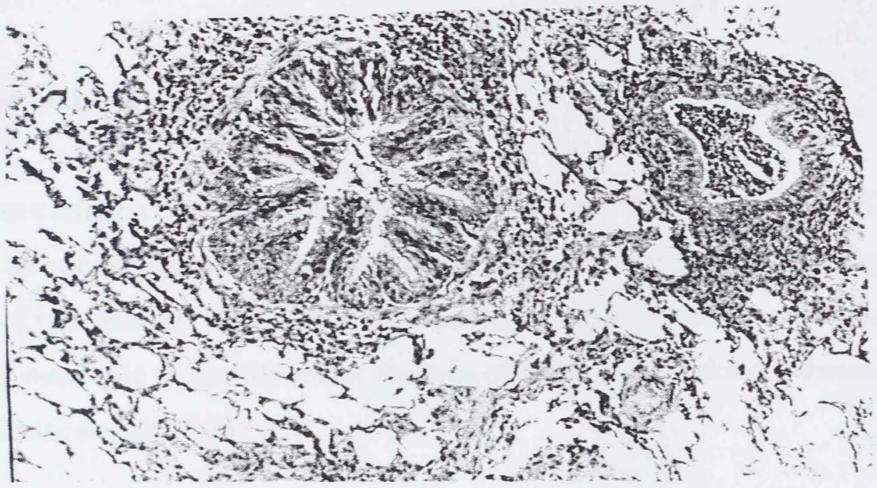


Fig.14. Peribronchiolar smooth muscle and lymphoid hyperplasia with polymorphic nuclear cells in the bronchiolar lumen

On a further case, there was interstitial mononuclear cell infiltration and alveoli filled with sections of adult and larvae parasitic worms (Fig.15) forming Maedi-verminous pneumonia complex.

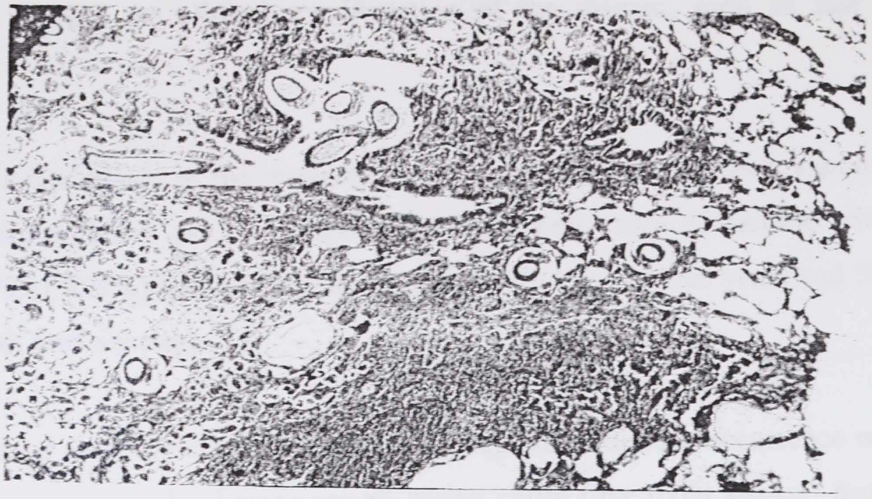


Fig.15. Interstitial mononuclear cell infiltration and lung worm sections in the lung

Tissues from mammary glands of non lactating ewes (n = 3) subjected for histopathology examinations had no microscopic lesion. From the brain samples of two animals, one showed necrotizing non-suppurative encephalitis. Necrotic changes showed loss of cell outline, kariolysis and mild gliosis and perivascular edema. Slight mononuclear cell infiltration was present, suspected of Visna infection.

4.6. Questionnaire Result

During serum sample collection a total of 65 stockowners were interviewed. Owners gave information on major sheep diseases, local names for respiratory diseases of sheep, causes and seasonality of the disease, frequent disease syndromes and animal recovery for treatments. Stockowners were able to identify major diseases of sheep and respiratory disease of different origin hold the first rank (Table 15).

Table 18. Rank for major sheep diseases according to their economic importance

Disease	Frequency	Rank
Respiratory	37	1
Gastro Internal Parasites	19	2
External parasites	9	3

Sheep owners in different localities use different local terms for respiratory diseases in sheep. The commonly used names are *Natew, Wozwuz, Engib* and *Gifaw*. All names have similar

meaning to express the clinical manifestation of the disease, such as fast and labored breathing.

Farmers associate causes of the respiratory disease mainly with the feed scarcity and drastic weather changes. 15/65 (23.1%) respondents associated cause of disease with cold/chilly weather, 20/65 (30.8%) with draught that occurs every two or three years and 30/65 (46.2%) associate with feed shortage.

The response of stockowners for season of the respiratory disease occurrence was divergent. 22/65(33.8%) stated as the disease occurs during chilly time (cold season) from October to January, 32/65 (49%) answered dry season (January to May) and 17.2% mentioned as the disease to occur any season of the year.

Owners were asked for clinical syndromes often occurred in their flocks. The response of 41.5% of the owners was respiratory signs. 37% of the respondents know both respiratory and nervous signs and 21.5% answered as the respiratory, nervous signs and lameness occurred in diseased sheep (Fig 16).

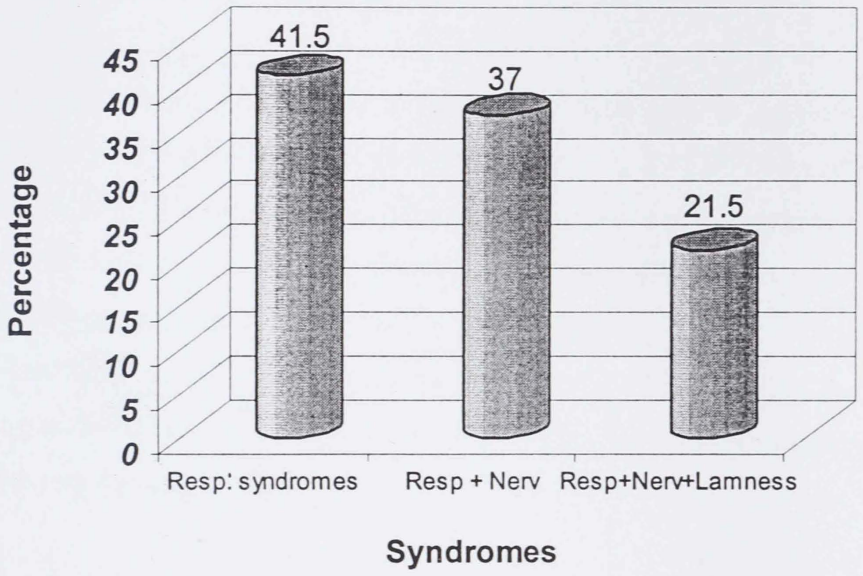


Fig.16. Proportion of respondents know disease syndromes in sheep

Concerning animal susceptibility difference to the respiratory disease, 15.4% owners answered as the disease affects young animals (<1year), 17% stated adults animals were affected and 67.6% answered both young and adult animals were equally affected. Regarding breeds, 38.5% of owners said that local animals were more affected and 13.8% witnessed

Awassi crosses were affected and 47.7% respond as there was no susceptibility difference between local and Awassi crossbreds.

Recovery rate of sheep after antibiotic and anthelmintic treatments for respiratory diseases was also asked. 87.7% of owners answered as treated animals do recover and 12.3% answered as animals usually die even after treatment.

5. DISCUSSION

5.1. Seroprevalence of Maedi-Visna in Smallholder Farms and Ranches

According to the serological results, the mean seroprevalence was found to be 6.6% in the smallholder farms and 30% in sheep breeding ranches with the flock seroprevalence estimate of 18.8% in *weredas* and 86.2% in ranches was indicative for the occurrence and wide distribution of MVV infection in both sheep management types in ANRS.

The seroprevalence difference between the ranches and smallholder farms could be associated with the management. The large flock size, confining animals for longer hours during cold seasons, mixing different breeds and age groups, keeping high proportion of older animals for longer years and other similar husbandry and management practices in ranches could have been considered conducive factors for the occurrence and transmission of MV in a higher rate. Baumgartner and others (1990) have also suggested unfavorable housing conditions such as insufficient room, bad climatic conditions and crowding behavior in some breeds of sheep promote a high incidence of the disease.

This seroprevalence findings in sheep ranches were not in agreement with the previous serological study in which 74% prevalence was reported (Woldemeskel *et al.*, 2002). The difference might be due to that the previous study was based on samples from morbid cases of sheep with high respiratory tract disease and could reflect a higher seropositivity than in the general sheep population. In addition to this, the primary infection flocks from Debre Berhan and Amd Guya ranches were removed a year before this study. The 6.6% seroprevalence finding in the smallholder farms is also higher than the previous report (3.7%) around Debre Berhan area (Ayelet *et al.*, 2001).

