Seroprevalence survey of *Chlamydophila abortus* infection in breeding goats on commercial farms in northern Namibia

By

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Submitted in partial fulfilment of the requirements for the degree:

Master of Science in Veterinary Tropical Diseases

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Declaration

I, *Alaster Samkange*, do hereby declare that during the course of this study the serology on the sera collected was done by Mrs. G. Tjipura-Zaire and her staff in the serology section of the Central Veterinary Laboratory in Windhoek. Except where acknowledgements indicate otherwise and the normal advice from my supervisors, this dissertation is my own original work. Neither the full dissertation nor any part of it has been, is being, or is to be submitted for another degree at this or any other University.

This dissertation is presented in partial fulfilment of the requirements for the degree of Master of Science (Veterinary Tropical Diseases) in the Department of Veterinary Tropical Diseases, University of Pretoria.

Signed……

Date……

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Abbreviations

OVD - Otavi Veterinary District

OEA - ovine enzootic abortion

DVS - Directorate of Veterinary Services

cELISA - competitive ELISA

FAT - fluorescent antibody test

EB - elementary body

RB - reticulate body

CFT - complement fixation test

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Abstract

A total of 1076 sera from breeding goats were randomly collected from 24 different farms and tested with CHEKIT®-ELISA (Dr. Bommeli AG-IDEXX, Switzerland) for antibodies against *Chlamydophila abortus*. The farms were divided into two categories of 12 farms each, depending on their level of observed abortions over the previous 12 months: those with insignificant (<5 %) levels of abortions and those with significant (≥5 %) levels of abortions. The farmers were also interviewed on their level of awareness about chlamydophilosis and whether or not they were doing regular preventive vaccination against the disease. The study determined the seroprevalence levels of 25 % at farm level and 8 % at individual animal level (at 95 % confidence level). A total of 6 out of 24 farms had at least one positive breeding animal. Only 5 out of the 24 (20.8 %) farmers interviewed were aware of chlamydophilosis and its zoonotic dangers. None of the 24 farmers interviewed practised any vaccination against chlamydophilosis. There was a significantly higher number of seropositive animals from farms with significant levels of abortions compared to those animals from farms with insignificant levels of abortions (P=0.0000). The study underscored the need for more farmer awareness and training on chlamydophilosis and its zoonotic dangers.

Key words: *Chlamydophila abortus*; chlamydophilosis; seroprevalence; CHEKIT®-ELISA; breeding goats; abortions; zoonosis; farmer awareness

Chapter 1

General introduction

Background

Since 2004 the Directorate of Veterinary Services (DVS) in Namibia embarked on a programme to test for *Brucella melitensis* infection in small stock on farms reporting abortions in the commercial farming areas of the country. The reason for this programme was to ensure that all meat from small stock (sheep and goats) destined for export markets was sourced from brucellosis-free flocks. However, a number of farms that were tested in the Otavi Veterinary District (OVD) were seronegative for *B. melitensis*, but seropositive for *Chlamydophila* when tested with an ELISA.

This discovery led to questions about the current status with regard to the prevalence of chlamydiosis in OVD. Therefore, this study aimed to explore this question further by determining the seroprevalence of *Chlamydophila abortus* infection in OVD.

Previous work in Namibia (Apel, Huebschle & Krauss 1989) indicated that *Chlamydophila abortus* infections were prevalent in all the geographical regions that were tested. These workers detected seroprevalence levels ranging from 12 % (Otjiwarongo) to as high as 50 % (Otavi) in goats. On average, 299/1185 (or 25.2 %) of caprine sera that was tested had chlamydophilial antibodies. However, the seroprevalence studies on *C. abortus* were last conducted over 16 years ago, before the country's independence. With the advent of independence in 1990, the country has undergone a lot of changes in livestock population dynamics. These changes are primarily attributed to the movement of previously disadvantaged groups of people from communal areas, in the extreme north of the country, into the commercial farming areas towards

the south of the country. Coupled with this is also the fact that small stock, especially goats, are constantly being moved from communal areas in the extreme north of the country, where no *Chlamydophila* seroprevalence studies have ever been done, into the commercial areas where previous studies had been done.

In light of the aforementioned, a more up-to-date study was therefore needed to determine the current *C. abortus* seroprevalence level, and to determine the level of farmer awareness of chlamydophilosis. Farmer awareness of this disease is very important since chlamydophilosis is an important zoonosis. Therefore, this study aimed to establish the current baseline information on the seroprevalence of *C. abortus* as well as to determine the level of farmer awareness of this disease.

Objectives of the study

The objectives of this study are summarised as follows:

1. To determine the seroprevalence of *C. abortus* in the breeding stock of goat herds with, and without a history of significant levels of abortions in the OVD.
2. To determine if there is a significant difference in seroprevalence of *C. abortus* between goat farms with significant levels (≥5 %) of abortions compared to farms with insignificant levels (<5 %) of abortions.
3. To determine if farmers are aware of *C. abortus* and its zoonotic potential.
4. To determine whether farmers are using vaccination as a means to control chlamydophilosis.

Chapter 2

Review of the literature

Introduction

Chlamydophila abortus (formerly *Chlamydia psittaci* serotype 1) is a zoonotic bacterium that commonly causes abortions in ruminants, referred to as ovine enzootic abortion (OEA) or simply, enzootic abortion. It is the most important infectious agent causing abortion in sheep and goats in numerous countries around the world (Smith & Sherman 1994, Aitken 2000, cited by Szeredi & Bacsadi 2002). OEA of sheep and goats is of major economic importance all over the world (Longbottom, Fairley, Chapman, Psarrou, Vretou & Livingstone 2002). It is estimated that in the United Kingdom, chlamydophilial abortion accounts for about 50 % of all diagnosed abortions, resulting in losses estimated to be in excess of £20 million annually (Longbottom *et al.* 2002). In the USA, *C. abortus* is the most common cause of infectious abortion in goats (Aiello & Mays 1998). Apart from causing abortion and foetal loss not only in sheep, cattle and goats, and also in humans who come into contact with aborting livestock (Ward 2006), *C. abortus* can also cause abortion in pigs (Woollen, Daniels, Yeary, Leipold & Phillips 1990, cited by Longbottom *et al.* 2002).

Aetiology

Chlamydophila abortus is a ubiquitous, small, Gram-negative bacterium. According to the current classification, which is based on ribosomal, biochemical, serological and DNA-DNA hybridisation data (Bush & Everett 2001), *C. abortus* belongs to the genus *Chlamydophila*, family Chlamydiaceae and order Chlamydiales as illustrated in FIG. 1 below. The genus *Chlamydophila* has five other species: *C. psittaci; C. felis; C. caviae; C. pecorum and C. pneumoniae*.

