STUDY ON THE EFFECTS OF A NATURAL MAEDI VISNA VIRUS INFECTION ON SHEEP PRODUCTIVITY

By

BAPTISTE DUNGU-KIMBENGA

Submitted in partial fulfilment of the requirements for the degree of Master in Science in the

Department of Veterinary Production and Ethology

Date submitted: February 2000

ACKNOWLEDGEMENTS

I wish to acknowledge and express my sincere appreciation to the following people and institutions:

Dr. Johan Vorster, with whom we have worked throughout the whole Maedi-visna project: thank you for your input, guidance and friendship.

Professor G. Bath, who served as promoter for this study, for guidance, wisdom and friendship.

Dr. D.W. Verwoerd, my co-promoter, for the insight, guidance, wisdom and for being a real inspiration in my research career.

The South African Meat Board, for funding this project.

The Onderstepoort Veterinary Institute for providing the facility and laboratories used throughout the experiment.

Dr. Leon Marais, from the Veterinary State Services, for full support, contribution and friendship.

The Agrimetric Institute of the Agricultural Research Council, for their assistance with the statistical analyses.

SUMMARY

STUDY ON THE EFFECTS OF A NATURAL MAEDI VISNA VIRUS INFECTION ON SHEEP PRODUCTIVITY

By

BAPTISTE DUNGU-KIMBENGA

Promoter: Prof. G Bath

Co-promoter: Dr. DW Verwoerd

Department: Department of Veterinary Production and Ethology

A cohort study was conducted in order to measure the effect of the chronic indurative lymphocytic mastitis caused by the South African strain of Maedi visna virus (SA-OMVV) on the pre-weaning growth of lambs born of naturally infected and uninfected ewes kept under similar conditions. 50 naturally infected ewes and 40 controls from an MVV-free source were purchased and kept separately. All ewes were of the same breed – the Dorper- and 3 to 4 years old. From the adaptation period, through mating, pregnancy and lactation periods they were monitored for MVV antibodies and managed under similar conditions. The lambs were weighed at birth and thereafter every two weeks until the age of 90 days, when they were weaned. The ewes were slaughtered, their udders examined histologically and the lesions were assessed by counting typical lymphocytic follicles. Although the observed values indicated a correlation between the number of follicles in the udder and the reduction in the growth rate of the lambs, this was not statistically significant. Similarly, despite higher counts of lymphoid follicles in the udder of sero-positive ewes as compared to sero-negatives and the observed lower ewe productivity indexes (EPI) in infected ewes, no statistically significant differences were found in the EPI of ewes in different follicle categories.
The present study was a first attempt to evaluate the effect of the SA-OMVV infection on sheep productivity in South Africa

TABLE OF CONTENTS

LIST OF TABLES

1 LITERATURE REVIEW

1.1 MAEDI VISNA VIRUS INFECTION

1.1.1 HISTORY

MAEDI-VISNA is a composite Icelandic name corresponding to two clinical entities caused by the same slow virus, a non-oncogenic ovine lentivirus (10,18,41). Maedi(meaning dyspnoea) is characterised by a chronic progressive pneumonia and visna (meaning wasting), is characterised by a meningoencephalitis of adult sheep which leads to weakness and progressive paresis of hind legs especially.

The disease is also known as ovine progressive pneumonia or Montana sheep disease in the USA (34), zwoegersiekte in the Netherlands, la bouhite in France and Graaff-Reinet disease in South Africa (10,32,41). The first pathological description of a condition in the lungs of sheep fitting the picture of Maedi was given in 1915 by Mitchell (35), in South Africa, as a chronic pneumonia of sheep. He regarded the condition as an aberrant form of jaagsiekte (sheep pulmonary adenomatosis). Marsh, in 1923, described a chronic progressive pneumonia of range sheep in the state of Montana, USA (34). The confusion with jaagsiekte caused by Mitchell's report was resolved by De Kock in 1929. He was the first to realise that he was

dealing with two distinct diseases, often co-existing in the same animal: the chronic progressive pneumonia was characterised by severe lymphocytic proliferation and secondary hyperplasia of the bronchiolar epithelium. He named this condition Graaff-Reinet disease, after the experimental station from which the diseased animals were obtained (13).

In Holland , a chronic pneumonia of sheep called "zwoegers" was recognised since 1918, and the close relationship of zwoegersiekte with Montana sheep disease and the condition described by Mitchell in South Africa was pointed out later by Koens, as quoted by Palsson (41).

Lucam described the condition in France in 1942, and he considered it to be similar to Montana sheep disease and Graaff-Reinet disease (32).

From various other countries, progressive pneumonias of sheep have been reported and found to be closely related to maedi-visna virus (MVV) after serological tests and virus isolation. As reviewed by Houwers, besides South Africa, Iceland, France, the United State of America and the Netherlands, MVV-infection has been recognised or reported in Germany, Switzerland, the United Kingdom, Belgium, Greece, Italy, Spain and Bulgaria. In the Middle East, pathological and serological evidence have been found in Turkey and in Israel. Reports have also been published from Russia, India, China, Canada, Morocco, Algeria, Kenya, Mexico and Peru (22).

Australia and New Zealand seem to be the only major MVV-free geographical area of the world (22,53). This is probably due to the fact that the sheep in these regions descended from small numbers of early 19th century imports, which were apparently free from MVV, but also due to their isolated geographical position and severe restrictions on further importation in the

20th century (22).

In Iceland, maedi and jaagsiekte were both introduced by importation of Karakul rams from Germany in 1933. Due to the insidious onset and long preclinical period, maedi had spread unnoticed to many flocks when first recognised six years after importation. Annual losses in sheep, caused by maedi were around 15-30% in flocks (41, 50). Most of the early work on maedi-visna was done in this country, where maedi and visna were initially considered to be two separate diseases.

Visna was observed in Iceland around 1940, in several flocks, but only where maedi had already been causing losses for some time. Usually only a small number of animals in the flock showed signs of visna, but in some flocks losses caused by visna exceeded those of maedi. An association between these two diseases was therefore considered likely from the very beginning (51, 52). This was later supported in animal experiments: in 1967, a single virus was shown by inoculation experiments to be the causative agent of both the Maedi and Visna sets of symptoms (18). Subsequently maedi and visna are accepted to be two different manifestations of an infection with the same virus.

Visna in sheep has since been reported in several countries: Denmark (21), USA (9), Norway (29), Holland (48), United Kingdom (45). Usually, however, only a small number of sheep within a flock show clinical signs of visna.

In recent years, clinical manifestations other than maedi and visna have been more frequently reported, mainly a chronic arthritis in mature sheep and a chronic nonfebrile mastitis. Olivier et al. in 1981 (39) reported chronic arthritis characterised by swelling of carpal and tarsal joints among mature sheep affected with progressive pneumonia. The maedi-visna virus

could be recovered from the affected joints and similar lesions were reproduced by inoculation of the virus by various routes (38). These lesions do not seem to occur in Dutch MVV-infected sheep (25).

The chronic nonfebrile mastitis affecting sheep suffering from MVV infection was first reported by Cross et al. (6). Later, Olivier et al. in 1981 (39), studying naturally occurring ovine progressive pneumonia, found some of the sheep affected with chronic indurative mastitis with massive lymphoid proliferation. In 1985, Van der Molen et al.(61) described a reduction in milk production and retarded growth of lambs from affected dams, associated with MVV infection. In 1987, Van der Molen and Houwers provided proof of the association of the lymphocytic mastitis with MVV infection in an experimental study (60). Subsequently, Houwers et al. (23), studying a flock severely affected with MVV, found lymphocytic lesions in 53 per cent of the mammary glands but distinct lung lesions in only 10 per cent of the ewes. These results, and later on the work by Pekelder et al. in 1994 (43), suggested that this lymphocytic mastitis and the resulting reduction in milk production develops before lung lesions and is the most important feature of maedi-visna in Dutch sheep (25).

1.1.2 EPIDEMIOLOGY

As mentioned earlier, with exception of Australia and New Zealand, ovine lentivirus infection have been reported in all the major sheep-producing countries.

Breed susceptibility or predisposition to infection as well as expression of MVV-induced disease appears to exist, although it is unclear whether there is genetic predisposition to this (9,25). No breed-associated resistance has, as yet, been demonstrated. Experimental

intrapulmonary infections have suggested the Icelandic sheep breed involved in the MVV outbreaks to be more susceptible to development of lesions than Texel sheep (12). Cutlip et al. (8), in a retrospective study, showed a consistent difference between Columbia and Border Leicester sheep after experimental as well as natural infections with MVV, i.e. significantly more Border Leicester sheep developed lesions and the lesions were more severe. In addition, their data suggest that the relative resistance of Columbia sheep was not associated with the use of a certain virus strain.