Among sheep ranches, there was statistically significant seroprevalence difference ($P<0.001$). The seropositivity was much higher in Sheno (87.4%) than Amed Guya (20.5%) and Debre Berhan (20%). The reason could be related to the removal of the primary infection flocks from Debre Berhan and Amed Guya and 90% of the flocks were substituted recently in the year 2002/3 by apparently free indigenous stock whereas the long years stayed sheep population in Sheno Agricultural Research Center (ShARC) has been kept undisturbed. The

lowest prevalence (3.1%) in Guguftu ranch may be related to frequent removal of sheep from the ranch and was replaced by younger sheep below 4 years.

The seropositivity difference among the different zones and *weredas* was found to be statistically significant ($p < 0.001$). The high seroprevalence (12.4%) in North Shewa zone and the respective *weredas*, Gera Keya (15.4%) and Basona-Worana (6.9%) might be the reflection of frequent contact or higher exposure to infected animals in sheep ranches (D/Berhan and Amed Guya) and research centers (ILRI and ShARC) located in these *weredas*. Sheep owners around ranches had frequent access to purchase rams and culled sheep from ranches. Besides loose fences of ranches allowed animal contacts between ranch and adjacent farms. This reality holds true for higher risk and pressure of MVV infection to flocks in Tsehaysina *kebele* (37.9% seropositivity) of Gera Keya *wereda*, which borders the Amed Guya ranch and Faji *kebele* (12.1%) that surrounds the previous ILRI sheep research centre that is also near to Dèbre Berhan sheep ranch.

The finding of 3.6% seroprevalence in North Wollo and 2.4% in South Wollo zone and their respective *weredas* is quiet interesting. These zones are geographically located far from severely affected sheep ranches and it is suggested that the disease might have spread along with the distribution of Awassi crossbred rams.

5.2. Risk factors for Maedi-Visna Virus Infection

The seroprevalence of MVV antibodies appeared to increase with increasing flock size ranging from 14% in flocks consisting of 1-10 animals/flock to 98.6% in flocks holding greater than 21 animals. Using logistic regression, the OR value for flock seropositivity indicated that the flock size of 11–20 animals was 4.6 times and flock size >21 animals was 9.6 times more likely to be seropositive than flock size of 1-10 animals. The OR value for flock size 11–20 animals was 0.5 times less compared to flock size (>21). The mean flock seroprevalence varied considerably between management types and found to be higher in ranches (82.6%) than smallholder farms (18.8%). This is in agreement with previous studies in Canada, the USA and some European countries. They have reported increased prevalence with increasing flock size and indicated an average flock seroprevalence of MV ranged from 19% to 97% (Kozaczynska *et al.*, 2002; Howers *et al.*, 1987; Constable *et al.*, 1996; Simard and Morley, 1991).

The larger flocks in sheep breeding ranches had been established over a longer period, thus permitting greater exposure of animals to horizontal transmission. The higher seroprevalence in ranches and some of the smallholder farms particularly in Gera Keya and Basonao-Worana *weredas* was partly a function of flock size. Scheer-czechowski and others (2000) indicated high risk of lactogenic transmission in severely affected flocks. In their study, lambs from seropositive ewes had 7.6 times higher risk to seroconvert within their first two years of life compared to those of seronegative ewes.

In this study, the breed related seroprevalence varied considerably from 16% in indigenous sheep to 77.6% in Awassi sheep. The difference was significant ($p < 0.001$). The OR value indicated that the Awassi sheep were 18.3 times and Awassi x Indigenous crosses 7.9 times more likely to be seropositive than the Indigenous sheep and Awassi crosses were 0.4 times less likely to be seropositive than the Awassi sheep. Differences in breed susceptibility to MVV infection was also indicated in previous studies (Houwers *et al.,* 1989; Snowder *et al.*, 1990). Schaller and others (2000) reported a prevalence difference varied between 0.4 and 36%. Simard and Morley (1991) found a prevalence that varied from 5.6% to 39.3% in Canadian sheep breeds. In contrast to this study, Woldemeskel and others (2002) found a significant difference among different breeds varying from 48% in Awassi to 92% in the Ethiopian Menz sheep in a study conducted on morbid cases.

According to Cutlip *et al.* (1988), all breeds of sheep are susceptible to MVV infection and the serological evidence of breed differences did not correlate with susceptibility. Houwers *et al.* (1989) found a difference in seroprevalence between Finnish landrace and Ile de France breeds of sheep where the latter remained negative. As the exposure was comparable, they concluded a breed associated difference in susceptibility to MVV infection, which may be related to the influence of traits of particular family lines, the strain of the virus and the result of one or more recessive genes. Nevertheless, complete breed associated resistance has not been demonstrated (Houwers *et al.,* 1990).

The age-specific seroprevalence finding was increased with the age of sheep. A significant seroprevalence difference among age groups was observed ($P<0.001$). The prevalence increased starting from 4 years and could probably be explained by the longer exposure of animals to horizontal transmission and the delay of seroconversion following infection. A decrease in prevalence in sheep older than 7 years could be explained by the losses through culling of morbid animals and deaths of sheep having clinical MVV following an infection

earlier in their life. The OR value for seropositivity among age categories indicated that age category 3<6yrs was 2.1 times and age category ≥6yrs was 7.8 times more likely to be seropositive than age category 1≤3yrs and age category 3<6yrs was 0.3 times less likely to be seropositive compared to age category ≥6yrs.

The age related seroprevalence finding in this study agrees with most of the studies in different countries that reported increased seroprevalence as age of animals increase. Cutlip *et al.* (1992) reported 4% prevalence at less than one year to 34% at 4 years but with variability associated with the breed type. Snowder and others (1990) determined the average seroprevalence to be 11% at one year of age and 93% in sheep 7 year and above. Kita *et al.* (1990) also reported prevalence of 7% at 1 year or less and 52% at 5 years older.

According to Houwers and others (1984), the period between exposure and the detection of the MVV antibodies varies with the route of infection, form of exposure and breed of sheep, and can vary from month to years. Although viral RNA can be detected from naturally infected animals less than 1 year of age by *in situ* hybridization, animals less than 1 year of age rarely show seropositivity when infected (Johnson *et al.*, 1992). The first appearance of antibodies following natural infection could range from 11 months to over 5 years and the majority of infected sheep show a seroresponse at 2-3 years of age (Houwers *et al.*, 1987).

Houwers and others (1989) found a statistically significant ewe to lamb relationship in the transmission of MVV. In their study seropositive dams had produced 36.6% positive progeny, where as the seronegative dams had 20%, indicating horizontal transmission. However, all age of sheep may apparently be infected by inhalation of droplets from the respiratory tract of infected individuals. De Boer *et al.* (1979) indicated that a contact of 10 hours between the infected dam and her progeny has a 28% probability of cross infection.

In this study, there was a statistically significant difference ($P<0.001$) in seroprevalence between sexes, higher in male (24.1%) than female sheep (14.4%). Similarly, Simard and Morley (1991) reported an association between sex and seropositivity. However, there is no other reported evidence of a difference in susceptibility to MVV infection between the sexes. The difference in this study could be related to the sheep management where large proportion of young female was recently introduced to the ranches.

The Awassi cross rams distributed from severely MVV infected sheep ranches to smallholder farms and the corresponding seroprevalence in each study *wereda* was not statistically significant ($p < 0.05$). However, the seropositively in smallholders flocks in *weredas* of North Wollo and North Shewa has increased with the number of rams distributed and may indicate the spread of MV diseases along with the distribution of rams.

The OR value of the seropositivity showed that the sheep in smallholder farms near ranches (Amed Guya and Debre Berhan) are 6.1 times more likely to be seropositive than those far away from the ranches.

Multivariate analysis using logistic regression adjusted for assumed risk factors; flock size, breed and age were found to be statistically significant ($p < 0.0001$) in ranches. The three independent variables (flock size, breed and age) in the logistic regression model were found to have significant effect on the out come (MV seropositivity) and can be assumed as risk factors for the occurrence and spread of the disease in flocks at ranches.

In the smallholder farms, the logistic regression model fitted with the assumed risk factors was significant ($p<0.0001$) for flock size, age, Awassi crossbred ram distribution and contact with ranches. Therefore, these factors could be considered as risk factors for the occurrence of the disease in the smallholder farms.

5.3. Comparison of Serological Tests

The concordance and kappa values were compared for ELISA and AGID tests for detecting MVV antibodies in sheep. The agreement found to be almost perfect. The concordance for various samples was found to be in the range of 95.9 - 98.4% in smallholder farms and 91.4 - 96.9% in ranches with a mean concordance of 96.6%. The kappa value was found in the range of 0.00-0.94 with mean value of 0.87. The indirect ELISA used in this study was 6.1% more sensitive than the AGID test. Although AGID has been widely used as a prescribed test for international sheep trade (OIE, 1996), it has limitations and various ELISA protocols have been described for routine diagnosis. Different works have been done comparing different ELISA formats for MVV with the conventional AGID and the ELISA test was found to be more sensitive than the AGID test. However, accurate serological diagnostics should be based on the combination of the tests (Kozaczynska *et al.*, 2002; Saman *et al.*, 1999).