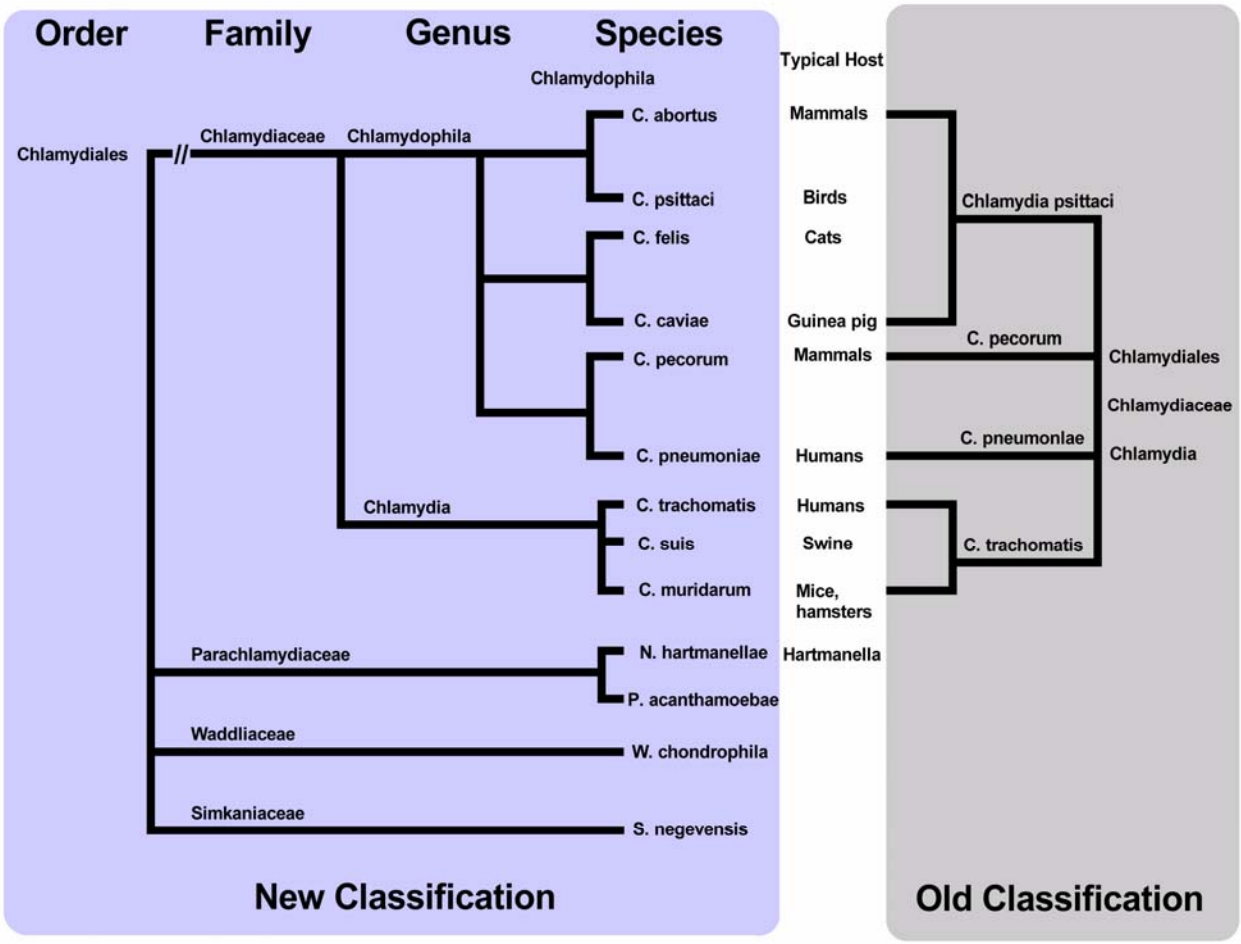

FIG. 1 Genetic classification of the order Chlamydiales. The tree on the left (blue) depicts the current classification of the Chlamydiales. Horizontal distances are roughly proportional to genetic distances as measured by 16S rRNA sequence data and DNA–DNA hybridization. A list of typical hosts illustrates the ecological heterogeneity of Chlamydiaceae species. Parachlamydiaceae are found in amoebae, and a second genus in this family, *Neochlamydia*, has recently been described. Host ranges of strains outside the Chlamydiaceae are not yet well resolved. (Grey) (Courtesy of Dr Robin M Bush and Prof Karin DE Everett (2001) & Michael Ward (2006), used with written permission).

Members of the Chlamydiales are all small, Gram-negative bacteria that are obligate intracellular parasites of eukaryotic cells and have a distinctive developmental cycle for their replication (Ward 2006). Chlamydiales are found within vertebrate cells and amoebae and many coexist in an asymptomatic state within their hosts (Ward 2006).

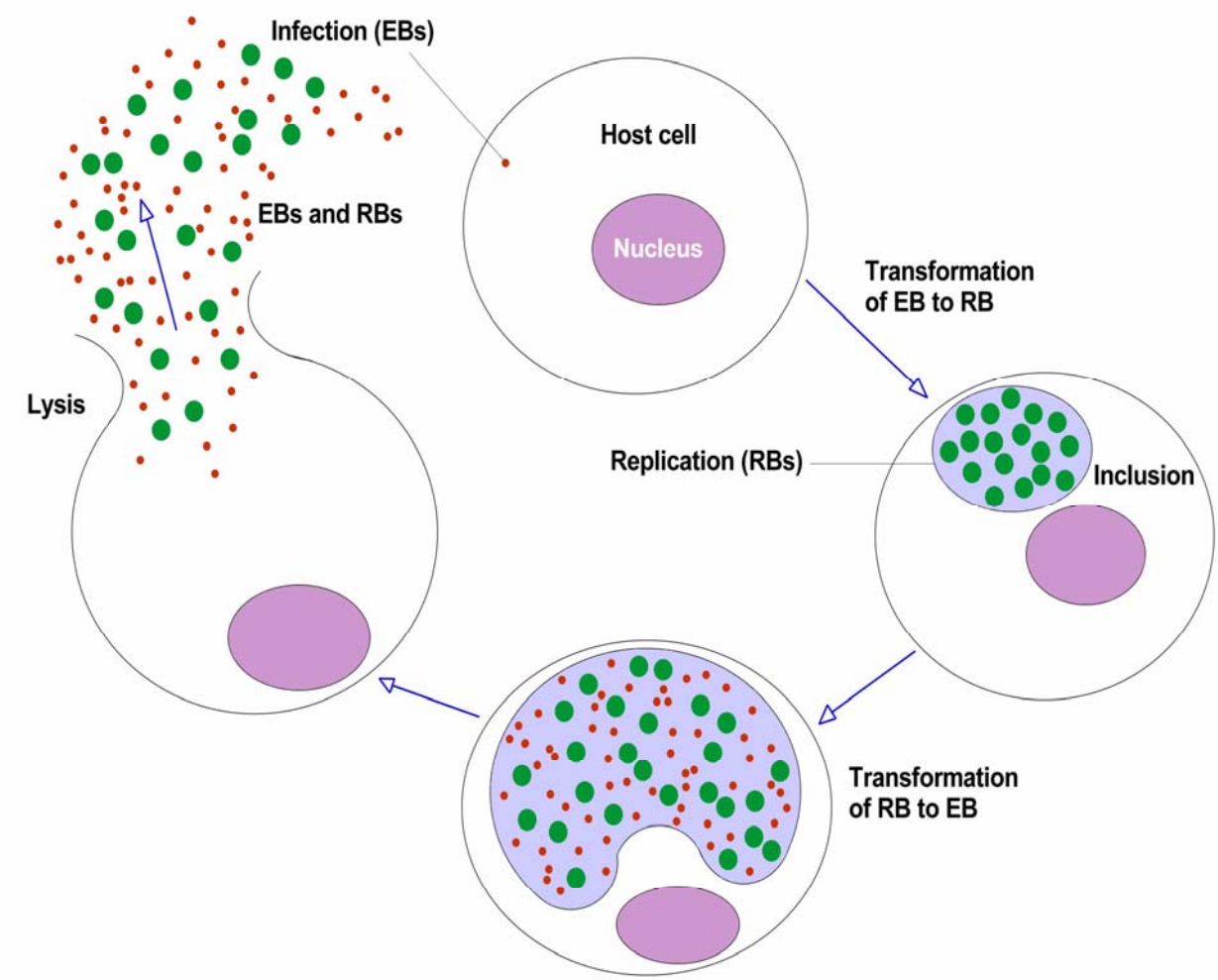

FIG. 2 Diagram of an idealised chlamydial developmental cycle. The small, infectious elementary bodies are in red; the larger, replicating reticulate bodies are in green. Chlamydial infection is initiated by attachment of a chlamydial elementary body (EB) to the host cell, followed by its entry into the cell. The chlamydial elementary bodies are internalised in tight, endocyctic vesicles, within which they differentiate into reticulate bodies (RB). (Courtesy of Prof Karin D Everett; used with written permission).