Transmission of the disease occurs under conditions of close contact (25). Droplet infection via the respiratory route has been implicated, especially between adult animals (40). There is no evidence of transplacental nor genetic transmission of the virus (25). The infection of lambs from the ewe has been shown to occur through the colostrum (25).

No study involving South African virus isolates or breeds has been conducted to date. The situation of MVV in South Africa is discussed in 1.4.

1.1.3 CLINICAL DESCRIPTION

Following MVV infection, there is a long incubation period of 3-4 years (10,41) and clinical signs are seen mainly in adult sheep. The clinical stage may last from 3 months to 2 years. Infection with MVV principally affects the lungs and udder, although the central nervous system and joints may also be affected (10,41,44). Deterioration in bodily condition, respiratory embarrassment and dyspnoea are common clinical signs, while the body temperature and the pulse rate remain normal. Lactating ewes may present decreased milk

yield, with weak and small lambs. Anaemia of the hypochromic type, in advanced cases, and prolonged lymphocytic leucocytosis can be demonstrated several months before the affected sheep eventually dies. Sheep may also succumb to an associated acute bacterial pneumonia. (10, 41, 44)

In most reports on the mammary gland's susceptibility to the infection, the effect on the udder is of the chronic, non-febrile type and usually difficult to diagnose, the signs being subclinical or inconclusive.

A bilateral and diffuse induration of the udder, which may be accompanied by nodular hardening of the ventral part, can sometimes be palpated. The accessory lymph nodes are only slightly enlarged. Milk consistency and appearance remain normal, but secretion in some instances seems poor. (1, 10, 43, 55, 61)

Visna is characterised by a very insidious onset, and under field conditions only mature sheep seem to be affected. Clinical manifestations such as sheep lagging behind the flock when driven and gradual weakness of the hind legs are the first manifestations to be seen. This may be followed by abnormal posture, stumbling and progressive limb paresis, culminating in a total paralysis (10, 12, 38, 39).

A chronic, non-suppurative arthritis has been described as a manifestation which is sometimes associated with maedi-visna (38, 39). The carpal and tarsal joints are most commonly affected, often bilaterally. The joints are swollen, painless and the joint capsules and synovial membranes are thick. Various degrees of lameness are observed. Arthritis of this type seems to be refractory to treatment.

MVV causes a lifelong infection. In acutely infected flocks, animals that do not succumb

from the disease or other opportunistic infections, becomes latently infected (41).

1.1.4 PATHOLOGY

The lungs of maedi-affected sheep do not collapse when removed from the thorax and often retain the impressions of the ribs. Both the lungs and their associated lymph nodes are increased in weight. The lesions are distributed throughout the lungs so that affected lungs are uniformly discoloured or mottled grey-brown and of a firm texture. The prime histological feature is infiltration of the interalveolar spaces by mononuclear cells often associated with peri-bronchiolar lymphoid hyperplasia and smooth muscle hypertrophy (16, 33, 51, 61). The mediastinal and tracheobronchial lymph nodes are characterised by a hyperplasia of their cortical and paracortical areas.

Udders affected by MVV are diffusely indurated and associated lymph nodes may be enlarged. The diffuse induration is seen on both udder halves, which differs from mastitis caused by bacterial infection, where according to a comparative study by van der Molen et al. (60) macroscopic changes are nodular and usually limited to one udder half. In the same study and others (9, 39, 61), it was found that the reduction in milk production was the consequence of the compression of the lactiferous sinuses. Histological examination reveals diffuse infiltration by lymphoid cells, periductal lymphoid proliferation and fibrosis (1, 9, 23, 61). The lymphoid proliferation often results in the formation of lymphoid nodules or follicles sometimes with active germinal centres. In the periductal area, they may protrude into the lumen and cause compression and distortion of the ducts, but are also found in the lobules.

The brain and spinal cord of sheep affected with visna appear normal on macroscopic examination. On microscopic examination however, an inflammatory infiltration of the leptomeninges of the brain is commonly observed and does frequently extend to the spinal meninges. The lesions are mainly distributed around the ventricular system affecting both grey and white matter. The meningitis is an early feature of the pathological lesions, and can be detected 1-2 weeks after infection. The inflammatory infiltrates consist of mononuclear cells, predominantly lymphocytes together with macrophages. The inflammatory changes can sometimes be severe resulting in necrotic foci, with destruction of the myelin sheath. The choroid plexus can be affected to varying degrees, sometimes with massive lymphoid proliferation. There is little destruction of neurones, and no changes have been seen in the peripheral nervous system (44, 48, 52).

Other pathological alterations, less commonly reported, involve the carpal joints and rarely the tarsal joints. Macroscopic lesions observed involve an increase in synovial fluid, which become more viscous and cloudy. Lymphoid follicles, with active germinal centres are found in the synovia on microscopic examination (9, 38, 39).

1.1.4. THE MAEDI VISNA VIRUS

The virus is a member of the lentivirus subgroup of the retrovirus family that includes the human immunodeficiency viruses, HIV 1 and 2, the caprine arthritis-encephalitis virus, and others. All of the lentiviruses cause persistent, lifelong infection and diseases which are characterised by long incubation periods and a chronic debilitating clinical course (37).

The Maedi-visna virus (MVV) genome, as in other lentiviruses, consists of a single stranded RNA molecule containing 3 major genes that encode the major structural proteins of the virus. These are the *env* gene that encodes the viral glycoproteins, the *gag* gene that codes for viral core proteins, and the *pol* gene that codes for the viral RNA-dependent DNA polymerase (reverse transcriptase) (44). The number of polypeptides in mature virions has been variously reported as 10 to 25 (37, 44). Of those only 4 comprise about 80% of the protein mass of the virion i.e. p25, p16, p14 and gp135. The p25 polypeptide is the main component of the virus core and comprises about 40% of the protein mass of the virion (37, 44).

Lentiviruses share nucleotide homology and serological properties: the ovine maedi-visna virus and the caprine arthritis-encephalitis have shown extensive homology in their gag-pol genes and gene products (20, 22, 27, 44, 65).

1.2 DIAGNOSIS OF MVV INFECTION

The clinical signs induced by MVV infection are not specific enough to enable a firm diagnosis to be made and confirmation must be attempted by laboratory procedures. Pathology and histological findings are not pathognomonic for the infection and the diagnosis has to be confirmed either by direct detection of the virus or viral components (viral RNA, proviral DNA, viral proteins), or indirectly by detection of antibodies against MVV (11, 17, 20, 49, 58). For the direct detection of the virus, virus isolation techniques by co-cultivation with permissive ovine cells, such as choroid plexus cells are most commonly used (10, 20, 42). Virus isolation is more commonly used to identify and confirm the presence of the MV

virus rather than as a large-scale diagnostic technique due to its laborious nature. Furthermore, the virus can be detected only in a very small proportion of infected animals, as only one in 10^6-10^7 leukocytes contains the viral information (44).

MVV components, such as viral RNA or proviral DNA, can now be detected by *in situ* hybridization (26, 50) and the polymerase chain reaction (PCR) (26, 66). Viral proteins can be detected in sections of infected tissues by means of the immunoperoxidase technique, using specific monoclonal antibodies. Although these techniques may in the near future increase the diagnostic possibilities for this infection, they are still not practical as large-scale diagnostic tools.

Sheep respond to infection with MVV by a persistent production of antibodies. Such antibodies can be detected using different techniques: virus neutralisation, complement fixation, immunofluorescence, agar gel immunodiffusion (AGID), passive hemagglutination and the enzyme linked immunosorbant assay (ELISA) (20).

In screening programmes aimed at rendering flocks free of MVV infection, the AGID and ELISA tests appear to be most appropriate (20). The AGID test, using antigen containing the glycoprotein gp 135 has been used in many screening programmes, being a simple and reliable flock test. However the reading of plates in this test is subjective, cannot be automated and experience is required (20, 31, 53). The AGID test is also known to be less sensitive than the ELISA, although its specificity might be higher (20, 53).

The first ELISA described for MVV was an indirect ELISA using disrupted and pelleted virus as antigen (24). However the cost of viral antigen needed in this test, which is produced in inefficient and time-consuming tissue culture systems, is prohibitive for its use in large

screening programmes (20, 31, 53). Indirect ELISA using recombinant proteins expressed from cloned specific genes coding for antigenic viral proteins have been successfully used in human HIV and in MVV (3, 30, 31, 65). With the expression of the major core protein of lentiviruses, p25 (17, 48, 65), the large-scale production of recombinant protein provides an economic, homogenous, and convenient source of immunoreactive antigen preparations.