5.4. Clinico-pathology

The investigated flocks for a variety of clinical problems (coughing, rapid and strained breathing, ill-thriftiness; uncoordinated gait, lameness and mammary indurations) were associated with high infection rate with MVV. There was statistically significant relationship ($P < 0.001$) between the clinical signs and the serological result. In this study, 85.3% of the clinical disease was observed in ranches. The finding of high percentage of clinical disease in ranches is in agreement with the observation made by Tibbo and others (2001) that reported 76% morbidity and 18% case fatality in adult sheep of Menz and Awassi crosses affected with respiratory disease complex.

The observed large number of seropositive sheep that develop overt clinical disease at ranches could probably be related to flock management where large proportion of animals are kept for longer years. According to Petursson *et al.* (1992), the incidence of clinical disease depends on how widespread the MVV infection is in a flock and it will take years to build up following the introduction of the infection. Sihvonen *et al.* (1999) also indicated a wide spread of MVV infection before clinical cases are detected when introduced into free areas.

The proportion of MVV infected sheep that developed clinical disease increased with age. This could be explained by the high level of MVV infection in flocks at ranches and chronic nature of the disease. Because of the long silent preclinical phase of the MVV infection, clinical signs are rarely seen in animals less than 3 years of age. According to Cutlip and others (1988), the percentage of infected sheep that eventually become clinically affected and the age of onset appear to be related to the level of infection in the flock. Baumgartner *et al.* (1990) also suggested unfavorable housing conditions; insufficient room, bad climatic conditions and breed specific behavior patterns (crowding of sheep) and high prevalence of MVV infection promote a high incidence of the clinical disease.

The clinical disease was significantly different among breeds ($P<0.05$). The percentage of clinical cases was higher in indigenous Menz sheep followed by the Awassi x Menz crosses. Although all breeds of sheep appear to be susceptible to MVV infection, the low resistance observed in indigenous sheep and their Awassi crosses may be explained by the duration of exposure to the disease because purchasing and introducing of new susceptible local sheep into longer years flocks at ranches is a frequent practice in ranches. Pedigree Awassi sheep in ranches were kept as parent stock for longer years for breeding purposes and seem relatively resistant to manifest the clinical disease. Cutlip *et al.* (1986) found experimentally infected

sheep of the Border Leicester breed to be significantly more susceptible to the signs and lesions of disease than were sheep of the Columbian breed. It was suggested that those findings might be related to differences in the pathogenicity of the strains of MVV and not solely a function of genotype of the sheep (Cutlip *et al.*, 1991).

Among the seropositive animals with overt clinical disease, the higher proportion was due to respiratory symptoms followed by combinations of ill-thriftiness and respiratory signs. Cutlip *et al.* (1988) reported a chronic pneumonia with signs of progressive respiratory failure and progressive emaciation as the most common clinical manifestation of MVV infection. Houwers and others (1983) also indicated increased number of progressively emaciated sheep and increased losses due to pneumonia were associated with the MVV infection.

Postmortem examination conducted in Awasi x Menz crosses revealed interstitial pneumonia, characteristic of MVV infection. Clinical and pathological findings suggested that MVV was the major cause of the respiratory disease in the ranches.

The intensity and distribution of typical lesions in organs were determined in sheep (n =12) with serological positivity to the MV disease. The peribronchial, perivascular mononuclear cell infiltration and lymphoid hyperplasia; prominent smooth muscle of the respiratory bronchioles, diffuse thickening of the alveolar septa due to mononuclear cells (macrophages and lymphocytes) indicated the progressive interstitial pneumonia characteristic of MVV infection. Georgsson and Palsson (1971) reported hyperplasia of the fibrous tissue and smooth muscle of the alveolar septa along with inflammatory mononuclear cell infiltration and accumulation of plasma cells, lymphocytes and macrophages. Prominent lymphoid aggregates with germinal centers often surrounding airways and blood vessels are associated with MVV interstitial pneumonia.

The findings of polymorphonuclear cells in lumens of air ways which is indicative of secondary bacterial complications and observations of some eosinophils together with lung worm infestations may be suggestive of the occurrence of concurrent infections. Bird and others (1990) indicated disturbances of immune functions of animals associated with MVV infection attributed to viral infection of macrophages and lymphocytes. However, severe immunodeficiency associated opportunistic infections characteristic of human AIDS is not common in small ruminant lentivirus infections.

The lungs were affected most frequently and brain to a lesser extent but none of the mammary glands were affected in this study. Although the brain lesion is not conclusive, severe lesions in the lungs and necrotizing encephalitis may indicate a virus strain with dual tropism. In general, those animals with clinical disease and gross lesions had histological pulmonary changes consistent with MVV infection. The high intensity of MVV associated lung lesions in Awassi and their crosses with lower expression of the clinical disease may indicate the relative resistance of the Awassi sheep associated with prolonged exposure to the MV disease.

5.5. Questionnaire Survey

Stockowners in study area use different terms for respiratory disease which express the clinical nature of the disease fast and labored breathing. Majority of owners, 46.2% and 30.8% associate causes of the respiratory disease with droughts and feed shortages respectively and 23.1% associate with chilly and cool weather.

According to the owners' response, the season of disease occurrence was in chilly and cold season (October - January) and in dry season (February - May). In highlands, these harsh seasons of the year are characterized by chilly and cool weather (extreme in October and January) and feed shortage that produce stress effects on animals and predispose to respiratory disease complex.

Stockowners' knowledge of disease was assessed based on clinical syndromes. 41.5 % of the respondents mentioned ill-thriftiness and respiratory symptoms as a frequent occurrence in their flocks and they associate the clinical picture with pastereullosis. Those respondents who mentioned nervous signs associate the problem with trauma and fluid pressure in the brain (coenurosis). They also associate lameness with foot rot. Although stockowners are familiar with many sheep diseases in their respective area, none of them were aware of MV disease except few owners in North Shewa around sheep ranches who were informed on the occurrence of MV in ranches.

The farmers' response on susceptibility of animals to respiratory diseases showed slight difference to the disease between breeds and age groups. Responses of diseased sheep for antibiotic and anthelmintic treatments may be indicating of the low incidence of clinical cases of MV infected animals in smallholder farms.

6. CONCLUSION AND RECOMMENDATIONS

The serological, clinical and pathological findings strongly suggested that the MVV infection is a major health problem in highland sheep of the ANRS.

The apparent seroprevalence of MVV infection, 6.6% in smallholder farms and 30% in sheep breeding ranches and the 18.8% and 86.2 % flock prevalence in smallholders and ranches, respectively is indicative for the wide occurrence and distribution of MVV infection in both sheep management types.

The ranch management and husbandry practice consisting of large flock size of different breeds and high proportion of older animals for longer years has contributed for the occurrence and spread of MVV infection in a higher rate in ranches than the smallholder farms.

The findings of this study and previous reports are suggestive of the introduction of MVV to Ethiopian highland sheep through importation of exotic sheep breeds from different countries without testing for the disease.

The high seroprevalence of MVV in smallholder farms especially in North Shewa where the state owned sheep ranches are found is suggestive for the sheep ranches to be considered as a home-base for MVV infection.

The finding of MVV antibodies in *weredas* hypothetically considered free of the disease is suggestive for the spread of MVV along with the distribution of Awassi crossbred rams from sheep ranches to smallholders and poor knowledge and misdiagnosing of the disease.

Flock size, breed and age in sheep ranches and flock size, age and contact with ranches in smallholder farms were identified as potential risk factors associated with the occurrence and spread of MVV infection in sheep in the ANRS.

The indirect ELISA test found to be 6.1% more sensitive than the AGID and the test agreement evaluated for both tests is almost perfect and this would be encouraging to use the two tests in combination for accurate serological diagnostics in the control of MVV infection.

Based upon the above conclusions the following recommendations are forwarded;

- Effective control measures should be implemented in ranches to keep flocks free from MVV. One means of controlling the spread of MVV infection is through annual or semi-annual testing and culling of all seropositive ewes and their progeny. European experiences for eradication of MVV from infected flocks relied on five consecutive serological tests at 12-16 months interval. The second method of controlling is removal of lambs at birth before taking colostrums and raising them artificially in isolation from the flock either on pasteurized milk or milk substitute. The second method is more effective and recommended for severely affected flocks in ranches because of the early establishment of MV free flock.

- All sheep lately introduced and any forthcoming animals into ranches for breeding should be kept separated and tested twice at 6 months interval using ELISA and AGID tests in combination and seropositives for MVV must be culled and slaughtered. Since the time required for seroconversion following MVV infection is relatively prolonged and unpredictable, positive flocks must be retested at 6-12 months interval until the flock is seronegative for 2 consecutive tests.