Members of the Chlamydiales have a unique cycle of development (FIG. 2) in which a resistant infectious form, the elementary body (EB) alternate with a metabolically active non-infectious form, the reticulate body (RB).

The EB attaches to the membrane of the host cell and promotes its own endocytosis in a membrane-limited vacuole called the inclusion. These inclusions do not fuse with lysosomes.

The EB transforms into a RB, which replicates by binary fission. After several divisions, the RBs, transform back into infectious EBs. These EBs are released through host cell lysis or extrusion of the inclusion out of the host cell (Moulder 1991, cited by Rodolakis 2001).

Chlamydophila abortus has many strains that can be differentiated by distinctive inclusion morphology, serotype-specific antibodies as well as distinct polypeptide and genomic profiles (Siarkou, Lambropoulos, Chrisafi, Kotsis & Papadopoulos 2002). However, four immunologically distinct groups were identified through cross-protection experiments (Siarkou 1992, cited by Siarkou *et al*. 2002) and the existence of subspecies variation within *C. abortus* strains was further demonstrated by PCR techniques (Siarkou *et al*. 2002).

Epidemiology

Chlamydophilosis has been described in many countries around the world and was reported for the first time in Germany in 1956. Since then it has been diagnosed in many other countries including the USA, India, United Kingdom, France, Japan, Chad, Switzerland, Jordan, Greece, Tunisia, South Africa, Namibia, and many others (Dawson 1988; Apel *et al.* 1989; Aiello & Mays 1998; Radostits, Blood & Gay 1994; Rodolakis 2001). In many countries chlamydophilial abortion is the second common cause of infectious abortions after brucellosis, and the main cause in most of the countries where brucellosis is controlled (Rodolakis 2001). In the USA *C. abortus* is the most common type of infectious abortion in goats and in herds where the disease is endemic, 25-60 % of primiparous does abort (Aiello & Mays 1998).

In a study by Al-Qudah, Sharif, Raouf, Hailat & Al-Domy (2004) on the seroprevalence of *C. abortus* in northern Jordan, it was reported that each of the 20 goat flocks in the study had at least one positive animal and that the overall infection rates ranged from 10.8 % to 11.8 %.

Apel *et al.* (1989) who did a study in Namibia using an IgG (heavy chain and light chain)-ELISA,

reported *Chlamydophila* antibody prevalence rates of up to 35 % in goat herds showing clinical signs indicative of chlamydophilial infections. They also reported that 86 % of all goat farms they tested had chlamydophilial antibodies. However, in herds without health problems antibody rates of 18 % were found. *C. abortus* antibody titres indicative of infections were found to be prevalent in all the Namibian geographic regions studied regardless of climatic differences.

According to Dawson (1988), the incubation period of enzootic abortion in goats, from challenge to abortion, may be as short as 2 weeks, compared to at least 6 weeks in sheep. At the time of kidding/lambing, infected does/ewes shed large numbers of *C. abortus* EBs in the placenta and foetal fluids (Rodolakis 2001). Goats, unlike sheep, discharge EBs in vaginal secretions for several days or even more than two weeks before and after an abortion (Rodolakis 2001; Dawson 1988). Dawson (1988) further noted that the combined effects of a shorter incubation period and early vaginal excretion contribute to a more rapid spread of infection and consequently, relative to sheep flocks, a higher proportion of the herd may be affected in an initial outbreak. In the same vein, Rodolakis (2001) also stated that it might explain the higher incidence of abortion in newly infected herds of goats, since the susceptibility to infection varies in relation to the physiological status of the animal. Goats that are less than 100 days pregnant are more susceptible than those at the end of gestation or those that are barren (Rodolakis 2001).

It has been shown in the case of chlamydophilosis in sheep that any surviving female progeny from infected ewes will become latently infected and may excrete the bacteria or display clinical signs of enzootic abortion if they are retained for breeding (Kadra & Balla 2006).

Milk, urine and faeces may also contain small amounts of *C. abortus* for several days after abortion. Young goats born from infected does may retain the infection in the herd or transmit it to other herds (Rodolakis 2001). Wildlife vectors like foxes and crows have been suspected of

carrying infections across farm boundaries (Dawson 1988). The role of venereal transmission of *C. abortus* by males still needs to be investigated (Rodolakis 2001).

In one study, Teankum, Pospischil, Jannet, Brugnera, Hoelzle, Hoelzle, Weilenmann, Zimmermann, Gerber, Polkinghorne & Borel (2007) investigated the prevalence of *C. abortus* infection in semen and male genital tracts of bulls, rams, and bucks. All the investigated male genital organs were negative for *Chlamydophila* whilst 20 out of 304 bull semen samples (6.6 %) were positive with PCR directed at the 16S ribosomal RNA. However, all the small ruminant semen samples were negative. The presence of *C. abortus* in semen samples from bulls indicates the possibility of venereal transmission, at least in bulls.

In previous studies however, *C. abortus* was isolated from the testes, epididymis and semen of bulls with seminal vesiculitis (Storz, Carroll, Ball & Faulkner 1968, cited by Teankum *et al.* 2007), and the organism was also recognised as a cause of epididymitis in rams (Lozano 1986, cited by Teankum *et al*. 2007). Other workers have also demonstrated that *C. abortus* can survive in cryopreserved semen (Storz *et al*. 1968, cited by Teankum *et al*. 2007), which could be of importance in artificial insemination (Teankum *et al.* 2007).

The *Chlamydophila* EBs that are excreted at abortion are the main source of infection for susceptible animals through ingestion or inhalation of uterine discharges (Vretou, Radouani, Psarrou, Kritikos, Xylouri & Mangana 2007). According to Dawson (1988), the contaminated lambing environment is the major source of infection for previously unexposed ewes and lambs. Mucous membranes of the upper respiratory tract, eyes and oropharynx are presumed to be the main routes of infection. Experimental studies with pregnant and non-pregnant ewes showed that chlamydophilial infection is followed by a brief bacteraemia during which *C. abortus* is distributed throughout the body. Apart from possible faecal shedding, infection remains unapparent until late gestation when *C. abortus* organisms are recovered from placental and

uterine tissue. Most of the abortions occur in the lambing season subsequent to that in which the infection was acquired (Dawson 1988).

Clinical signs

C. abortus infections in ruminants can cause a variety of clinical signs including polyarthritis, conjunctivitis, pneumonia and abortion (Storz, Shupe, Smart & Thornly 1966, White 1965, Cox, Hoyt, Poston, Snider, Lemarchand & O'Reilly 1998 & Shewen 1980, cited by Teankum *et al.* 2007). In goats, chlamydophilosis is clinically characterised by abortion during the last months of pregnancy. Stillbirths or premature birth of weak kids with low birth weight also occurs. Abortions may occur without prior clinical signs. Abortion rates of 25-90 % have been described, and goats that have aborted may later succumb to metritis or respiratory disease (Dawson 1988; Radostits *et al*. 1994). Ewes appear to suffer no systemic effects but retained placenta and metritis are common sequelae in does (Radostits *et al*. 1994). There may be rapid recovery after an abortion (Eugster, Jones & Gayle 1977, cited by Rodolakis, 2001). . Some goats may develop a brown vaginal discharge, a persistent cough without dyspnoea, or arthritis and keratoconjunctivitis (Rodolakis 2001).