The p25 protein is among the most conserved of MVV proteins (49) and has group-specific reactivity, whereas neutralising antibodies, directed mainly against the envelope glycoprotein gp135 are thought to be strain-specific due to the high variability of the *env* gene (44, 31).

Studies conducted mainly with HIV have shown that both the gag p25 and the transmembrane epitope are needed to produce a sensitive and specific screening assay (28, 31). During infection, antibodies raised against these two antigens are detectable. However, as the infection progresses to the clinical disease, there is a shift of the immune response from initially being mainly anti-p25, to predominantly TM specific. Therefore, an ELISA test was developed at OVI using these two antigens, cloned and expressed as a single fusion protein, TM/p25, called COM-2 (2).

The COM-2 ELISA has been evaluated in comparative studies against commercially available tests and has proven to be very sensitive and specific (2).

1.3 EFFECT ON SHEEP PRODUCTIVITY

D.J. Houwers (25) summarises the overall effect of MVV infection on sheep productivity as

follows:

a) Direct effects:

- Loss of sheep that die from the disease-complex or are culled in an advanced stage of emaciation.
- Increased culling rate resulting in increased replacement rate.
- Decreased pre-weaning growth rate of lambs due to mastitis in ewes

b) Indirect effects:

- Affected animals are prone to secondary infection
- Low market value of breeding sheep and reduced chance of sale in international export markets.

Studies on the effect of clinical and subclinical MVV infection on sheep productivity, however, have shown that assessing the damage caused by MVV infections is a complicated task. Factors such as husbandry, management and the structure of the local sheep industry have to be taken into account (4, 14, 22, 56, 64). Although in some studies no statistically significant differences could be found in lamb weight between infected and non infected animals (14, 56), the reduction in milking potential of ewes, due to the indurative lymphocytic mastitis has been shown to generally result in poor pre-weaning growth. There is also a possibility of an increased mortality rate of the lambs (1, 11, 14, 22, 55). The gross output of an infected flock can thus be affected.

In a study conducted in Holland, the effect of the indurative lymphocytic mastitis caused by infection with MVV was quantified by comparing the pre-weaning growth of lambs from

infected and uninfected ewes under the same conditions. Histological changes in the udder were correlated to the growth of lambs. A statistically significant association was found between the number of follicles in the udder and the reduction in the growth rate of lambs. Lambs from ewes with the mean number of follicles weighed 1.7 kg less at weaning (43).

It is important to emphasise that the strains of the virus found in Holland, and in Europe in general, may be much more virulent than the South African ovine lentivirus (43, 62). This is suggested by the same European study, where sero-positive ewes were mixed with non-infected ewes, and during the 9 months of the experiment, 76% of the latter seroconverted and amongst those which seroconverted, 90% showed maedi lesions in the udder. Some ewes died after showing clinical signs of maedi(43).

1.4 THE SITUATION OF MVV INFECTION IN SOUTH AFRICA

The effect of MVV infection on South African sheep production is uncertain as no specific study has yet been conducted. The disease was first described in South Africa by Mitchell (35) in 1915, who confused the condition with jaagsiekte. De Kock was the first in 1929 to realise that he was dealing with two distinct diseases, often co-existing in the same animal: he named the chronic pneumonia Graaff-Reinet disease (13). The disease seemed to disappear, and South Africa was thought to be free of maedi-visna until a lentivirus was isolated in 1986 from lungs of sheep suffering from jaagsiekte (42).

A significant genetic divergence was demonstrated between the virulent MV virus isolated in Iceland, the caprine arthritis-encephalitis virus (CAEV), and the SA-OMV viruses by means

of endonuclease restriction analysis, nucleic acid hybridisation techniques and nucleotide sequence studies (46). This study demonstrated that the SA-OMVV was more closely related to MVV than to CAEV, but was sufficiently distinct to be regarded as a separate subgroup. A phylogenetic history, based on the divergence of nucleotide sequences, suggested that the two ovine lentiviruses have been evolving independently for at least 42 years (46).

A limited survey was performed and it was found that a number of areas were affected: in Smithfield (Free State Province), for example, 29% of 79 sheep were positive for Maedi (42). Although this small serological survey revealed a wide distribution of the virus, it seemed to have lost its pathogenicity (42). Because of the prevalence of sero-positive flocks, it could be hypothesised that a local milder strain of MVV is causing economic losses as an erosive subclinical disease rather than the classical clinical forms of Maedi visna.

Due to the costs involved in importing the diagnostic kits, no large surveys were carried out. The need for developing local diagnostic kits, and studying the effect of MVV on sheep productivity, caused the Onderstepoort Veterinary Institute (OVI) to initiate projects studying different aspects of the disease, including:

- The development of diagnostic techniques such as serology and viral or viral particle detection assays.(2, 65)
- Epidemiological surveys, based on serology, aimed at determining the distribution and prevalence of the infection in South Africa.
- Abattoir surveys to study the pathology of concurrent infections in South African sheep.
- The effect of the SA-OMVV infection on sheep productivity and the economics of

eradication strategies.

The present study is therefore part of a larger OVI program which is planned to run for a much longer period.

Using the expressed fusion protein p25 as antigen in a Western blot test and in an ELISA test (65), sera from a closed flock of 2400 sheep with a history of lung problems (the Goedemoed prison flock in Aliwal North – Free State Province) were tested. A seroprevalance for MVV antibodies of 80% was established (York and Dungu, 1992; unpublished data). Since then MVV infection has been confirmed clinically and histologically (62), as well as with commercially available test kits. The results of this pilot study lead to the design of this cohort study on the effect of South African natural MVV infection on sheep productivity.

1.5 PRODUCTIVITY PARAMETERS

Various production factors influence the relative economic efficiency of a sheep enterprise. They also provide a practical way of assessing the economic impact of clinical and subclinical diseases on a sheep production unit. Some critical factors include the number and the weight of lambs weaned. In commercial mutton units, weight gain from birth to weaning is usually the most convenient production factor to use, in order to assess the economic impact of both clinical and subclinical disease (15, 36, 43). Birth weights are genetically correlated with average daily gains and weaning weights.

Ewe productivity, defined as number (or total weight) of lambs weaned per ewe mated, is a

composite trait with multiple contributing factors such as fertility, litter size, neonatal survival, preweaning survival and lamb growth (15).

Besides genetic influence, the ewe productivity will be largely dependent on the ability of the ewe to produce sufficient milk of appropriate quality, in order to insure the optimal growth of their progeny.

2 OBJECTIVES OF THE STUDY

2.1 HYPOTHESIS

Maedi-visna, caused by SA-OMVV has a detrimental effect on sheep productivity due to reduced weight gain in lambs, resulting from the associated subclinical mastitis.

2.2 AIM OF THE STUDY

To determine whether the subclinical mastitis, with proliferation of lymphoid follicle in udder tissue, due to the infection with SA-OMVV, has a detrimental effect on sheep productivity.

3 MATERIALS AND METHODS

3.1 EXPERIMENTAL DESIGN

Fifty Dorper ewes from the heavily infected closed flock of Goedemoed prison in the South-east Free State were selected (see section 1.4). They were three to four years old (6 or early 8 tooth) and confirmed to be sero-positive for MVV by two ELISA results with the OVI ELISA (2, 65), and a commercially available test, i.e. a French ELISA test kit [1*]. The preclinical period of Maedi-visna is generally 3 to 4 years' (41), although antibodies to MVV are present earlier. This age group, besides being the most productive in a sheep enterprise, is more likely to be in the critical period of developing the disease, and subsequently should be best suited for a study of the effect of the disease on sheep productivity.

The control group consisted of forty Dorper ewes of the same age. They were selected from a

---

[1*] KIT ELISA Visna Maedi, Institut Pourquier - Montpellier

flock with no history of lung problems, and confirmed sero-negative to MVV antibodies by two serological tests, conducted on the flock basis, with the same OVI ELISA and the commercial test kit.

More sero-positive ewes than sero-negative were used in order to prevent any difficulty in the comparison of results, in the event of loss of infected ewes due to Maedi visna or any opportunistic disease that could be associated with MVV infection.

The two sero-groups were brought to OVI and kept in two widely separated groups of pens. Two pens were used for each sero-group, with 20 sero-negative ewes in each for the control ewes, and 25 ewes in each for the sero-positive ewes. They were allowed an adaptation period of two months, during which they were monitored for any condition that could affect the study.