- Elimination of MVV from infected flocks in the smallholder farms would be very difficult, expensive and time consuming. It is therefore of great importance to prevent further spread of infection into free populations through restriction of movement of sheep from infected flocks, particularly from severely infected sheep ranches.

- The spread of MVV in smallholder farms would increase with time if no measures are undertaken to control the disease. Hence, targeted plans and programs should be designed to undertake control measures.

- Information dissemination and trainings are necessary for field animal health personnel and stockowners to increase the level of awareness about the disease.

- The status of MV outside the study area is not known. Therefore, national survey is required to determine the national picture of the disease and for the establishment of centralized MV disease reporting system.

- The MVV targets the cells of the immune system and probably contributes to the development of most other concomitant infectious diseases. Therefore, further studies on the occurrence of concurrent infections and their impacts are required.

7. REFERENCES

Ayelet, G., Roger, F., Tibbo, M., Tembely, S. (2001): Survey of Maedi-Visna in the Ethiopian highland sheep. *Vet. Rec.* **161**, 208-210.

Baumgartner, W., Reckinger, M., Pernthaner, A., Leitold, B. (1990): The occurrence and distribution of Maedi-Visna virus infection in lower Australian sheep breeding establishments. *Dtsch. Tierar. Wochen.* **97,** 465-469.

Beaussoleil, S., Bosgiraud, C., Nicolas, J. A. (1989): Animal lentivirus replication and reverse transcriptase inhibitors. *Rev. Infect. Dis.* **11,** 732-740.

Bird, P., Allen, D., Reyburn, H., Watt, N., Mc Connell, I. (1990): Immunological studies in sheep naturally infected with Maedi-Visna virus. European Federation of Immunological Societies. 10th meeting. Edinburgh. Abstract.

BoA. (2000): Report on the preliminary investigation of sheep disease at sheep ranches and ram distribution sites in North Shewa. Disease investigation report, Bahir Dar.

BoA. (2001): Control and management of Maedi-Visna in sheep at ranches in North Shewa. Proceeding of workshop held in 1-2nd June 2001. Bahir Dar.

Braun, M.J., Clements J.E., Gonda, M.A. (1987): The Visna virus genome: evidence for a hypervariable site in the *env* gene and sequence homology among lentivirus envelope proteins. *J. Virol.* **61,** 4046-4054.

Brodie, S.J., Bickle, H.M., DeMartini, J.C. (1995b): Ovine lentivirus associated subclinical encephalomyelitis: virological markers in cerebro-spinal fluid are predictive of central nervous system disease. *Clin. Immunol. Immunopathol.* **77,** 14-18.

Brodie, S.J., de la Concha-Bermejillo, A. Snowder, G.D., DeMartini, J.C. (1998): Current concepts in the epizootology, diagnosis, and economic importance of ovine progressive pneumonia in Northern America: a review. *Small Rum. Res.* **27,** 1-17.

Brodie, S.J., de la Concha-Bermejillo, A., Koeing, G., Snowder, G.D., DeMartini, J.C. (1994): Maternal factors associated with prenatal transmission of ovine lentivirus. *J. Infecct. Dis.* **169,** 653-657.

Brodie, S.J., Pearson, I.D., Snowder, G.D., DeMartini, J.C. (1993): Host-virus interaction as defined by amplification of viral DNA and serology in lentivirus infected sheep. *Arch. Virol.* **130,** 413-428.

Brodie, S.J., Pearson, I.D., Zink, M.C., Bickle, H.M., Anderson, B.C., Marcom, K.A. DeMartini, J.C. (1995a): Ovine lentivirus expression and disease: virus replication but not entry is restricted to macrophages of specific tissues. *Am. J. Pathol.* **146,** 250-263.

CACC. (2003): Statistical report on livestock and farm implements. Ethiopian Agricultural Sample Enumeration, 2001/2. Addis Ababa.

Carey, N., Dalziel, R. G. (1993): The biology of Maedi-Visna virus: an overview. *Br. Vet. J.* **149,** 437-450.

Clements, J.E., Gabuzda, D. (1990): Cellular regulation of Visna virus gene expression and its role in pathogenesis. *Dev. Biol. Stand.* **72,** 207-212.

Clements, J.E., Gidovin, S.I., Monteclato, R.C., Narayan, O. (1988): Antigenic variation in lentiviral diseases. *Ann. Rev. Immunol.* **6,** 139-159.

Clements, J.E., Zink, M.C. (1996): Molecular biology and pathogenesis of animal lentivirus infections. *Clin. Microbiol. Rev.* **9,** 100-117.

Clements, J.E., Narayan, O., Griffin, D.E., Johnson, R.T. (1979): The synthesis and structure of Visna virus DNA. *Virology.* **93,** 377-387.

Constable, P.D., Meier, W.A., Foley, G.L., Morin, D., Cutlip, R.C., Zachary, J.F. (1996): Visna-like disease in a ram with chronic demylinating-encephalomylitis. *J. Am. Vet. Med. Assoc.* **208,** 117-120.

Crowford, T.B., Adams, D.S., Cheevers, W.P., Cork, L.C. (1980): Chronic arthritis in goats caused by a retrovirus. *Science.* **207**, 997-9.

Cutlip, R.C., Houward, D., Lehmkuhl, H.D., Schmerr, M.J., Brogden, K.A. (1988): Ovine progressive pneumonia, Maedi-Visna in sheep. *Vet. Microbiol.* **17**, 237-250.

Cutlip, R.C., Lehmkuhl, H.D. (1986): Eradication of progressive ovine pneumonia from sheep flocks. *J. Am. Vet. Med. Assoc.* **188**, 1026-1027.

Cutlip, R.C., Lehmkuhl, H.D., Brogden, K.A., Schmerr, M.J. (1987): Failure of experimental vaccines to protect against infection with ovine progressive pneumonia, MV virus. *Vet. Microbiol.* **13**, 201-204.

Cutlip, R.C., Lehmkuhl, H.D., Brogden, K.A., Schmerr, M.J. (1991): Seroprevalence of ovine progressive pneumonia virus in various domestic and wild animal species, and species susceptible to the virus. *Am. J. Vet. Res.* **52**, 189-191.

Cutlip, R.C., Lehmkuhl, H.D., Sacks, J.M., Weaver, A.L. (1992): Seroprevalence of ovine progressive pneumonia virus in sheep in the United States as assessed by analysis of voluntarily submitted samples. *Am. J. Vet. Res.* **53**, 976-979.

Dawson, M., Birront, P., Houwers, D.J. (1982): Comparison of serological tests used in three state veterinary laboratories to identify Maedi-Visna infection. *Vet. Rec.* **111**, 432-434.

Dawson, M. (1987): Pathogenesis of Maedi-Visna. *Vet. Rec.* **120**, 451-454.

Dawson, M., Done, S.H., Venables, C. Jenkins, C.E. (1990): Maedi-Visna and sheep pulmonary adenomatosis: a study of concurrent infection. *Brit. Vet. J.* **146**, 531-538.

De Boer G.F., Trpstra, C., Houweres, D.J. (1979): Studies in epidemiology of Maedi-Visna in sheep. *Res. in Vet. Science* **26**, 202-208.

de la Concha-Bermejillo, A., Juste, R.A., Ott, T.I., Kwang, J., Bazer, F.W. (1995b): Recombinant interferon-γ inhibits ovine lentivirus replication. Proc. Am. Sco. Virol. 14th Annual Meeting. p.192 (Abstract).

de la Concha-Bermejillo, A., Magnus-corral, S., Brodie, S.J., Bowen, R.A., DeMartini, J.C. (1995a): Pathologic and serological responses of isogenic twin lambs to phenotypically distinct ovine lentiviruses. *J. Acquir. Immun. Defic. Syndr. Human Retrovir.* **8**, 116-123.

de la Concha-Bermejillo, A., Magnus-corral, S., Brodie, S.J., DeMartini, J.C. (1996): Venereal shedding of ovine lentivirus in infected rams. *Am. J. Vet. Res.* **57**, 684-688.

de la Concha-Bermejillo, A., Shelton, M., Magnus-Corral, S. (1994): Seroprevalence of ovine progressive pneumonia in texas sheep. Texas Agric. Exp. St. Res. Reports. PR. **5223**, 34-35.

De Boer, G.F., Jackson, T.A., Lehmkuhl, H.D. (1979): Lesions of ovine progressive pneumonia: interstitial pneumonitis and encephalitis. *Am. J. Vet. Res.* **40**. 1370-1734.

DeMartini, J.C., Bowen, R.A., Carlson, J.O. de la Concha-Bermejillo, A. (1991) : Strategies for the genetic control of ovine lentiviral infections. In: Anford R.I.E. (Ed.) Breeding for Disease Resistance in Farm Animals. pp. 293-314.