In a newly infected flock, up to 90 % of pregnant does may abort and milk production may decrease. The high rate of abortion is observed for 2 or 3 years after which the disease takes on a cyclical nature with about 10 % of pregnant females aborting yearly for several years until a new outbreak occurs and then all the yearlings will abort. It is exceptional for a goat to abort twice (Rodolakis 2001).

A brown material may cover kids delivered close to term. The following are also often observed: clear or bloodstained diffuse oedema and bloodstained fluids in abdominal and pleural cavities. There may also be petechiae on the tongue, in the buccal cavity and on the hooves (Rodolakis, 2001).

Zoonotic implications

Chlamydophila abortus is zoonotic, and although most human infections are mild and often unnoticed, pregnant women can develop severe, life-threatening illness and abort (Jorgensen, 1997; Buxton 1986, cited by Garcia de la Fuente, Gutierrez-Martin, Ortega, Rodriquez-Ferri, del Rio, Gonzalez & Salinas 2004). People get infected through contact with infected sheep or goats, especially during lambing (Ward 2006), and possibly through ingestion of food or water contaminated by infected abortion materials. Inhalation of infected material from sheep or goats can also result in severe chlamydophilial respiratory disease in humans (Ward 2006).

The consumption of unpasteurised milk has also been suggested as another possible risk factor for humans (Dawson 1988). However, other authors point out that the possibility that humans could become infected by ingesting the organism in dairy products manufactured from infected ewe's milk has been discounted due to the failure to detect the organism in milk, but contamination of milk by EBs in vaginal discharge seems to be a serious risk (Radositis *et al*. 1994). Some abattoir workers, vaccine manufacturing workers, and laboratory scientists have also been reported to have developed the disease after coming into contact with *C. abortus* from aborting sheep (Ward 2006).

Five confirmed human cases of abortion due to *C. abortus* infection from sheep have been published. The clinical signs in the mother included thrombocytopaenia with disseminated intravascular coagulation, renal failure and hepatic dysfunction during the late second and early third trimester of pregnancy. The outcome for the foetus was usually fatal and the infection in the mother resolved after delivery (Helm, Smart, Cumming, Lambie, Gray, MacAulay & Smith 1989, cited by Kadra & Balla 2006).

Laboratory diagnosis

Smears and tissue sections

Enzootic abortion can be diagnosed in the laboratory by identification of the causal agent in stained smears or impressions made from the placenta, especially the affected chorionic villi or the adjacent chorion (Rodolakis 2001; Aitken & Longbottom 2004; Radostits *et al*. 1994). If placental material is not available, smears can be made from vaginal swabs from females that have aborted within the previous 24 hours, or from the moist fleece of a freshly aborted kid that has not been cleaned by its mother (Aitken & Longbottom 2004).

A number of staining procedures can be used, for instance modified Machiavello; Giemsa; Brucella differential or Ziehl-Neelsen stains (Aitken & Longbottom 2004) as well as the streptavidin-biotin method and Stamp's staining technique (Szeredi & Bacsadi 2002). Intracellular *Chlamydophila* inclusions can also be demonstrated by Giemsa staining of thin (4 µm) sections taken from target tissues that have been suitably fixed in solutions such as Bouin or Carnoy (Aitken & Longbottom 2004). The organism can be seen in the cytoplasm of trophoblast cells covering or already detached from the villi, and sometimes in the cytoplasm of the inflammatory cells, forming one or more inclusion bodies (FIG. 3 & FIG. 4) (Szeredi & Bacsadi, 2002).

Chlamydophila abortus resembles *Brucella* and the rickettsia *Coxiella burnetti* in morphology and staining characteristics, and therefore requires an experienced person to differentiate them (Rodolakis 2001). However, the three can be distinguished serologically, or by the placental pathology that characterises chlamydophilosis.

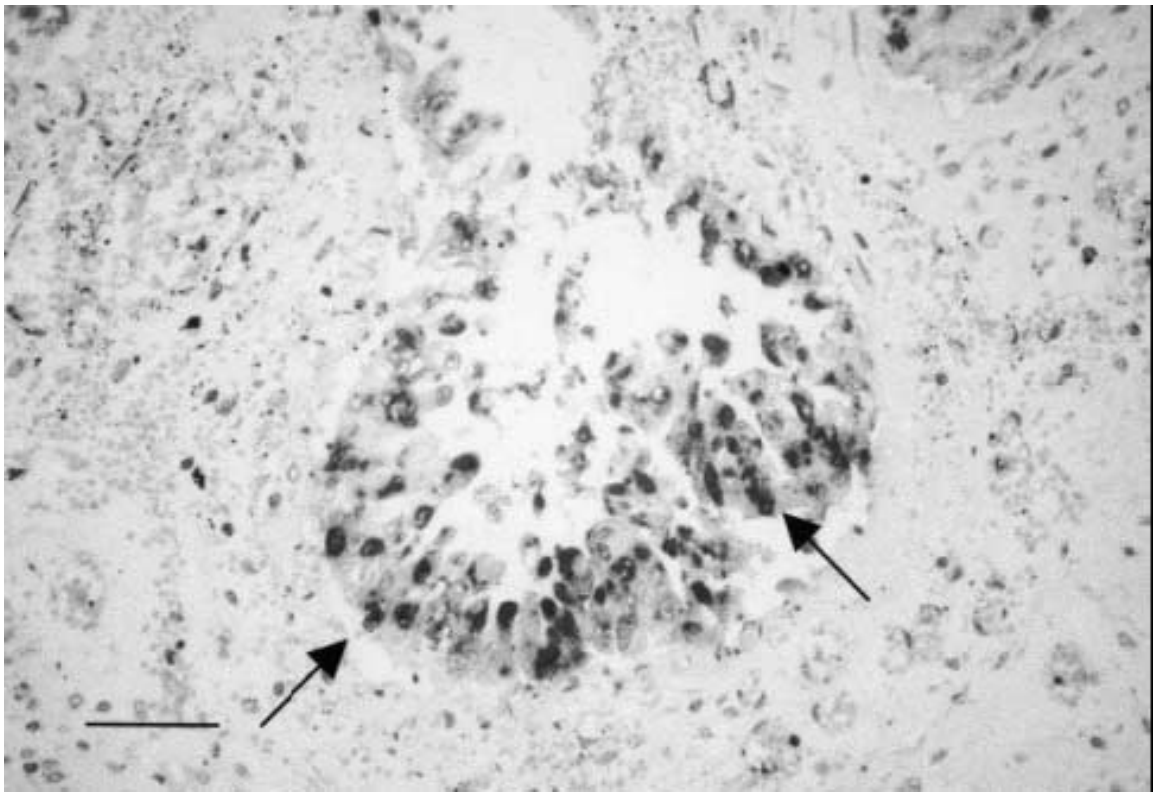

FIG. 3 Ovine cotyledon section. *C. abortus* in cytoplasmic inclusions in the trophoblast cells bordering the lacunae (arrows). Labelled streptavidin-biotin method, counterstaining with Mayer's haematoxylin. Bar, 30 µm. (Courtesy of Szeredi & Bacsadi 2002, used with written permission).

Szeredi & Bacsadi (2002) made a comparison between immunohistochemical examination of cotyledons fixed in formalin and embedded in paraffin wax, immunocytochemical examination of smears made from the surface of foetal membranes and light microscopical examination of smears stained by Stamp's method for the diagnosis of *C. abortus*. They reported that the immunohistochemical method detected the highest number of cases, followed by the immunocytochemical method. The light microscopical examination of smears stained by Stamp's method detected the least number of cases.