No common facilities were shared by the two sero-groups. Different shepherds attended to each sero-group and the sheep were fed ad lib on the standard OVI sheep diet (addendum 1).

Four Dorper rams of the same genetic background, i.e. the progeny of one sire, were purchased from a flock with no history of lung problems. Before selecting the rams, this flock was also serologically screened for MVV antibodies and no sero-positive result was recorded. The four rams were brought to OVI, dewormed and sampled for faecal egg count (47)and vaccinated for pulpy kidney (Onderstepoort Enterotoxaemia Vaccine, Onderstepoort Biological Products, Pretoria). In collaboration with the Department of Theriogenology, Faculty of Veterinary Science, University of Pretoria, the rams were examined, evaluated and certified sound for breeding purposes. They were initially kept far away from the ewes.

3.1.1 ADAPTATION PERIOD

On the day of their arrival at OVI, all selected sheep were weighed, condition scored and re-tagged according to the OVI numbering system. In collaboration with the Helminthology section of the OVI, faecal samples were collected from all animals and they were dewormed with Rafoxanide(Ranide®, Logos Agvet). They were monitored for faecal egg count and retreated if necessary, until their egg counts were negative or acceptably low (egg per gram of faeces less than 100)

All sheep were vaccinated for pulpy kidney and for blue tongue with the Onderstepoort vaccines, according to the producer's recommendations (see addendum 2).

The weight, recorded with a scale of 0.1kg accuracy, and the condition score of all ewes were monitored every second week. They were also bled every month and their sera tested for MVV antibodies.

3.1.2 BREEDING

Two weeks prior to joining, the rams were brought in close proximity to the ewes, in order to generate biostimulation, by the so-called "ram effect" (5).

All ewes were weighed on the joining day.

Two rams were used for each sero-group. They were left with the ewes for a period of 35 days in one pen, and then moved for the same period to the other pen of the same sero-group.

3.1.3 POSTBREEDING AND PREGNANCY PERIOD

Seventy days after joining, a pregnancy diagnosis using trans-abdominal ultrasound (Toshiba® Sonarlayer SAL-32A) was performed on all ewes.

All ewes were monitored monthly during the first 3 months of pregnancy for faecal egg count, weight, condition score and serology. A routine clinical examination was carried out weekly.

3.1.4 LAMBING AND LACTATION PERIOD

Lambing was monitored daily and all new-born lambs were weighed and eartagged within two days of birth. To monitor the growth of the lambs, their weights were recorded every two weeks, using a scale of 0.1kg accuracy. All lambs or ewes with clinical conditions other than those which could be associated with MVV infection, were attended to and, if necessary, eliminated from the study (see table 4.3.4).

3.1.5 MICROBIOLOGICAL AND CYTOLOGICAL EXAMINATION OF MILK SAMPLES

Milk samples were collected from each ewe three times, in early, mid and late lactation. Cytological and bacteriological examinations were conducted on each sample. For the cytological examination, the somatic cell count (SCC) was performed in order to determine the level of non-specific disturbance associated with mechanical mastitis. Mastitis associated with bacteria was evaluated by a combination of the SCC and the total bacterial count on agar plates.

3.1.6 WEANING

Lambs were weaned at the age of approximately 90 days. On the weaning day, blood samples were collected from the lambs for serological tests and their weight recorded. Milk and blood samples were collected from the ewes, and their weights and condition scores recorded.

3.1.7 SLAUGHTERING OF EWES

Approximately three weeks after weaning, the ewes were slaughtered. Their udders were collected and histologically examined by the pathology department, OVI.

3.1.8 HISTOLOGICAL EXAMINATION OF THE UDDER

Udder tissues from all ewes were collected at slaughter. Histological sections were prepared from the udder glandular tissue sampled at two sites, one central and one from the tissue

adjoining the lactiferous sinus. The tissues were fixed in 10 per cent buffered formalin and embedded in paraffin. Once prepared, the histological sections were stained with haematoxylin and eosin and examined for typical MV lesions (63). The extent of the lesions was measured by counting the number of lymphoid follicles per tissue section of standard size and summing the counts from the four sections from each udder, according to a modification of the method described by Pekelder and others (44). Based on these total follicle counts, the udders were divided into three classes: class 1 udders with 0 to 5 follicles; class 2 udders with 6 to 15 follicles; and class 3 with more than 15 follicles.

3.2 OBSERVATIONS / ANALYTICAL PROCEDURES

The weight of all experimental ewes and their lambs was recorded and stored in a database for further analysis. For ewes, the mating and weaning weights were used for calculations, although they were weighed several times (see experimental design). The lambs' birthweights and weaning weights were used for calculation of different rates described below.

The rates calculated were:

1. Conception rate (number of ewes lambed divided by the number of ewes mated).
2. Lambing percentage (number of lambs born divided by the number of ewes mated).

3. Fecundity (number of lambs born divided by the number of ewes lambed).
4. Perinatal lamb mortality (number of lambs died divided by the number of lambs born).
5. The average daily gain (ADG) of lambs for the pre-weaning period. The pre-weaning ADG was determined by dividing the mass gained from birth to weaning by the number of days for the same period.
6. The age and sex corrected weaning weight (ASWW). The corrected weaning weight for 100 days is first determined:

   The birth weight plus hundred times the ADG [Birth weight +(ADG x 100)]

   The least square mean is used to determine the correction factors for the ram and ewe lambs. These correction factors are then subtracted from the age corrected weaning weight to give the ASWW (15).
7. The ewe productivity index. For each ewe the ASWW of all her weaned lambs are added to get the total weaning weight per ewe (TWW). The ewe productivity index is then calculated as follows:

   (TWW / average TWW for all ewes) X 100

8. The weaning percentage (number of lambs weaned divided by the number of ewes mated).

3.3 STATISTICAL ANALYSIS OF DATA

Differences between the two groups, i.e. sero-positive versus sero-negative, and the association between different variables and rates were statistically studied by analysis of covariance and f-probability, using Gynstat 5 Release 3.2. (57).

Using regression analysis, the lambs' corrected weaning weights and pre-weaning average daily gains were compared between the infected and the control groups. Mean variates were compared in order to determine if the differences found in the means of these values were statistically significant between the groups.

Regression analysis was also used to determine the relationship between the number of lymphoid follicles in the dam's udder and the lamb's pre-weaning ADG on the one hand, and the ewe's productivity indexes (EPI) and the number of follicles on the other hand. Mean variates were compared in order to determine if the differences found in the means of these values were statistically significant between the follicle categories.

All collected data were stored in a data base programme, dBase IV. Different files were created, in order to allow all the calculations to be performed.

Special computer applications, obtained from the Grootfontein Agriculture Development Institute, were used to calculate the lamb age and sex corrected weaning weight, as well as the ewe productivity index.

4 RESULTS

4.1 SEROLOGICAL MONITORING

All sero-positive ewes were selected on the basis of their previous results, during different serological screenings conducted for the whole Goedemoed flock. Each ewe was tested at least 4 times. The sero-negative ewes were also selected after consistent negative results in their flock of origin. The flock of origin was screened twice, and the selected ewes were tested one more time before their relocation to Onderstepoort.

Blood samples were collected monthly during the adaptation period, every second month during pregnancy, at weaning and on the day of slaughter. ELISA tests were performed on those samples.

Control ewes (sero-negative group) remained sero-negative throughout the whole study period.

Serological results of ewes in the sero-positive group are given in table 4.1.1.

Table 4.1.1: SUMMARY OF SEROLOGICAL RESULTS IN THE MVV SERO-POSITIVE GROUP.

Blood collection	ELISA test used	Number of animals tested	Positive serology	Dubious serology	Negative serology
1st test on arrival	COM-2	50	45	4	1
1st test on arrival	Pourquier	50	45	4	1
2nd bleeding/ adaptation period	COM-2	49	41	5	3
Mating time	COM-2	47	42	4	1
Mid-gestation	COM-2	47	40	6	1
Weaning period	COM-2	36	35	1	0

4.2 MATING AND LAMBING

After the adaptation period, 38 and 47 sero-negative and sero-positive ewes respectively were mated. The mating and lambing results are given in table 4.2.1.

During the adaptation period, 5 ewes died or were culled for different reasons (see adendum 3), 3 in the sero-positive group and 2 in the sero-negative group. Although one of the 3 sero-positive ewes showed lesions of MV on post-mortem evaluation, in none of the 5 ewes the cause of death or the reason for culling could be associated with MVV infection.