DeMartini, J.C., Rosadio, R.H., Sharp, J.M., Russell, H.I., Laimore, M.D. (1987): Experimental conduction of type D retrovirus associated pulmonary carcinoma and lentivirus associated lymphoid interstitial pneumonia in lambs. *J. Nat. cancer inst.* **79**, 167-177.

Desrosiers, R.S. (2001): Nonhuman Lentiviruses. In: D.M. Knipe and P.M. Howley (ed.), Fields Virology II, 4th ed. LippinotCott Williams and Wilkins, Philadelphia, USA.

Dohoo, I.R., Curtis, R.A., Finley, G.G. (1985): A survey of sheep diseases in Canada. *Can. J. Comp. Med.* **49**, 239-247.

Dohoo, I.R., Heaney, D.P., Stevenson, R.G., Samagh, B.S. Rhodes, C.S. (1987): The effects of Maedi-Visna virus infection on productivity in ewes. *Prev. Vet. Med.* **4**, 471-484.

Gelderblom, H.R.., Ozel, M., Gheysen, D., Reupke, H., Winkel, T., Herz, U., Grund, C., Pauli, G. (1990): Morphogenesis and fine structure of lentiviruses In: Schellekens H. and Horzinek M.C(eds.) *Animal Models in AIDS.* Elsevier, Amsterdam/Newyork/Oxford, pp. 1-26.

Georgsson, G., Houwers, D.J., Palsson, P.A., Petursson, G. (1989): Expression of viral antigens in the central nervous system of Visna-infected sheep: An immunohistochemical study in expermental visna induced by virus strains of increase neurovirulence. *Acta Neuropathologica* (Berlin). **77**, 299-306.

Georgsson, G., Palsson, P.A. (1971): The histopathology of Maedi: a slow viral pneumonia of sheep. *Vet. Pathol.* **8**, 63-80.

Gonda, M.A., Braun, M.J., Clements, J.E., Pyper, J.M., Casey, J.W., Wongstaal, F., Gallo, R.C., Gilden, R.V.(1986): HTLV-III shares sequence homology with a family of pathogenic lentiviruses. Proceedings of the National Academy of Sciences, USA. **83,** 4007-4011.

Griffin, D.E., Narayan, O., Adams, R.J. (1978): Early immune responses in Visna, a slow viral disease of sheep. *J. Infect. Dis.* **138**, 340-350.

Gurovich, I. (1989): Acquired immunodeficiency syndrome; realistic concerns and appropriate precautions. *Heart Lung.* **18**,107-112.

Haase, A.T., Stowring, L., Harris, J.D., Traynor, B., Ventura, P., Peluso, R., Brahic, M. (1982): Visna DNA synthesis and the tempo of infection in vitro. *Virology.* **119**, 399-410.

Haase, A.T. (1986): Pathogenesis of lentivirus infections. *Nature.* **322**, 130-136.

Houwers, D.J., DeBoer, G.F. (1984): Maedi-Visna control in sheep: half yearly serological testing with culling of positive ewes and progeny. *Vet. Microbiol.* **9**, 445-451.

Houwers, D.J., Konig, D.W., DeBoer, M.J., Pekelder, J.J., Vallenia, P. de Varies, G. (1987): MV control in sheep III. Results and evaluations of a voluntary control program in the Netherlands over a period of four years. *Vet. Q.* **9**, 29-36.

Houwers, D.J., Nauta, I.M. (1989): Immunoblot analysis of the antibody response to ovine lentivirus infections. *Vet. Microbiol.* **19**, 127-139.

Houwers, D.J., Van der Molen, E.J. (1987): Active year serological study of natural transmission of Maedi-Visna virus in a flock of sheep completed with post mortem investigation. *J. Vet. Med.* **34**, 412-431.

Houwers D.J., Konig C.D.W., De Boer G.F. Schaake J. (1983): Maedi-Visna Control in Sheep. I. Artificial Rearing of Colostrum-Deprived Lambs. *Vet. Microbiol.* **8**,179-185.

Johnson L.K., Meyer A.L., Zink M.C. (1992): Detection of ovine lentivirus in seronegative sheep by in situ hybridization. PCR and cocultivation with susceptible cells. *Clin. Immunol. immunopathol.* **65**, 254-260.

Juste R.A., Kwang, J., de la Concha-Bermejillo, A. (1995): Comparative evaluation of the agar gel immunodiffusion test and recombinant ELISA for the diagnosis of ovine progressive pneumonia. Proceeding, 99th Annual Meeting United States Animal Association (Abstract 536).

Juste, R.A., Ott, T.L., Kwang, J., Bazer, F.W., de la Concha-Bermejillo A. (2000): Effect of recombinanat ovine interferon – τ on ovine lentivirus replication and progression of disease. *J. Gen. Virol.* **81**, 525-532.

Kajikawa, O., Lairmore, M.D., DeMartini, J.C.(1990): Analysis of antibody responses to phenotypically distinct lentiviruses. *J. Clin. Microbiol.* **28**,764-770.

Kimberling, C.V. (1988): Ovine progressive pneumonia. In: Jensen and Swift's diseases of sheep. 3rd ed. Philadelphia, Lea and Febiger, pp. 274-280.

Kita, J., Cutlip, R.C., Kempski, W., Sacks, J. (1990): Survey for antibodies against Maedi-Visna in sheep in Poland. *Pol. Arch. Weter.* **30**, 5-11.

Knowles, J.R., Cheevers, W., MacGuire, T., Stem, T., Gorham, J. (1990): Severity of arthritis is predicted by antibody responses to Gp135 in chronic infection with Caprine Arthritis Encephalitis Virus. *J. Virol.* **64,** 2396-8.

Kozaczynska, B., Kuzmak, J., Rola, M. (2002): Application and evaluation of serological methods in the diagnosis of Maedi-Visna virus infection in sheep from Poland. *Med. Weter.* (Poland). **58**, 444-447.

Kwang, J., Cutlip, R. C. (1992): Detection of antibodies to ovine lentivirus using a recombinant antigen derived from the *Env* gene. *Biochem. Biophys. Res. Commun.* **183**, 1040-1046.

Kwang, J., Rosati, S., Yang, S., Juste, R.A. de la Concha-Bermejillo, A. (1996): Recognition of lentiviruses *gag* gene products by serum from infected sheep. *Vet. Immunol. Immunopathol.* **55**, 107-114.

Lairmore, M.D., Butera, S.T., Callahan, G.N., DeMartini, J.C. (1988): Spontaneous interferon production by pulmonary leukocytes is associated with lentivirus-induced lymphoid interstitial pneumonia. *J. Immunol.* **140**, 779-785.

Lairmore, M.D., Rosadio, R.H., DeMartini, J.C. (1986): Ovine lentivirus lymphoid interstitial pneumonia rapid induction in neonatal lambs. *Am. J. Pathol.* **125**, 173-181.

Leeson, T.S., Leeson, C. R. (1981): Histology. 4th ed. Philadelphia, W.B. Saunders Company. pp 7-18.

Leroux, C., Greenland, T. Mornex, J.F., (1996): Molecular characterization of field isolates of lentiviruses of small ruminants. *AIDS Res. Hum. Retrovir.* **12,** 427-429.

Marcom, K.A., Pearson, L.D., Chung, C.S., Poulson, J.M., DeMartini, J.C. (1991): Epitope analysis of capsid and matrix proteins of North American ovine lentivirus field isolates. *J. Clin. Microbiol.* **29**, 1472-1479.

McGuire, T.C., O'Rourke, K.I., Knowles, D.P., Cheevers W.P. (1990): Caprine Arthritis Encephalitis Lentivirus. Transmission and Disease. Current Topics in Microbiology and Immunology. **160**, 61-75.

McGuire, T.C., Adams, D.S., Johnson, G.C., Klevjer-Anderson, P., Barbee, D.D., Gorham, J.R. (1986): Acute arthritis in Caprine Arthritis–Encephalitis virus challenge exposure of vaccinated or persistently infected goats. *Am. J. Vet. Res.* **47**, 537-540.

Narayan, O. (1990): Lentiviruses are etiological agents of chronic diseases in animals and acquired immunodeficiency syndrome in humans. *Can. J. Vet. Res.* **54,** 42-48.

Narayan, O., Clements, J.E. (1989): Biology and pathogenesis of lentiviruses. *J. Gene. Virol.* **70**, 1617-1639.

Narayan, O., Sheffer, D., Clements, J.E., Tennekoon, G. (1985): Restricted replication of lentiviruses. Visna viruses induce a unique interferon during interaction between lymphocytes and infected macrophages. *J. Exp. Med.* **162**, 1954-1639.

Narayan, O., Silverstein, A.M., Price, D., Johnson. R.T. (1974): Visna virus infection of American lambs. *Science* **183**, 1202-3.

OIE (1996): Caprine Arthritis-Encephalitis and Maedi-Visna. Manual of Standards for Diagnostic Tests and Vaccines. pp 369-373

Oliver, R.E., Gorham, J.R., Perryman, I.E., Spencer, G.R. (1981): Ovine progressive pneumonia: experimental intrathoracic, intracerebral, and intra-articular infections. *Am. J. Vet. Res.* **42,** 1560-1564.