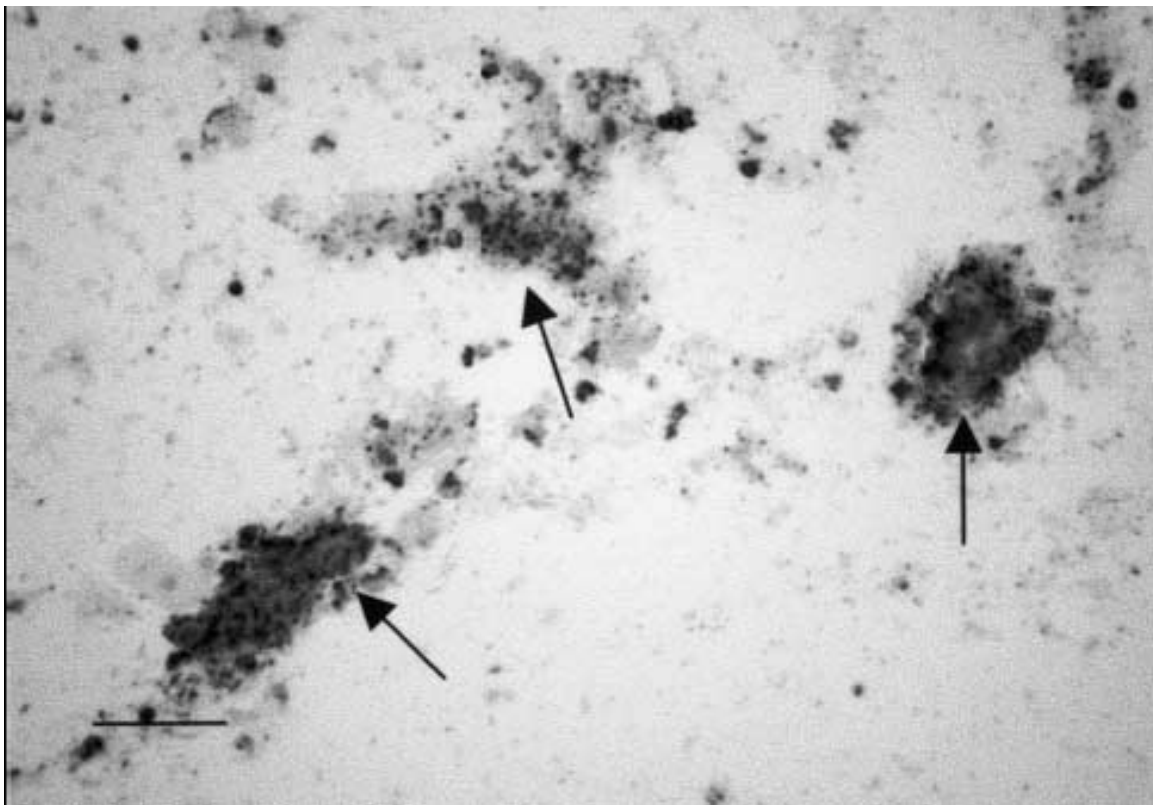

FIG. 4 Smear made from the surface of an ovine cotyledon. *C. abortus* can be seen in inclusion bodies and as small dots in intra- and extra-cellular locations (arrows). Labelled streptavidin-biotin method, counterstaining with Labelled streptavidin-biotin method, counterstaining with Mayer's haematoxylin. Bar, 30 µm. (Courtesy of Szeredi & Bacsadi 2002, used with written permission)

Antigen detection

There are a number of commercially available genus-level antigen detection tests that include ELISA and fluorescent antibody tests (FATs), with the former being more sensitive (Wood & Timms 1992, cited by Aitken & Longbottom 2004). FATs using a specific antiserum or monoclonal antibody may be used for *C. abortus* identification in smears (Aitken & Longbottom 2004). The presence of *Chlamydophila* antigens in ground placenta or vaginal swabs sampled just after abortion may be detected by ELISAs developed for human *Chlamydia trachomatis* infections (Amin & Wilsmore 1994 & Wilsmore & Davidson 1991, cited by Rodolakis 2001). These ELISAs are group-specific due to the antigenic cross-reactivity of the chlamydial lipopolysaccharide (LPS) which is present in all chlamydiae (Everett 2000, cited by Hoelzle,

Hoelzle & Wittenbrink 2004). This means that ELISA cannot identify the causative chlamydial species (Hoelzle *et al*. 2004).

Nucleic acid based methods

In human medicine, polymerase chain reaction or its variation, ligase chain reaction are considered the most sensitive diagnostic tests available for diagnosis of *Chlamydia* (Rodolakis, 2001). Several primers common to all species of *Chlamydophila*, such as *Omp*1, the gene coding for the major outer membrane protein, or specific for *C. abortus* or *C. pecorum* have been developed for veterinary application (Rodolakis, 2001).

Isolation of the agent

Aitken and Longbottom (2004) described how *Chlamydophila abortus* could be isolated in embryonated chicken eggs and in cell cultures, with the latter being the preferred method for the isolation of new strains. Tissue samples such as diseased cotyledons, placental membranes, foetal lung or liver, vaginal swabs are ideal for *C. abortus* isolation. If there is going to be any delay before isolation procedures begin, transport medium such as sucrose/phosphate/glutamate medium supplemented with 10 % foetal bovine serum, antibiotic (streptomycin and gentamycin are suitable, but not penicillin), and a fungal inhibitor (Spencer & Johnson 1983, cited by Aitken & Longbottom 2004). A tissue to medium ratio of 1:10 is commonly used or, alternatively, approximately 1g of tissue is ground with sterile sand in 8ml of transport medium (Aitken & Longbottom 2004).

For *C. abortus* isolation in chicken embryos, test samples are prepared as 10 % suspensions in nutrient broth containing streptomycin (not penicillin) (200 µg/ml). 0.2 ml of suspension is inoculated into the yolk sac of 6-8-day old embryos, which are then further incubated at 37 °C. Infected embryos die between 4 and 13 days after inoculation. Smears prepared from their vascularised yolk sac membranes reveal large numbers of elementary bodies (Aitken & Longbottom 2004).

Chlamydophila abortus can be isolated in a variety of cell types although McCoy, BGM or baby hamster kidney (BHK) cells are commonly used. For confirmatory diagnosis, cultured monolayers are suspended in growth medium at a concentration of 2 x 10^5 cells/ml. Aliquots of 2 ml of the suspension are dispensed into flat-bottomed glass Universal bottles, each containing a single 16mm cover-slip. Confluent cover-slip monolayers are achieved after incubation for 24 hours at 37 °C. The growth medium is removed and replaced by 2 ml of test inoculum, which is then centrifuged at 2500 *g* for 30 minutes on to the cover-slip monolayer to promote infection. After further incubation for 2-3 days, the cover-slip monolayers are fixed in methanol and stained with Giemsa or according the method of Gimenez (Arens & Weingarten 1981 and Gimenez 1964, cited by Aitken & Longbottom 2004). Infected cultures contain basophilic (Giemsa) or eosinophilic (Gimenez) intracytoplasmic inclusions (Aitken & Longbottom 2004).

Chlamydophilial growth can be further enhanced by chemical treatment of cultured cells, before or during infection. These treatments include: cycloheximide (0.5 µg/ml) in the maintenance medium, emetine (1 µg/ml) for 5 minutes before infection, and 5-iodo-2-deoxyuridine (780 µg/ml) for 3 days prior to infection (Aitken & Longbottom 2004).