Table 4.2.1: MATING AND LAMBING RESULTS

	Sero-negative groups	Sero-positive groups
Ewes at the beginning of the study	40	50
Ewes mated	38	47
Average mating weight of ewes	53.83 kg	52.38 kg
Ewes diagnosed pregnant	36	43
Ewes lambed	36	43
Lambs born alive	40	48
Stillborn	1	1
Single lambs	30	36
Sets of twins	5	6
Average birth weight of lambs	4.67 kg	4.52 kg

Table 4.2.2: REPRODUCTIVE RATES UP TO LAMBING

RATES	SERO-NEGATIVE EWES	SERO-POSITIVE EWES
Conception rate	94.73%	91.49%
Lambing percentage	105.26%	102.12%
Twinning rate	23.8%	25.0%
Fecundity	11.11%	11.16%

4.3 LACTATION AND WEANING PERIODS

The lambs were weaned at the approximate age of 90 days. On weaning day, they were weighed and a blood sample was collected from each of them for serological examination. Serological results of lambs and ewes at weaning are given in table 4.3.1. Productivity data and rates are given in table 4.3.2 and 4.3.3 respectively.

Table 4.3.1: SUMMARY OF SEROLOGICAL RESULTS AT WEANING

GROUP	Number of animals	Positive ELISA	Dubious ELISA	Negative ELISA
Sero-positive ewes	36	35	1	0
Sero-negative ewes	29	0	0	29
Lambs of sero-positive ewes	38	0	1	37
Lambs of sero-negative ewes	33	0	0	33

Table 4.3.2: SUMMARY OF PRODUCTIVITY DATA DURING LACTATION

Category	Sero-negative ewes	Sero-positive ewes
Ewes weaning lambs	29	36
Lamb loss before weaning	5	10
Lambs weaned	33 (1)	38
ASWW (corrected for age and sex)	26.636 kg	25.176 kg
ALWW (corrected for age and birth status)	26.888 kg	25.029 kg

(1) One pair of twin lambs was not monitored further due to an unrelated health problem.

Table 4.3.3: SUMMARY OF PRODUCTIVITY RATES DURING LACTATION

	Sero-negative group	Sero-positive group.
Weaning percentage	91.66	88.37
Pre-weaning lamb mortality	12.5%	20.8%
Pre-weaning average daily gain (gram per day)	219.636	208.132
Average ewe productivity index	112.71	93.42

Table 4.3.4: CAUSES OF MORTALITY IN LAMBS DURING LACTATION

	Sero-negative group	**Sero-positive group**
Environmental factors during lambing period:		
• Ewe not bonding with lamb during bad weather	1	1
• Trauma	1 (broken leg)	2 (1 broken leg, 1 ruptured liver)
• Other		**4 (1 spinal cord abscess, 1 paralysis, 1 cloudburst, 1 undetermined)**
Sudden death	1	2
Problem with the ewe (sick dam)	2 (1 sick dam, 1 dam with no milk)	
Other		1 (during tail docking)
Total	**5**	**10**

4.3.1 STATISTICAL ANALYSIS

Using regression analysis, the lambs' corrected weaning weights and pre-weaning average daily gains were compared between the infected and the control groups. Mean variates were compared in order to determine if the differences found in the means of these values were statistically significant between the groups.

Corrected weaning weight	Group	Mean of variate	Standard error
	Sero-negative	26.331	0.624
	Sero-positive	25.532	0.589

F-probability ($P<0.01$): 0.17780 i.e. not significant

Average daily gain	Group	Mean of variate	Standard error
	Sero-negative	217.18	6.15
	Sero-positive	211.59	5.81

F-probability ($P<0.01$): 0.2560 i.e. not significant

There were therefore no statistically significant ($P<0.01$) differences in the average daily gain and the corrected weaning weight between lambs born from infected and non infected ewes.

4.4 MONITORING OF EWES UDDERS AND CORRELATION WITH LAMB GROWTH

4.4.1 MICROBIOLOGICAL AND CYTOLOGICAL EXAMINATION OF MILK SAMPLES

Table 4.4.1 gives a summary of results obtained after the evaluation of milk samples collected 3 times during the lactation period from each ewe lactating. In the majority of cases, bacterial growth were not specific enough to be associated with the high somatic cell count (SCC). The bacteria identified in these cases were considered to be contaminants. We therefore interpreted the somatic cell count without discriminating between a mechanical and a bacterial cause.

Table 4.4.1: SUMMARY OF MILK EVALUATION: MASTITIS CASES ACCORDING TO SCC WITH OR WITHOUT BACTERIA (number of cases / number of ewes tested in the group)

		Sero-negative ewes	Sero-positive ewes
Early lactation	Both half udders	4/33 (12.1%)	2/28 (7.1%)
	One half udder	3/33 (9.1%)	1/28 (3.6%)
Mid lactation	Both half udders	3/32 (9.3%)	3/38 (7.9%)
	One half udder	5/32 (15.6%)	7/38 (18.4%)
End lactation	Both half udders	2/29 (6.9%)	2/37 (5.4%)
	One half udder	5/29 (17.2%)	2/37 (5.4%)

- One ewe in the positive group (no.3602) showed mastitis in both halves for all three examinations.
- One ewe from the negative group (no.3345) showed mastitis in one or both halves in all three examinations.
- Six ewes, 3 sero-positives and 3 sero-negatives, showed mastitis in one or both halves in 2 of the examinations.

4.5 POST MORTEM EVALUATION OF UDDERS AND CORRELATION WITH LAMB GROWTH

Approximately three weeks after weaning, ewes were slaughtered and their udders evaluated as described earlier (see materials and methods). Depending on the number of lymphoid follicles found, they were classified in 3 categories, as illustrated in table 4.5.1.

Table 4.5.1: DISTRIBUTION OF THE EWE GROUPS ACCORDING TO UDDER CLASSES

Udder class	**Sero-negative ewes n=29**	**Sero-positive ewes n=37**	**Total**
Class 1 (0 to 5 follicles)	28 (96.6%)	18 (48.6%)	46 (69.7%)
Class 2 (6 to 15 follicles)	1 (3.4%)	8 (21.6%)	9 (13.6%)
Class 3 (more than 15 follicles)	0 (0%)	11 (29.7%)	11 (16.7%)

Table 4.5.2: DISTRIBUTION OF LAMBS ACCORDING TO UDDER CLASSES OF EWES

(number of lambs and the percentage in the sero-group)

Class	Lambs in sero-negative group	Lambs in sero-positive group
Class 1 (0-5 follicles)	31 (93.9%)	18 (47.36%)
Class 2 (6-15 follicles)	2 (6%)	8 (21.05%)
Class 3(more than 15 follicles)	0	12 (31.57%)

Table 4.5.3: AVERAGE DAILY GAIN OF LAMBS FOR EACH UDDER CLASS

Class	Average daily gain of lambs (g/day)
Class 1 (0-5 follicles)	222.2
Class 2 (6-15 follicles)	199.7
Class 3 (more than 15 follicles)	198.3

4.5.1 STATISTICAL ANALYSIS

Using regression analysis, the relationship between the number of lymphoid follicles in the dam's udder and the lamb's pre-weaning ADG on the one hand, and the ewe's productivity indexes (EPI) and the number of follicles on the other hand were calculated for all animals in the study. Mean variates were compared in order to determine whether the differences found in the means of these values were statistically significant between the follicle categories.

Table 4.5.4: STATISTICAL ANALYSIS: correlation follicle class-ADG-EPI

Follicle category	**Mean variates: EPI**	**Standard error**	**Mean variates: ADG**	**Standard error**
1	102.8	2.46	216.49	5.19
2	95.09	5.57	208.61	11.34
3	92.29	5.04	209.88	10.46

F-probability (P<0.01): EPI, 0.118

ADG, 0.751

The differences in the ADG of lambs born from ewes in different follicle categories were not statistically different.

No statistically significant differences were found in the EPI of ewes in different follicle categories.

5 DISCUSSION

To the best of our knowledge, no study has ever been conducted to determine the effect of MVV on the productivity of South African sheep. This study was therefore aimed at determining whether the SA-OMVV would cause the low lamb pre-weaning growth observed in sheep infected with MVV in other parts of the world. A mutton breed, the Dorper, which represents the largest mutton breed in South Africa, was used for the study and its outcome would thus more likely be useful to mutton rather than wool producers. The Goedemoed flock, which is made up exclusively of Dorper sheep, provided an ideal opportunity to select naturally infected ewes since 80% of the 3500 sheep in the flock showed antibodies to MVV using an ELISA test. Due to the differences in breed susceptibility to MVV infection observed in other parts of the world (9,12), the results of this study cannot necessarily be extrapolated to other South African breeds. However, the fact that the infection occurs in South African sheep, without any apparent clinical effects suggests that the effect of SA-OMVV on South African breeds is milder than that seen in Europe.