Palfi, V., Glavits, R., Hojtos, I. (1989): Testicular lesions in rams infected by Maedi-Visna virus. *Acta Vet. Hung.* **37,** 97-102.

Pasick, J. (1998): Maedi-Visna virus and Caprine-Arthritis-Encephalitis virus: distinct species or quasispecies and its implications for laboratory diagnosis. *Can. J. Vet. Res.* **62,** 241-244.

Payne, A.L., York, D.F., Devilliers, E.M., Verwoerd, D.W., Querat, G., Barban, V., Sauze, N., Vigne, R. (1986): Isolation and identification of a South African lentivirus from Jaagsiekte lungs. *Onderstepoort J. Vet. Res.* **53,** 55-62.

Pearson, L.D., Poss, M.L., DeMartini, J.C. (1989): Animal lentivirus vaccines: problems and prospects. *Vet. Immunopathol.* **20,** 183-212.

Peklelder, J.J., Veenink, G.J., Akkersmans, J.P., Van Eldik, P., Elving, E., Houwers, D.J. (1994): Ovine lentivirus-induced indurative lymphocytic mastitis and its effect on the growth of lambs. *Vet. Rec.* **134,** 348-350.

Pepin, M., Vitu, C., Russo, P., Mornex, J.F., Peterhans, E. (1998): Maedi-Visna infection in sheep: a review. *Vet. Res.* **29,** 341-367.

Perk, K., Yaniv, A., Gazit, A., DeMartini, J.C. (1996): Evaluation of vaccines for ovine lentivirus infection. *AIDS Res. Hum. Retrovir.* **12,** 425-426.

Petursson, G., Andresdottir, U., Andresson O.S., Georgsson, G., Palsson, P.A., Rafnar B., Torsteinsdottir, S. (1992): Lentivirus diseases of sheep and goats: Maedi-Visna and Caprine Arthritis- Encephalitis. In: Speedy A.W. (ed.). Progress in sheep and goat research. C.A.B International. Redwood, UK. PP 85-107.

Petursson, G., Hoff-Jorgensson, R. (1990): Developments in veterinary immunology : Maedi-Visna and related diseases. Kluwer Acadamic Publisher.

Petursson G., Nathanson N., Georgsson G., Panitch H., Palsson P.A.(1976): Pathogenesis of Visna I. Sequential virologic, serological and pathologic studies. *Lab. Invest.* **35,** 402-0412.

Philipon, V., Vellutinni, C., Gambarelli, D., Harkiss, G., Arbuthnott, G., Metzger, D., Roubin, R., Philippi, P. (1994): The basic domain of the lentiviral *tat* protein is responsible for damages in mouse brain: involvement of cytokines. *Virology.* **205,** 519-529.

Querat, G., Barban, V., Sauze, N., Filippi, P., Vigne, R., Russo, P., Vitu, C. (1984): Highly lytic and persistent lentiviruses naturally present in sheep with progressive pneumonia are genetically distinct. *J. Virol.* **52**, 672-679.

Radostits, O.M., Blood, D.C., Gay, C.C. (2000): Ovine progressive pneumonia (Maedi-Visna). In: Veterinary Medicine. A Textbook of Cattle, Sheep, Goats, Pigs and Horses, 8th Ed. London, Bailliere Tindall, pp. 1071-1075.

Reyburn, H.T., Roy, D.J., Blacklaws, B.A., Sargan, D.R., Watt, N.J., McConnell, I. (1992): Characteristics of the T cell-mediated immune response to Maedi-Visna virus. *Virology* **191**, 1009-1912.

Saman, E., Van Eynde, G., Lujan, L., Extramiana, B., Harkiss, G., Tolari, F., Gonzalez, L., Amorena, B., Watt, N., Badiola (1999): A new sensitive serological assay for detection of lentivirus infections in small ruminants. *J. Clin. Diagn. Lab. Immunol.* **6**, 734-740.

Sargan, D.R., Sutton, K.A., Bennet, I.D., McConnell, I., Harkiss G.D. (1995): Sequence and repeat structure variants in the Long Terminal Repeat of Maedi-Visna virus EVI. *Virology* **208**, 343-348.

Schaller, P., Vogt, H.R., Strasser, M., Nettleton, P.F., Peterhans, E., Zanoni, R.(2000): Seroprevalence of Maedi-Visna and border disease in Switzerland. *Schw. Arch. Teirh.* **42**, 145-153

Scheer-czechowski, P., Vogt, H.R., Tontis, A., Peterhanse, E., Zanoni, R. Schweiz, A.T. (2000): Pilot Project for Eradicating Maedi-Visna in Walliser Blacknose Sheep. *Apr.* **142**, 155-164.

Sigurdsson, B., Grimsson, H., Palsson, P.A. (1952): Maedi, a chronic progressive infection of sheep lungs. *J. Infect. Dis.* **90**, 233-241.

Sigurdsson, B. (1954): Observations on the slow infections of sheep. *Br. Vet. J.* **110**, 255-270.

Sigurdsson, B., Palsson, P.A., Grimsson, H. (1957): Visna, a demyelinating transmissible disease of sheep. *J. Neuropathol. Exp. Neurol.* **16**, 389-403.

Sihvonen, L, Hirvela-Koski, V., Nuotio L., Kokkonen, U.M. (1999): Serological Survey and Epidemiological Investigation of Maedi-Visna in Sheep in Finland. *Vet. Microbiol.* **65**, 265-270.

Sihvonen, L., Nuotio, L., Rickula U., Hirvela-Koski, V., Kokkonen, U.M (2000): Preventing the Spread of Maedi-Visna in Sheep Through a Voluntary Control Program in Finland. *Prev. Vet. Med.* **47**, 213-220.

Sihvonen, L. (1981): Early immune responses in experimental Maedi. *Res. Vet. Sci.* **30**, 217-222.

Sihvonen, L., Estola, T., Tuomi, J. (1980): Experimental Maedi infection in sheep. I. Detection. *Acta Vet. Scanda.* **21**, 113-123.

Simard, C.I., Briscoe, M.R. (1990a): An enzyme linked immunosorbent assay for detection of antibodies to Maedi-Visna virus in sheep. I. A simple technique for production of antigen using sodium dodecyl sulfate treatment. *Can. J. Vet. Res.* **54**, 446-450.

Simard, C.I., Briscoe, M.R. (1990b): An enzyme linked immunosorbent assay for detection of antibodies for Maedi-Visna virus in sheep. II. Comparison to conventional agar gel immunodiffussion test. *Can. J. Vet. Res.* **54**, 451-456.

Simard, C.I., Morley, R.S. (1991): Seroprevalence of Maedi-Visna in Canadian sheep. *Can. J. Vet. Res.* **55**, 269-273.

Simon, M.A., Brodie, S.J., Sasseville, V.G., Chalifoux, L.V., Disrosiers R.C., Ringler, D.J. (1994): Immunopathogenesis of SIVmac. *Virus Res.* **32**, 227-251.

Snowder G.D., Gates N.L., Glimp H.A., Gorham J.R. (1990): Prevalence and effect of subclinical ovine progressive pneumonia virus infection on ewe wool and lamb production. *J. Am. Vet. Med. Assoc.* 197, 475-479.

Stata Corp. (2001): Stata statistical software. Release 7.0. Lakeway Drive, College station, Texas.

Tibbo, M., Woldemeskel, M., Gopilo, A. (2001): An outbreak of respiratory disease complex in sheep in central Ethiopia. *Trop. Anim. Helth. prod.* **33**, 355-365.

Thrusfield, M. (1995): Veterinary Epidemiology. 2nd ed. Cambridge, Blackwell Science, pp. 183.

Valas, S., Benoit, C., Guionaud, C., Perrin, G., Mamoun, R. Z. (1997): North American and French Caprine Arthritis-Encephalitis viruses emergence from ovine Maedi-Visna viruses. *Virology* **237,** 307-318.

Van der Molen, E. J., Vecht, U. Houwers, D. J. (1985): A chronic indurative mastitis in sheep associated with MV virus infection. *Vet. Q.* **7,** 112-119.

Wiliams-Fulton, N. R., Simard, C.L. (1989): Evaluation of two management procedures for the control of MV. *Can. J. Vet. Res.* **53,** 419-423.

Win Episcope (2000): Version 5.1.2600 Microsoft Corp.

Woldemeskel, M., Tibbo, M., Potgieter, L N. (2002), Ovine progressive pneumonia (Maedi-isna): an emerging respiratory disease of sheep in Ethiopia. *Dtsch Tiera. Wochen.* **109,** 486-488.

Zanoni, R., Pauli, U., Peterhans, E. (1990): Detection of Caprine Arthritis Encephalitis and Maedi-Visna viruses using the polymerase chain reaction. *Experimentia* **46**, 316-319.