Although culture has long been considered the gold standard, it has significant disadvantages. A cold chain is required to protect the viability of the organism during specimen transport. These organisms are also extremely difficult to grow and specimens are often too contaminated to allow their isolation. Moreover modern molecular methods of diagnosis based on nucleic acid amplification are potentially much more sensitive towards the detection of antigen than either culture or ELISA (Ward 2006).

Serological tests

The complement fixation test (CFT) is the most widely used test for detecting *C. abortus* infection (Aitken & Longbottom 2004; Longbottom *et al*. 2002; Vretou *et al*. 2007) and it is also

the test recommended by the World Organisation for Animal Health for the purpose of international trade of sheep and goats. However, CFT is not very sensitive and not specific because the test uses an antigen shared with *C. pecorum*, which most goats harbour in their intestine (Rodolakis, 2001). The cross-reactive, genus-specific antibodies so produced interfere with the interpretation of the CFT results. This poses a particular problem in sera from animals infected with strains of both species.

Positive reactions with titres between 1:10 and 1:40 are therefore not specific for abortion and may relate to an intestinal infection with *C. pecorum*. It is therefore recommended that the CFT be done 3 to 6 weeks after abortion or lambing, when the antibody response is at its maximal level (Rodolakis, 2001). According to some workers, CFT cannot be used for diagnosis of individual or young animals or to detect infection in males (Dawson 1988; Rodolakis *et al.* 1998, cited by Rodolakis, 2001), presumably because of its low sensitivity and specificity (Buendia, Cuello, Del Rio, Gallego, Caro & Salinas 2001) which would imply that these categories of animals are unlikely to have high enough antibody titres for the CFT to detect.

The serological responses to *C. abortus* and *C. pecorum* can be resolved by indirect micro-immunofluorescence, but the procedure is too time-consuming for routine diagnostic purposes (Aitken & Longbottom 2004). ELISAs have higher sensitivities than CFT (Aitken & Longbottom 2004) and several ELISAs have been reported for the diagnosis of chlamydophilosis (Ward 2006). The rOMP90-3 and rOMP90-4 ELISAs were found to be more sensitive and specific than CFT for differentiating animals infected with *C. abortus* from those infected with *C. pecorum* (Longbottom *et al.* 2002). Studies by various other workers comparing different ELISAs with CFT also found the former to be more sensitive and specific for detection of *C. abortus* antibodies (Buendia *et al*. 2001; Vretou, Radouani *et al*. 2007; Anderson, Tan, Jones & Herring 1990, Anderson, Herring, Jones, Low & Greig 1995, Gajdosova, Kovacova, Kazar, Kolcunova & Sabo

1994, Pospisil, Veznik, Hirt, Svecova, Diblikova & Pejcoch 1996, Veznik & Pospisil 1997, cited by Tràvnicek, Kovacova, Bhide, Zubricky & Cislakova 2002).

In one such study Vretou *et al*. (2007) evaluated two commercial ELISAs, the CHEKIT®-CHLAMYDIA (produced by Dr. Bommeli AG-IDEXX, Switzerland), which uses the inactivated *Chlamydophila psittaci* antigen, and the *Chlamydophila abortus* ELISA (produced by the institute Pourquier, Montpellier, France), which uses a recombinant fragment of the 80-90 kDa protein. The results were then compared to those obtained by the CFT and the "in house" competitive ELISA [cELISA] (Salti-Montesanto, Tsoli, Papavassiliou, Psarrou, Markey, Jones & Vretou 1997, cited by Vretou *et al*. 2007). The CFT lacks specificity because it makes use of an antigen mainly consisting of the heat-resistant lipopolysaccharide (LPS), which is common to all members of the Chlamydiaceae family (Brade, Brade & Nano 1987, cited by Vretou *et al*. 2007). The authors did not specify which competitive antibodies they used in their "in house" cELISA or the antigen employed. The tests were assessed with a panel of 17 serum samples from specific pathogen-free lambs experimentally infected with various subtypes of *C. pecorum*; sera from 45 *C. abortus*-infected pregnant sheep and sera from 54 sheep free of OEA. The 4 assays were further evaluated with a total of 254 sera from flocks with documented OEA (97 samples), from flocks with no history of abortion (69 samples), OEA free flocks with suspected *C. pecorum* infection (26 sera) and from animals after abortion of unknown cause (62 sera).

The study reported the sensitivity and specificity (S/P) of the 4 assays as follows (%): Pourquier ELISA – 80/100; CHEKIT-ELISA – 73.3/96.3; CFT – 68.8/88.9 and cELISA – 77.7/98.1. The CFT was therefore found to be the least sensitive and specific of all the 4 assays evaluated.

Treatment and control

In affected flocks, recommended control measures include prompt disposal of contagious

materials (e.g. placentas), disinfection of the area and the isolation of aborted does until uterine discharges have ceased, thus limiting further spread of infection (Dawson 1988; Kadra & Balla 2006). In unaffected flocks, female stock intended for use as breeding replacements should be purchased from *Chlamydophila*-free flocks (Dawson 1988). This should prevent the introduction of latently infected does that may be destined to abort, thus spreading the infection in the new flock.

Since tetracyclines interfere with chlamydophilial replication they are effective in preventing abortions, but they do not suppress bacterial excretion at birth or control the level of infection in the flock (Rodolakis 2001; Maurin & Raoult 1999, cited by Kadra & Balla 2006; Rekiki, Bodier, Berri & Rodolakis 2006). Intramuscular oxytetracycline injections at a dosage of 20 mg/kg given at 105 and 120 days of pregnancy can prevent abortions without preventing *Chlamydophila* shedding at kidding (Rodolakis 2001). Dawson (1988) recommends that oxytetracycline injections be repeated at 10 - 14 day intervals after 100 days of gestation. In situations where serious problems are anticipated, for instance in the face of an outbreak, the metaphylactic administration of intramuscular or oral oxytetracycline to the whole flock can reduce clinical losses without eliminating infection (Dawson 1988; Aiello & Mays 1998).

However, Rekiki *et al*. (2006) warn against repeated tetracycline treatments, which could result in the development of antibiotic resistance. The authors further point out that the use of antibiotics for prevention of chlamydophilosis should be discouraged, and that effective control of the disease with the aid of vaccination to prevent abortion and vaginal excretion at kidding, is the recommended control measure.

In the United Kingdom, an inactivated egg-derived vaccine has been available since the mid-1950s and after its introduction the disease stopped being a significant problem for several years. However, the disease steadily increased from the late 1970s until it got to a stage where it

was responsible for about 20 % of all investigated abortions in the late 1980s. This breakdown in the immunity observed in vaccinated flocks could have been due to antigenic variation in the bacteria (Dawson 1988).

Killed vaccines can reduce the incidence of abortion as well as the shedding of *C. abortus* at kidding, but unfortunately they do not completely stop *Chlamydophila* shedding, which leads to endemic cycles of infection that have serious consequences regarding the epidemiology of chlamydophilosis (Rodolakis 2001; Rodolakis & Souriau 1983, cited by Garcia de la Fuente *et al*. 2004). Abortion induces a very strong immunity capable of withstanding later challenges (Rodolakis *et al*. 1980, cited by Rodolakis 2001).

Work by Caro *et al*. (2001, cited by Garcia de la Fuente *et al*. 2004) demonstrated that none of the inactivated vaccines that were commercially available in Spain at that time were able to provide an acceptable degree of protection against *C. abortus* in a murine model. Garcia de la Fuente *et al*. (2004) then compared the protective efficacy of two inactivated commercially available vaccines with two experimental inactivated vaccines (M7 and QS). The experimental vaccines induced considerably better protection than the two commercial ones. The new vaccine especially M7, prevented abortions, showed a good antibody response, the highest newborn lamb weights and the lowest level of *C. abortus* shedding at lambing.