Genetic factors are known to influence ewe productivity. In order to circumvent or minimize that bias, we used the same breed of sheep as controls. The rams used in the study were also selected for their genetic background: they were all the progeny of one sire. The adaptation period gave us the opportunity to adapt these genetically similar ewes, but coming from two

different parts of the country, into similar management and husbandry conditions. Data obtained at the end of this period did not show any discrepancies between the ewes in the 2 groups (see table 4.2.1).

5.1 SEROLOGICAL MONITORING

Maedi visna virus causes a persistent and lifelong infection in sheep. However, fluctuation in the level of detectable antibodies has been reported by many authors (2, 11, 28, 31). Although a lot of research aimed at understanding the immune response to different antigens of the human lentivirus HIV has provided valuable information, there are still many aspects in the MVV immune response that are unclear. Following a long incubation period, the drop in antibody response to capsid antigens, such as the p25, toward the onset of the clinical disease has been well established in HIV, and is assumed to also occur in MVV infection (2, 30, 31). This factor, added to others such as the restriction in viral replication during the latent phase of the infection, the high mutation rate resulting in antigenic variation of the virus in MVV-infected sheep (44), and the limitation of antibody detection assays used currently in MVV serology, could explain the recorded fluctuations in antibody titres.

It must be stressed that a fluctuation in serological results of sero-positive ewes was not observed in specific animals. When re-tested a few months later, these animals clearly showed a positive titre again. Another factor to be taken in account in the interpretation of serological results is the cut-off value (the test result value selected for distinguishing between negative and positive results). Low serological titres were sometimes recorded and

were usually difficult to interpret. In routine serology, these cases (referred to as dubious in our study) require a re-submission of sera from the same animals after a certain period and a second testing. In our study, dubious results which mostly occurred in the sero-positive group, were assumed to be sero-positive.

Lambs were serologically tested for MVV antibodies at weaning (see table 4.3.1) and on the day of slaughter (data not shown). The negative serological results observed in lambs born from infected ewes were in accordance to previous observations (11,25). Unlike some lentiviral infections such as HIV in humans, there is no evidence of *in utero* transmission of MVV from the ewe to the lamb (25). Transmission from ewe to lamb has been shown to occur mainly through the colostrum, presumably by means of infected monocytes (25). Transmission of the virus under conditions of close contact remains one of the most important routes of natural infection, however. Sero-conversion of the lambs (or the production of detectable antibodies) did not seem to occur by the age of 3 months, when they were slaughtered. One lamb born from a sero-positive ewe had a very low titre of antibodies to MVV antigen.

Sero-conversion has been reported to occur between 5 weeks and 11 months or more after birth (25). It was difficult in our study to assess whether lambs born from infected ewes were lactogenically infected or not, as they did not show antibodies after 3 months. Considering results obtained in their flock of origin, Goedemoed, where sero-prevalence in lambs born from infected ewes was extremely low under the age of one year (unpublished data), it could be speculated that the lactogenic mode of transmission was not predominant in our study. Horizontal transmission seems to be the main route for infection in the Goedemoed flock.

5.2 PRE-LAMBING PERIOD

Although 5 out of 47 ewes mated in the sero-positive group (10.6%) did not conceive, as compared to 2 out of 38 in the sero-negative group (5.26%), no evidence of a causal association between these conception rates and the presence of MVV infection could be found. None of the 5 non-pregnant sero-positive ewes had signs of MVV infection, either clinically or after post-mortem evaluation. In general, the conception rates in both groups were within the norms of the breed and no statistically significant difference was found. Studies by different authors have shown no correlation between conception rate and MVV infection (14, 43, 56), and the pathogenesis of MVV infection does not indicate a possibility of a drop in the conception rate unless due to the loss of general condition of animals caused by clinical disease. The same authors also did not see a statistically significant difference in the lambing percentage, the twinning rate and the fecundity of the ewes. The low pre-weanning lamb growth associated with MVV infection seems to be the consequence of a drop in milk availability, resulting from the induced lymphocytic mastitis, rather than a compromised ability of the dam to give birth to a healthy lamb. In our study, no statistically significant differences were found between the two sero-groups for these rates.

5.3 LAMBING AND LACTATION PERIOD

Any mortality occurring during this period was accurately recorded and a thorough post-mortem evaluation performed. The high lamb mortality rate observed especially during the

first month post-lambing, could principally be attributed to unforeseen reasons not related to MVV infection. The lambing season unfortunately coincided with abnormally heavy rains. As the animals were kept in open pens, it was not always possible for the dams to protect their offspring in bad weather. It was also difficult for the personnel involved in the study to intervene when bad conditions prevailed during the night and over weekends. Most of the mortalities occurred during such times. Of the 15 lamb mortalities recorded, 9 (or 60 %) could be associated with bad weather conditions (Table 4.3.4). Management-related conditions could be associated with 4 fatalities (26.6 %): 3 cases of sudden death, and one death associated with tail docking. Two lamb mortalities and one pair of twins were removed from the study for reasons related to the dam: one ewe had a liver abscess and two ewes suffered from chronic weight loss and produced very little milk. No definite diagnosis could be made on postmortem and histopathological examinations of these 2 ewes and no antibodies to MVV were detected (the 2 ewes were in the sero-negative group). In summary, no lamb mortality in either of the groups could be attributed to MVV infection (table 4.3.4). Productivity parameters and rates such as the EPI, the lamb ADG and the corrected weaning weight are critical for assessing the viability of a sheep production unit. They were used in this study to assess the possible effect of reduced milk production due to MVV infection on ewe productivity measured, in this case, by lamb growth. Although the observed means of most rates tended to be higher in the control group, no statistically significant ($P<0.01$) differences were found between the two groups for critical variables such as the ADG and the corrected weaning weight of lambs. It is important to note that in both cases the mean values were quite close and the calculated standard errors fairly high. This could be due to the small sample sizes in the study. For practical reasons, it was difficult to obtain bigger study groups

for the experiment. Although not statistically significant, the observed differences between these values cannot however be summarily dismissed. They seem to be similar to results obtained in a study by Pekelder et al. (43). In their study, the incubation period was short and the majority of infected animals developed an acute maedi visna. The resulting loss in ewe productivity could be directly correlated to the severity of the infection. The picture looks different in South Africa. The lesions observed in the pathological survey conducted on slaughtered sheep from the heavily infected Goedemoed flock were typical of MVV (62). The clinical picture was unequivocally less severe than what is observed in Pekelder et al.'s study, however, despite the high sero-prevalence (80% of the 3000 sheep in the flock). As a consequence, this mild maedi visna strain seems to cause a less severe reduction in milk production. It would therefore be unwise to entirely discount the deleterious effect of MVV infection on lamb production in South Africa. When applied to a bigger flock and over a longer period of time, the mild effect observed in this study could be multiplied and result in a bigger loss for the farmer.

5.4 MONITORING OF EWES' UDDER HEALTH AND CORRELATION WITH LAMB GROWTH

In this study, the effect of MVV infection on the udder tissue could not be evaluated by means of the traditional udder health evaluation procedures, using somatic cell counts. The indurative lymphocytic mastitis caused by MVV infection has been reported as not producing a "classical" mastitis with high levels of somatic cells, and affecting the milk quality (1, 23,

33). The main consequence of these udder lesions is the reduction of milk production as a consequence of reduced acinar space.

No statistically significant association could be found between the somatic cell counts and the respective serological status of the two serological groups.

The histological results of this study (see table 4.5.1) indicated that 19 out of 37 sero-positive ewes (51.35%) had more than 5 lymphoid follicles in their udder tissues, compared to only one out of 29 (3.4%) in the sero-negative group. This ewe (no. 3345) raised twins and had high somatic cell counts in several samples taken, without bacterial growth. It can therefore be concluded that the lymphoid proliferation observed in this sero-negative ewe could have been caused by other factors than MVV infection.

The mastitis caused by MVV infection is known to be of a chronic nature, resulting in a lymphoid proliferation in the udder tissue as outlined in the literature review. Studies by Houwers (23) have shown that the lymphoid proliferation in the udder of infected ewes seems to appear in the early stages of the infection, before the development of other lesions. The finding that a high proportion of sero-positive ewes had heavily infiltrated udders, as compared to sero-negative ewes, could be linked to MVV infection. These results are in accordance with the observation of the pathological survey of the Goedemoed flock, where MVV lesions were commonly seen unaccompanied by clinical signs in sero-positive sheep (62).