Zhang, Z., Guo, J., Ermel, R.W., Bazer, F.W., Giavedoni, L., de la concha-Bermejillo, A. (2000): Construction and characterization of a recombinant ovine lentivirus carrying the optimized green flourescent protein gene at the dUTPase locus. Am. Soc. Virol.. 19th Ann. Meet. W52-5, 144.

Zink, M.C., Johnsson, L.K. (1994): Pathobiology of lentivirus infections of sheep and goats. *Virus Res.* **32**,139-154.

9. ANNEXES

Annex 1: Procedures used for indirect-Enzyme Linked Immunosorbant Assay test

The test procedure used was according to the manufacturers' recommendation (Pourquier Institute, France).

1. Deposit of the sera

- Control and sample sera were diluted to 1:20
- 190µl of serum dilution buffer was dispensed per well.
- 10µl of undiluted negative control serum in A1 and A2 and 10µl of undiluted positive control serum in B1 and B2 and in replicates was dispensed.
- 10µl of each undiluted serum sample was dispensed in even and odd-numbered columns and homogenized by delicate shaking.
- The plate was covered with a lid or aluminium foil and incubated for 1 hour (± 5m.n) at 37^0C (±3^0C).

2. Washing

- The content of the plate was emptied by returning manually,
- All the wells on the plate were filled with the wash solution and emptied using automatic washing system (a total of 3 washes).

3. Depositing the conjugate

- The conjugate was diluted to 1:100 with the conjugate dilution buffer.
- 100µl of this solution was dispensed per well.
- The plate was covered with a lid/ aluminium foil and incubated for 30 minutes (± 3min) at 37^0C (±3^0C).

4. Washing

- The content of the plate was emptied by returning manually, and washed 3 times using wash solution.
- All the wells on the plate were filled with the 'wash solution' and then emptied using the automatic washing system (total of 3 washes).

5. Revelation

- 100µl of revelation solution, TMB (enzyme substrate) ready to use was dispensed per well.
- The plate was incubated at 21^0C ($\pm 5^0C$) for 20 minutes (away from the light).
- 100µl of stop solution (H_2SO_4, 0.5M) was dispensed per well.
- The plate was gently shaken until the coloured solution was homogenized.

6. Reading

- The optical densities were read at 450nm (OD. 450). The photometer was first blanked on air.
- The corrected OD 450 was calculated for each serum: by subtracting the OD 450 value obtained from the uncoated well from the OD 450 of the coated well.

Validation criteria:

The results were considered reliable when;

- The positive control serum has a minimal uncorrected OD 450 value of 0.350 and
- A ratio between the corrected OD 450 value of the positive control and corrected OD 450 value of the negative control was greater or equal to 3.5.

Annex 2: Procedures used for Agar Gel Immuno Diffusion test

The AGID kit was used to detect antibodies to the MV viral envelop glycoprotein (gp 135) using the agar gel immuno-diffusion technique.

Kit contents include:

- 1 x 3 ml vial of freeze-dried antigen to be reconstituted in 3 ml distilled water
- 2 x 4.5 ml vials of freeze-dried positive control serum to be reconstituted in 4.5 ml distilled water
- 1 x 0.5 ml of freeze-dried positive limit control serum to be reconstituted in 0.5 ml distilled water
- 1 x 0.5 ml of freeze-dried negative control serum to be reconstituted in 0.5 ml distilled water

Agar gel preparation

The gel medium is 0.7-1% agarose in 0.05M Tris buffer, PH 7.2, with 8.0% NaCl. In this particular study ready-to-use agar gel supplied with the AGID kit has been used.

- The ready to use agar gel in bottle wass immersed in a boiling water bath until the medium was completely liquefied and clear for about 30 minutes
- The agar gel was cooled down to about 60^0C to avoid excessive evaporation at pouring.
- With a preheated pipette, 50 ml of agar gel was poured in a Petri dishes (120 x 120mm) or 32ml in 90x90mm Petri dishes and kept in a horizontal position until the medium has cooled off and solidified. It is some times necessary to increase the agar gel volume slightly to avoid reagent overflow.
- Petri dishes were stored upside down for up to 3 days at 2-8^0C in a humid atmosphere but preparing one day prior to testing was recommended.
- A metal gel cutter of seven cylinders was used to cut seven wells of 5mm diameter for sample sera and 3.5mm diameter for control sera and antigen deposition (Fig 2).

Reagent deposition

- The antigen was placed in the central well and the sera to be tested were placed in the 5 mm wells around the antigen and positive control serum in 3.5mm wells.
- The Petri dishes were kept in a humid atmosphere at room temperature (18-23^{0}C).
- Results were read after 3-4 days
- The reading was standardized by adding a positive limit and negative control sera in each series of test analysis.

Validation

- A zone of equivalence between gp 135 antigens and antibodies in the serum tested was demonstrated by a continuous precipitin line between the control serum and the wells containing the serum tested.

Annex 3. Procedures used for histopathological examination

Tissue samples collected were processed and examined following histopathological procedures, according to Leeson and Leeson, (1981).

1. **Fixation:**
   - Tissues were fixed in buffered formalin solution as soon as collected and submitted to laboratory.
2. **Embedding:**
   - Prior to embedding, each tissue is washed to remove excess fixative.
   - The tissues were infiltrated with the embedding agent (paraffin) and solidified.
3. **Sectioning:**
   - Tissues embedded in paraffin were sliced 3-10 micrometer thickness.
   - Each section was transferred to a clean slide on which a little egg albumen has been smeared.
4. **Staining:**
   - Paraffin was removed using paraffin solvents.
   - The sections were passed through descending strengths of alcohol.
   - The sections were stained with hematoxylin and eosin combination dye.
5. **Mounting:**
   - After staining, excess dye was removed by washing with alcohol
   - The sections were dehydrated through ascending grades of alcohol and cleaned.
   - A drop of mounting medium is placed on each section and covered with a cover slip and allowed to dry.

Examination and interpretation of tissue sections: hematoxylin and eosin stains were used and nuclear structures of cells are stained dark purple or blue, and practically all cytoplasmic structures and intercellular substances are stained pink. Then stained tissue sections were examined for characteristic lesions of MVV infection, which is mononuclear cell infiltration (lymphocytes and macrophages).

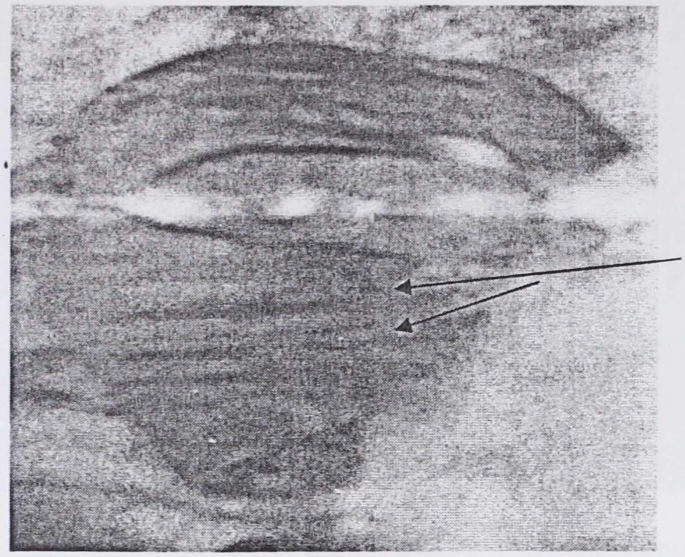
Annex 4. Format for questionnaire survey

1. Location: Zone ..... Date .....
   Wereda.....
   Kebele/PA..... .....
   Village .....
2. Serial number .....
3. Respondent name ..... Status: Owner .....
   Worker .....
4. Housing system: 'Indoor ....., Outdoor .....
5. Introduction of crossbred ram: Yes ..... If yes, year .....
   No .....
6. Presence of disease syndrome with:
   Emaciation and weakness .....
   Laboured breathing and cough .....
   Enlargement of udder .....
   Lameness and swelling on joints .....
   Circling and paralysis of hind legs .....
7. Season of the disease occurrence? Month .....
   Year .....
8. Breed of sheep frequently affected? Local .....
   Exotic ....
   Crossbred .....
9. Age group of animals affected? < 1year .....
   1 year and above .....
10. Response of animals for treatment: Yes/No .....
11. Number of animals died ..... year of death .....
12. When do you know the disease? year .....