Some workers developed a live vaccine containing temperature sensitive mutants of *C. abortus* (Rodolakis 1983, cited by Rodolakis 2001), which was shown to protect goats against abortion and chlamydophilial shedding at kidding when administered before mating (Rodolakis *et al*. 1986, cited by Rodolakis 2001; Chalmers, Simpson, Lee & Baxendale 1997, cited by Garcia de la Fuente *et al*. 2004). However, when all goats in an infected herd are vaccinated the first year and all replacements are vaccinated on subsequent years, it can take up to 3 years before abortions stop. This was attributed to latent infection in goats that were infected before

vaccination but may not have aborted, since vaccination does not change the course of latent infection. As long as there are goats with a latent infection in a herd it is not advisable to stop vaccination, or else abortions would start anew (Rodolakis 2001). However, the potential danger of attenuated vaccines makes them a less attractive solution, particularly since *C. abortus* can cause serious disease in immunocompromised persons and abortions in pregnant women (Buxton 1986, cited by Garcia de la Fuente *et al*. 2004).

In both South Africa and Namibia there are two vaccines registered for use in sheep against OEA. Ovine Enzootic Abortion Vaccine For Sheep (Onderstepoort, South Africa) is an inactivated vaccine, which according to the manufacturer, can be administered to ewes of any age, at least four weeks prior to the first mating. A second booster is recommended before the next breeding season, and protection is claimed to last for several years. Ovilis® Enzovax (Intervet, The Netherlands) is an attenuated live vaccine that is recommended for administration every two years from the age of 5 months. Shearlings and older ewes should be vaccinated during the 4-month period prior to mating.

Chapter 3

Materials and methods

Study area

This study was conducted in OVD, which is located in a commercial farming area in northern Namibia (FIG. 5). It is surrounded by three other veterinary districts namely, Grootfontein to the east, Ondangwa to the north, Outjo to the west, and Otjiwarongo to the south.

The extreme northern veterinary districts of the country namely Katima Mulilo, Opuwo, Ondangwa, and Rundu are all in communal areas. The latter three districts are separated from the rest of the southern part of the country by a game-proof fence referred to as the Veterinary Cordon Fence, which cuts across the whole width of the country and simultaneously forms the northern borders of Grootfontein, Otavi and Outjo districts.

There are a total of 313 farms with goats (among other livestock) in the OVD, with a total goat population of about 35 000.

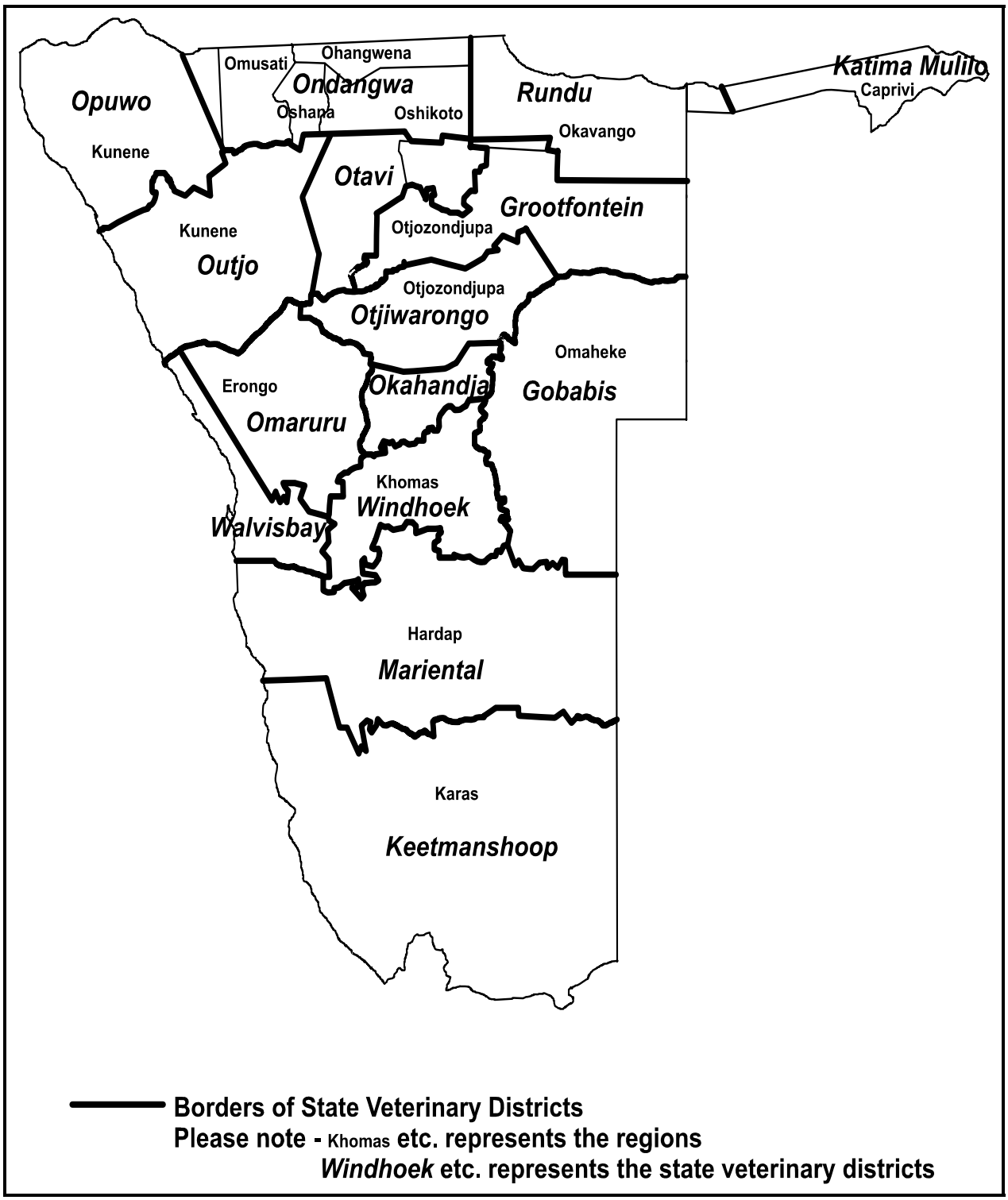

FIG. 5 Map showing the veterinary districts of Namibia

Sampling methods

A multi-stage sampling strategy was used for selecting the farms and the individual goats to be sampled. Firstly, a total of 24 farms with goats were randomly selected. Although goat flocks that were vaccinated against chlamydophilosis were to be excluded from this study in order to avoid interference with the serology results (Gerber, Thoma, Vretou, Psarrou, Kaiser, Doherr, Zimmermann, Polkinghorne, Pospischil & Borel 2007), none of the randomly selected farms practised vaccination against chlamydophilosis. For this reason, in the end there was no selected farm that ended up being excluded from the study.

The selected farms were divided into two categories: (1) farms with a history of significant levels of abortions (≥5 %) during the previous 12 months and (2) farms with an insignificant level of abortions (<5 %) during the same period. Various literature sources state that it is normal for up to 5 % of ewes and does in a flock to abort (Schoenian 2000; Robinson 1951, Quinlivan, Martin, Taylor & Cairney 1966, Edey 1969, Kelly 1984 & 1986, cited by FAO 2006). It is on this basis that the 5 % level of abortion was used as a cut-off point in determining whether or not the levels of abortions observed in a given flock were regarded as significant or not.