In our study, due to the low number of animals used, the difference in the average daily gains of lambs born from ewes in different udder classes was not statistically significant. However the observed average daily gains of lambs (table 4.5.3) were clearly dropping proportionally

to the number of follicles found in the dam's udder. It can be deduced that the correlation found in European studies with highly virulent strains of MVV (43), may be present to a lesser extent with the South African strain. The subtle drop in lamb growth observed in the study could result in bigger losses in the long run in an infected flock.

The significant genetic divergence between the South African ovine lentivirus (SA-OMVV) and other ovine lentiviruses (46) and the low pathogenicity observed in the infection with SA-OMVV (42, 59) would suggest that the effect of the indurative mastitis caused by this virus may be milder than what is seen in Europe. The results of this study appear to confirm these findings.

Although no statistically significant difference could be found in the growth of lambs, the proportion of sero-positive ewes found with high lymphoid proliferation in the udder tissue should be considered as potential cause of a reduced milk production, and subsequent drop in growth rate of lambs born from infected ewes.

MVV infection being lifelong, it can be predicted that MVV associated udder lesions, once developed, would proliferate with time, as the ewe undergoes more lactations. This would result in a definite drop in milk production by the ewe and a cumulative negative effect on lamb production. This could be observed if a longer study, covering more lambing seasons, was conducted.

6 CONCLUSION

The present study was a first attempt to evaluate the effect of the SA-OMVV infection on sheep productivity in South Africa.

A lymphoid proliferation in the udder tissue could be observed in MVV infected ewes. Despite an apparent correlation between the extent of the lymphoid proliferation in the udder tissue and the average daily gain of the lambs, no statistically significant effect of the infection on lamb growth could be established.

The slight differences observed in the udder of MVV infected ewes as compared to the control groups, and the subsequent slight difference in lamb growth would suggest that a longer study need to be conducted. It would then be possible to assess if the cumulative effect of the life long MVV infection will result in a much bigger drop in milk availability for the lamb born from infected ewes. Such a study will give more information on the possible financial implication of the infection for a farming unit. It will therefore be possible to assess whether it will be wise for a farmer to embark in an eradication campaign or to live with the infection, in case the effect is mild, up to a certain age of the ewe.

The course of MVV infection in South African flocks suggests that either the local viral strains are mild or the local sheep breeds have a lower susceptibility to the infection, or both.

7 REFERENCES

1) Anderson B.C., Bulgin M.S., Adams S., Duelke B.(1985): Firm udder in periparturient ewes with lymphocytic accumulations, retrovirus infection, and milk unavailable at the teat. *J Am Vet Med Assoc.*186:391-393.

2) Boshoff C.H., Dungu B., Williams R., Vorster J., Conradie J.D., Verwoerd D.W. and York D.F. (1997): Detection of Maedi-Visna virus antibodies using a single fusion transmembrane-core p25 recombinant protein ELISA and a modified receiver-operating characteristic analysis to determine cut-off values. *J Virol Methods* 63: 47-56.

3) Cabradilla C.D, Groopman J.E, Lanigan J (1986): Serodiagnosis of antibodies to the human AIDS retrovirus with a bacterially synthesized env polypeptide. *Bio/Technology* 4: 128-133.

4) Campbell J.R., Menzies P.I., Waltner-Toews D.,Walton J.S., Buckrell B.C. and Thorsen J. (1994): The seroprevalance of maedi-visna in Ontario sheep flocks and its relationship to flock demographics and management practices. *Can Vet J* 35: 39-44.

5) Chemineau P., Cagnié Y.(1992): Training manual on artificial insemination in sheep and goats. Rome: Food and Agriculture Organization of the United Nations publishers.

6) Cross R.F., Smith C.K. and Moorhead P.D. (1975): Vertical transmission of progressive pneumonia of sheep. *Am J Vet Res* 36:465-468

7) Cutlip R.C., Lehmkuhl H., Brogden K., Bolin S.R.(1985): Mastitis associated with ovine progressive pneumonia virus infection in sheep. *Am J Vet Res* 46:326-328.

8) Cutlip R.C., Jackson T.A. and Lehmkuhl H.D. (1979): Lesions of ovine progressive pneumonia: Interstitial pneumonitis and encephalitis. *Am J Vet Res* 173: 1578-1579.

9) Dawson M. (1987): Pathogenesis of maedi-visna. *Vet Rec* 120: 451-454.

10) Dawson M (1980): Maedi visna: a review. *Vet Rec*, 106: 212-216.

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

12) De Boer G.F. (1975): Zwoegerziekte virus, the causative agent for progressive interstitial pneumonia (maedi) and meningo-leucoencephalitis (visna) in sheep. *Res Vet Sci* 18: 15-25.

13) De Kock G.(1929): Are lesions of jaagsiekte in sheep of the nature of neoplasm? *Fifteenth Annual Report of the Director of Veterinary Services, Union of South Africa*. 611-641.

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

15) Forgaty N.M. (1995): Genetic parameters for live weight, fat and muscle measurements, wool production and reproduction in sheep: a review. *Anim Breed Abstr* 63(3):101-143

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

17) Gilmour J.E.M., Senior J.M., Burns N.R. (1989): A novel method for the purification of HIV-1 p24 protein from hybrid Ty virus like particles (Ty-VLP's). *AIDS* 3:717-723.

18) Gudnadottir M. & Palsson P.A. (1967): Transmission of maedi by inoculation of a virus grown in tissue culture from maedi-affected lungs. *J Infect Dis* 117,1-6.

19) Gudnadottir M., and Kristinsdottir K. (1967): Complement fixing antibodies in sera of sheep affected with visna and maedi. *J Immunol*, 98:663-667.

20) Hoff-Jorgensen R.(1990): Diagnostic methods. *In* Maedi-visna and related diseases. Dordrecht:Kluwer Academic Publishers.

21) Hoff-Jorgensen R. (1978): Maedi-visna in Danish sheep. *Bull Off Int Epizoot*, 89: 527-530.

22) Houwers D.J. (1990): Economic importance, epidemiology and control of MVV infection. in Maedi-visna and related diseases. Dordrecht:Kluwer Academic Publishers.

23) Houwers D.J., Pekelder J.J., Akkermans J.P.W.M., Van der Molen E.J. and Schreuder B.E.C. (1988): Incidence of indurative lymphocytic mastitis in a flock of sheep infected with maedi-visna virus. *Vet Rec* 122:435-437

24) Houwers D.J., Gielkens A.L.J., Schaake J. (1982): An indirect enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to maedi-visna virus. *Vet Microbiol* 7: 209-219

25) Houwers D.J. (1988): Ovine lentivirus infections: aspects of immunology, pathology, epidemiology and control. PhD thesis. University of Utrecht.

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

27) Jolly P.E. and Narayan O. (1989): Evidence of interference, coinfections and intertypic virus enhancement of infection by ovine-caprine lentiviruses. *J Virol* 63:4682-4688.

28) Kajikawa O., Lairmore M.D. and DeMartini J. (1990): Analysis of antibody responses to phenotypically distinct lentiviruses. *J Clin Microbiol* 28(4): 764-770.

29) Krogsrud J. and Udnes H. (1978): Maedi. Diagnosis, epizootiology, prevention and control programme in Norway. *Bull Off Int Epizoot* 89: 451-464.

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

31) Kwang J., Keen J., Cutlip R.C. and Littledike E.T. (1993): Evaluation of an ELISA for detection of ovine progressive pneumonia antibodies using a recombinant transmembrane envelope protein. *J Vet Diagn Invest* 5: 189-193

32) Lucam F. (1942): La Bouhite ou lymphomatose pulmonaire maligne du mouton. *Recueil de Med Vet* 118.273.

33) Lujan L., Garcia Marin J.F., Fernandez de Luco D., Vargas A. and Badiola J.J. (1991): Pathological changes in the lungs and mammary glands of sheep and their

relationship with maedi-visna infection. *Vet Rec*, 129:51-54.

34) Marsh H. (1923): Progressive pneumonia in sheep. *J Am Vet Med Assoc* 62:458-473

35) Mitchell D.T.(1915): Investigation into Jaagsiekte or chronic catarrhal-pneumonia of sheep. *Third and Fourth Reports of the Director of Veterinary Research, Union of South Africa*, 585-614.

36) Morris J., Farver T.B., Glenn J.S. and Hird D.W. (1984): Influence of commonly recorded variables on weaning weight of lambs – a necessary consideration before assessing the effects of subclinical disease on production. *Prev Vet Med* 3: 143-149.