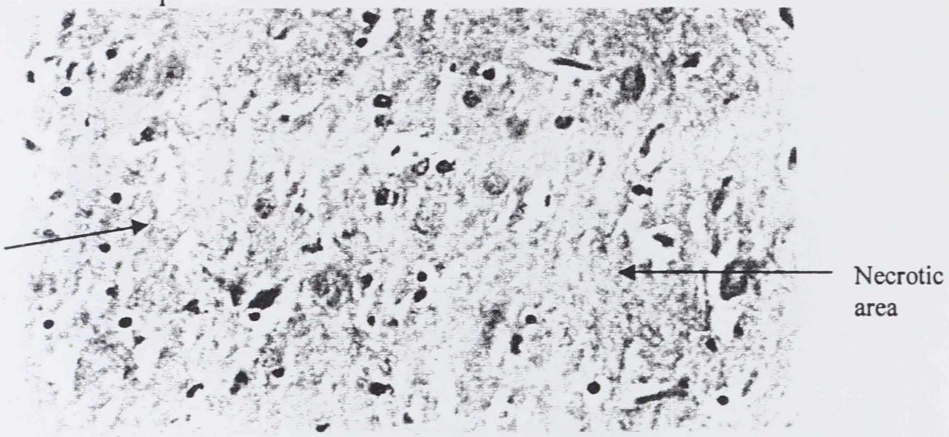
Annex 5. Maedi-Visna seroprevalence based on ELISA and AGID tests

Zone	Wereda	Kebele	Sera Tested	ELISA Positive	Seropos-itivity(%)	AGID Positive	Seropos-itivity (%)
North Shewa	Basona-worana	Faji	99	12	12.1	12	12.1
		Angolela	103	2	1.9	0	0.0
	Gera-Keya	Tsehay Sina	116	44	37.9	43	37.1
		Sarmidir	121	3	2.5	3	2.5
		Sinamba	127	9	7.1	8	6.3
North Wollo	Meket	Akat	136	4	2.9	1	0.7
		Estayish	123	3	2.4	1	0.8
	Wadila	Gashena	124	4	3.2	1	0.8
		Kuana	121	7	5.8	2	1.7
South Wollo	Desie Zuria	Gelsha	119	2	1.7	0	0.0
		Gerado	121	4	3.3	4	3.3
	Legambo	Chiro	91	2	2.2	0	0.0
		Segnogebiya	90	2	2.2	0	0.0
Sub total			**1491**	**98**	**6.6**	**75**	**5.0**
Sheep breeding ranches		Guguftu	65	2	3.1	0	0.0
		Amed Guya	375	77	20.5	73	19.5
		D/Berhan	335	67	20.0	66	19.7
		Sheno ARC	151	132	87.4	139	92.1
Sub total			**926**	**278**	**30.0**	**278**	**30.0**
Total			**2417**	**376**	**15.6**	**353**	**14.6**

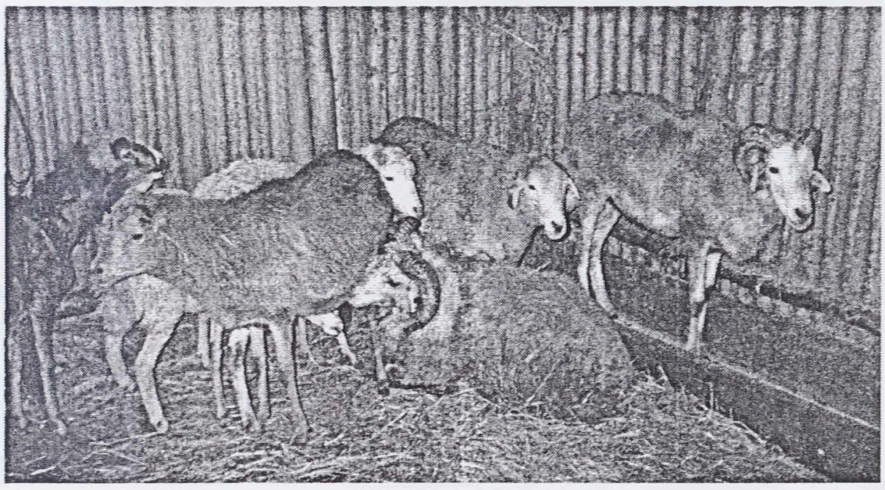
Annex 6. Clinical and pathological pictures



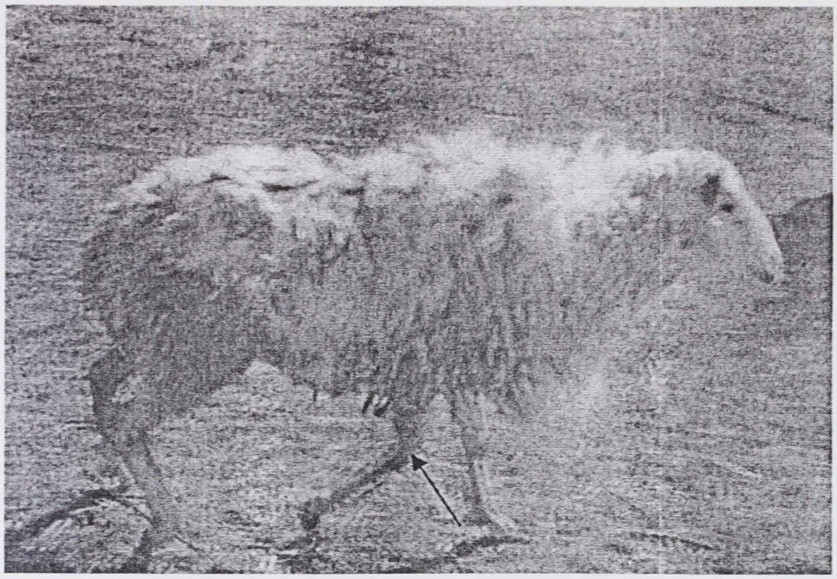
1. Enlarged lung with diffused gray-brown consolidation and rows of rib attachments in Maedi-Visna infected sheep



2. Necrotic-encephalitis of the brain in Maedi-Visna seropositive sheep



3. Maedi-Visna seropositive and clinically diseased Menz sheep (Sheno ARC)



4.Progressively emaciated, lamed and MV seropositive Menz male sheep (Sheno ARC)

5. Maedi-Visna seropositive Awassi ewe with moderately enlarged and firm udder (Amed Guya ranch)

6. Maedi-Visna seropositive Awassi ewes and their Menz crosses (Debre Berhan ranch)

7. Maedi-Visna seropositive Awassi and Awassi x Menz crossed male sheep (Amed Guya ranch)

10. CURRICULUM VITAE

1. Personal data

Name:	Tsegaw Fentie Kassa
Date of Birth:	March 16, 1958
Nationality:	Ethiopian
Marital status:	Married
Children:	3 aged 11, 9 and 7years
Religion:	Christian
Position:	Head, Kombolcha Regional Veterinary Laboratory, BoA
Membership:	Ethiopian Veterinary Association
Office contact:	Kombolcha Regional Veterinary Laboratory
	P.O.Box, 9, Kombolcha
	Telephone: 03-510020/510264
	E-mail: ts0348@yahoo.com

2. Educational background

Sept. 1963- July1966 EC:	Primary school, Gondar
Sept. 1967- July 1968 EC:	Junior secondary, Fasiledes, Gondar
Sept.1969, 1973-July1975EC	Senior secondary, Fasiledes comprehensive, Gondar
	Achievement: ESLCE
Sept. 1976- July1981 EC	University of Addis Ababa, Faculty of Veterinary Medicine.
	Achievement: Doctor of Veterinary Medicine (DVM)

3. Work experience

Sept. 1982 – Dec.1985 EC	Field Vet. Officer, Ministry of Agriculture
Dec.1985 – July,1989 EC	Team leader, Animal and Fisheries Resource, Agricultural Development Office, South Gondar zone
July, 1989- June, 1990EC	Department head, Agricultural Development Office, South Gondar zone
July 1990 – Oct. 1991	Research officer, Kombolcha Reg.Vet. Laboratory, Bureau of Agriculture, Amhara Region
Oct. 1991- Sept. 2002	Head, Regional Vet. Lab., Kombolcha, BoA

4. **Research Papers**

1. Selection of bait systems for Glossina morsitans submorsitans and control at the higher elevations, DVM thesis (1990), FVM, AAU (unpublished).
2. Study on the ethnoveterinary knowledge and practices in South Wollo zone. Proceeding of 15th Ethiopian Veterinary Association conference, 2001.
3. Epidemiological and clinico-pathological study of Maedi-Visna virus infection in sheep in selected districts of the Amhara National Regional State, MSc thesis (2004), FVM, AAU.

6. **Additional trainings:**

1. Computer literacy: Word processing, Spread sheet and Data base management (April1 -30, 1999)
2. Research project planning, monitoring and evaluation. Ethiopian Agricultural Research Organization (Sept. 24-30, 2001)
3. Seminar on health surveillance and emerging diseases held in Addis Ababa, Ethiopia. Office International des Epizooties (OIE), (28-30 Januar. 2002).

SIGNED DECLARATION SHEET

The thesis my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name T Segaw Fenkie

Signature

Date of submission June 15, 2004

The thesis has been submitted for examination with our approval as university advisor.

Epidemiological & Clinico pathological study of Maedi-visna virus infections in sheep in selected districts of the Amhara National Regional state

Tsegaw Fentie