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

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

39) Olivier R.E., Gorham J.R., Parish S.F., Hadlow W.J. and Narayan O. (1981): Ovine progressive pneumonia: pathologic and virologic studies on the naturally occuring disease. *Am J Vet Res* 42: 1554-1559

40) Palsson P.A.(1976): Maedi and visna in sheep. In: Kimberlin,R.H.,(ed.).Slow Virus Diseases of Animals and Man. Amsterdam: North Holland Publishing Company.

41) Palsson P.A. (1990): Maedi-visna. History and Clinical Description. in Maedi-visna and Related Diseases. Dordrecht:Kluwer Academic Publishers.

42) Payne A.L., York D.F., De Villiers E.M., Verwoerd D.W., Querat G., Barban V., Sauze N. and Vigne R. (1986): Isolation and identification of a South African

Lentivirus from Jaagsiekte lungs. *Onderstepoort J Vet Res*. 53: 55-62

43) Pekelder J.J., Veenink G.J., Akkermans J.P.W.M., van Eldik P., Elving L. and Houwers D.J. (1994): Ovine lentivirus induced indurative lymphocytic mastitis and its effect on the growth of lambs. *Vet Rec*.134: 348-350

44) Petursson G. and Hoff-Jørgensen(1990): Maedi-visna and related diseases. Dordrecht:Kluwer Academic Publishers.

45) Pritchard G.C., Spence J.B., Arthur M.J. and Dawson M. (1984): Maedi-visna virus in commercial flocks of indigenious sheep in Britain. *Vet Rec* 115, 427-429.

46) Querat G., Barban V., Sauze N., Vigne R., Payne A., York D.F., De Villier, E. and Verwoerd,D.W.(1987):Characteristics of a novel lentivirus from South African sheep with pulmonary adenocarcinoma (jaagsiekte). *Virology* 158:158-167.

47) Reinecke, R.K. (1983): Veterinary helminthology. Butterworths, Durban.

48) Ressang, A.A., Stam, F.C. and Boer, G.F. (1966): A meningoleukoencephalomyelitis resembling visna in Dutch zwoeger sheep. *Path Vet* 3: 401-411.

49) Reyburn H.T., Roy D.J., Blacklaws B.A., Sargan D.R. and McConnell I.(1992): Expression of maedi-visna virus major core protein, p25: development of a sensitive p25 antigen detection assay. *J Virol Methods*. 37:305-320

50) Roy D.J., Watt N.J., Ingman T., Houwers D.J., Sargan D.R. and McConnell I. (1992): A simplified method for the detection of maedi-visna virus RNA by in situ hybridisation. *J Virol. Methods* 36: 1-11.

51) Sigurdsson B., P.A. Palsson and L. van Bogaert (1962): Pathology of Visna, Transmissible demyelinating disease in sheep in Iceland. *Acta Neuropathol* 1:343-

362.

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

53) Simard C.L., Briscoe M.R.(1990): An enzyme-linked immunosorbent assay for detection of antibodies to maedi-visna virus in sheep. II. Comparison to conventional agar gel immunodiffusion test. *Can J Vet Res* 54: 451-456

54) Smith, V.W., Dickson, J., Coackley, Carman, H. (1985): Response of Merino sheep to inoculation with a caprine retrovirus. *Vet Rec* 117, 61-63

55) Snowder G.D, Glimp H.A (1989): Effect of ovine progressive pneumonia on ewe milk production. *J Anim Sci* 67:171

56) 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.

57) Steel R.G.D. and Torrie J.H.(1980): Principles and Procedures of statistics, 2nd edn, McGraw-Hill Book Company, New York.

58) Thormar H., (1963): Neutralization of visna virus by antisera from sheep. *J. Immunol.*,90:185-192.

59) Tustin,R.C.(1969): Ovine jaagsiekte. *J S Afr Vet Med Assoc* 1: 3-23.

60) Van der Molen E.J. and Houwers D.J. (1988): Indurative lymphocytic mastitis in sheep after experimental infection with maedi-visna virus. *Vet Quart* 9:193-202

61) Van der Molen E.J., Vecht U., and Houwers D.J.(1985): A chronic indurative mastitis in sheep, associated with maedi/visna virus infection. *Vet Quart* 7:112-119.

62) Vorster J.H., Dungu B., Marais L.C., York D.F., Williams R. and Boshoff C.H. (1996): A perspective of Maedi-visna in South Africa. *J S Afr Vet Ass* 67(1): 2-3.

63) Watt N.J, MacIntyre N., Collie D., Sargan D., McConnell I. (1992): Phenotypic analysis of lymphocyte populations in the lungs and regional lymphoid tissue of sheep naturally infected with maedi-visna virus. *Clin Exp Immunol* 90:204-208

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

65) York D., Dungu B. and Du Plessis D.H. (1993): The cloning and expression of a maedi-visna virus cross reacting antigen and its use in a sensitive diagnostic assay to detect MVV affected sheep. Proceeding: Biotech SA '93, poster 6.

66) Zanoni R., Pauli U. and Peterhans E. (1990): Detection of caprine arthritis-encephalitis and maedi-visna viruses using the polymerase chain reaction. *Experientia* 46: 316-319.

ADDENDUM 1:

FEEDING OF SHEEP AT OVI

All sheep were fed ad lib a ration worked out so that each sheep could approximately receive daily 0.5kg of hay and 0.8kg of concentrate.

The concentrate given consists of:

1.	Lucerne	40%
2.	Molasses	10%
3.	Mealiemeal	32%
4.	Brans	10%
5.	Sunflower cake meal	6%
6.	NaCl	1%
7.	Di-calcium phosphate	1%
8.	Vitamins and trace elements:	
	Mg	400g
	Cu	10g
	Fe	50g
	Mn	57g
	Co	1g
	Na	1g
	Zn	50g
	Vitamin A	6 000 0000g I.E

Se

ADDENDUM 2

DAY TO DAY OPERATIONS IN THE RUNNING OF THE PROJECT

The study was conducted through the following different periods:

1. Adaptation period.
2. Mating period.
3. Gestation period.
4. Lambing period.
5. Pre-weaning period or lactation.
6. Weaning
7. Post-weaning.

Day -60: Arrival of sheep.

Numbering, weight, condition score, age determination.

Day -58: Vaccinations: Blue tongue A

Pulpy kidney.

Faeces samples for egg count (EPG).

Day -56: Dosing.

Day -46: Weight and condition score.

Day -39: Vaccination: Blue tongue B and C.

Pulpy kidney.

Enzootic abortion.

Day -32: Weight and condition score.

Day -30: Beginning of flush feeding and dosing.

Day -18: Weight and condition score.

Day -14: Rams brought close to ewes ("Ram effect").

Day - 4: Weight and condition score

Day 0: Introduction of rams in ewe flocks.

Day 10: Weight and condition score.

Day 30: Withdrawal of rams.

Day 50: Scanning of all ewes.

...

Up to day 150: Monthly weight and condition score.

Every lamb born: Identification related to dam, weight within two days and then weighed every second week.

ADDENDUM 3: CULLINGS AND DEATH OF EWES DURING THE STUDY

Ewe Number (Sero-group)	*Cause Of Death/Reason For Culling*
3339 (n)	Culled for joint problem during adaptation period.
3384 (n)	Euthanised due to chronic weight loss and condition. Adaptation period.
3584 (p)	Euthanised. Vaginal prolapse.
3593 (p)	Chronic weight loss. Died. On post-mortem examination: Severe abscessation, caused by Crynebacterium in the kidney, liver, lungs and mediastinal lymph nodes. Maedi lesions as well as Jaagsiekte lesions were present in the lung.
3681 (p)	Died. No definite diagnosis was made.
3344 (n)	Culled. Stillborn lamb.
3685 (p)	Culled. Stillborn lamb.
3382 (n)	Culled. Mismothering.
3688 (p)	Culled. Lost her lamb due to bad weather conditions during lambing.
3662 (p)	Culled. Lost her lamb due to bad weather conditions during lambing.
3398 (n)	Culled. Lamb died (broken ribs).
3617 (p)	Culled. Lamb died (ruptured liver).
3696 (p)	Culled. Lamb died (broken leg).

3393 (n)	Culled. No milk.
3392 (n)	Culled. Lamb died (sudden death).
3603 (p)	Culled. Lamb died (sudden death).
3595 (p)	Culled. Lamb died during tail docking.
3356 (n)	Culled. Empty.
3387 (n)	Culled. Empty.
3592 (p)	Culled. Empty.
3667 (p)	Culled. Empty.
3590 (p)	Culled. Empty.
3578 (p)	Culled. Empty.
3693 (p)	Culled. Empty.