At farm level, stratified random sampling was used to select the individual goats to be sampled. The target group for the study included adult breeding does and bucks. The number of serum samples collected on each farm was calculated according to the sample size formula for determining the prevalence of a disease (Martin, Meek & Willeberg 1987), assuming an expected prevalence of 10 % (Thrusfield 1995, cited by Al-Qudah *et al.* 2004) at 95 % confidence as illustrated in Annexure 2. A total of 1076 sera were collected from 24 different farms in the study area.

The goats were bled from the jugular vein using 20-gauge needles, needle holders and sterile

evacuated tubes (BD Vacutainer Systems, Pre-Analytical Solutions, United Kingdom). Sera were collected, and then stored at –20 °C until they were tested at the Central Veterinary Laboratory in Windhoek.

Laboratory testing

The commercially available ELISA kit CHEKIT®-CHLAMYDIA (Dr. Bommeli AG-IDEXX, Switzerland) referred to in this paper as CHEKIT-ELISA, was used to test the goat sera for chlamydophilial antibodies. The CHEKIT-ELISA is a direct ELISA that uses an inactivated *Chlamydophila psittaci* antigen containing lipopolysaccharide (Vretou *et al*. 2007). Since the lipopolysaccharide is common to all chlamydiae (Everett 2000, cited by Hoelzle *et al*. 2004), the CHEKIT-ELISA is therefore a group-specific test that is designed to detect antibodies against *C. psittaci* in serum and plasma samples of ruminants.

The CHEKIT-ELISA was performed according to the manufacturer's instructions. Briefly, the kit includes microtitre plates that are pre-coated with inactivated *C. psittaci* antigen. Dilutions (1:400) of the samples to be tested were incubated for 90 minutes at room temperature (18 °C to 30 °C), in a humid chamber, in the wells of the microtitre plates to allow for the binding of any *C. psittaci*-reactive antibody in the test sample to the antigen in the wells. After incubation each microtitre plate was washed twice with CHEKIT-Washing & Dilution Solution. Hundred microlitres of diluted (1:200) monoclonal anti-ruminant-IgG conjugate, labelled with horseradish peroxidase, was added into each well and incubated again for 90 minutes as before. After a further two washing steps 100 µl of substrate-containing CHEKIT-Chromogen, pre-warmed to 25°C, was added to each well. The reaction was stopped with CHEKIT-Stopping Solution within 10 to 30 minutes after addition of the chromogen, as soon as the difference in optical density (OD) between the negative and positive controls is at least 0.3.

The degree of colour change that develops (optical density measured at 405 nm) is directly proportional to the amount of antibody specific for *C. psittaci* in the sample. The results were standardised using the positive and negative control sera and were expressed as a percentage proportion of the optical density (OD) of the sample (OD_{sample}) divided by the OD of the positive control (OD_{pos}), which were both corrected by subtracting the OD of the negative control (OD_{neg}) according to the following formula:

$$\text{Value (\%)} = (OD_{sample} - OD_{neg}) / (OD_{pos} - OD_{neg}) \times 100$$

Sera with a value below 30 % were considered negative, sera with values between 30 % and 40 % were ambiguous and were therefore also considered negative. Sera with values equal to or above 40 % were considered positive for antibodies to *C. abortus*.

Questionnaire

A questionnaire (Annexure 1) was used to collect information on the goat flocks that were sampled in this survey. The questionnaire was composed of five sections, from Section A through to Section E. The information provided in each section is briefly described:

Section A

This section solicited the general information about the farms that were sampled. This included the magisterial and veterinary districts in which the sampled farms were situated, farm name, owner's name and telephone number, and the date of sampling. This information was for identification of the farm and its owner, and to record the date on which the sampling took place.

Section B

This section was meant to collect information regarding the composition of the goat population of the farms sampled. This included the total number of goats on the farm and the numbers of

breeding does and bucks. These figures were then used to calculate the number of breeding goats to be sampled at that particular farm. The number of samples collected was also indicated in this section.

Section C

Section C was designed to provide information on the clinical history of the goats on the farm, particularly the clinical signs suggestive of chlamydophilosis. The farmer was asked to indicate whether or not he had experienced any incidences of the following eight clinical conditions: abortions; stillbirths; kids born weak; retained placentas; eye problems; arthritis; coughing and cases of orchitis or epididymitis in bucks during the previous 12 months. The total numbers of abortions and stillbirths were used to calculate the percentage level of abortions. This percentage was then used to categorise the sampled farms as to whether or not they had significant levels of abortions.

The clinical conditions were also recorded using a scoring system. The presence of any one of the eight clinical conditions listed in this section was indicated by a score of “1” whereas the absence thereof was indicated by a score of “0”. Therefore, each farm could potentially score a maximum score of 8. The scores for the two categories of farms sampled would be separately averaged and compared in order to see which category of farms had a higher average score.

Section D

This section was used to record the vaccination practises for chlamydophilosis on the farm. Any farm that practised vaccination against chlamydophilosis was to be excluded from the study.

Section E

Section E was meant to provide information pertaining to the level of awareness of chlamydophilosis by farmers. The farmers were asked to indicate whether they had ever heard

of enzootic abortion and the fact that it is a zoonosis.

Statistical analysis

The data collected during this study included the serology results from the laboratory as well as information collected via the questionnaires that were completed at the time of sample collection. Ambiguous or suspicious serology results were treated as negative. The questionnaires were analysed for completeness and only data fields that were complete for all the forms were taken into consideration. Both the serology results and the data from questionnaires were then captured on a Microsoft Excel® spreadsheet.

The clinical conditions that were recorded in the questionnaire were captured as scores for each individual farm. A simple descriptive statistic, the mean score, for each farm category was used to analyse and compare this data.

Descriptive statistics were used to describe both the study population as well as the seroprevalence of *C. abortus* up to farm level. The Chi-square test was used to compare the seroprevalence of *C. abortus* on farms with significant abortions (≥5 %) to farms with insignificant abortions (<5 %). Odds ratios were calculated to estimate the comparative risk between the two categories of farms that were studied. It compared the likelihood of farms having significant levels of abortions being seropositive to the likelihood of farms having insignificant levels of abortions being seropositive. All statistical analyses were performed using Intercooled Stata 7 (Stata Corporation, USA) software.

Chapter 4

Results

In this study, a total of 24 farms were randomly selected from a pool of 313 goat farms in OVD. A total of 1076 of the 1960 goats in the study population were tested for antibodies to *C. abortus*. None of the randomly selected farms practised vaccination against chlamydophilosis.

TABLE 1 A tabulated summary of the seroprevalence results of *C. abortus* in the Otavi Veterinary District. The table shows the breeding goat population on each farm tested, the number of sera collected from each farm, the percentage of positive sera, the farmer awareness of chlamydophilosis, and it also indicates on which farms there was a significant number (≥5 %) of abortions.

Farm number.	Breeding goat population	Number tested	Number positive	Percentage positive	Farmer awareness of chlamydophilosis	Farm with significant abortions
1	267	63	25	39.7	N	**Y**
2	100	57	0	0.0	**Y**	**Y**
3	201	49	13	26.5	N	**Y**
4	65	23	0	0.0	N	**Y**
5	127	26	0	0.0	N	N
6	129	87	14	16.1	N	**Y**
7	94	69	0	0.0	N	**Y**
8	53	25	0	0.0	**Y**	N
9	80	24	0	0.0	**Y**	**Y**
10	150	50	27	54.0	N	**Y**
11	60	52	0	0.0	N	N
12	26	25	0	0.0	N	N
13	25	29	5	17.2	N	**Y**
14	46	40	0	0.0	N	**Y**
15	38	37	0	0.0	N	**Y**
16	100	99	0	0.0	N	N
17	72	42	0	0.0	N	N
18	12	12	0	0.0	N	N
19	56	44	0	0.0	**Y**	N
20	47	45	0	0.0	**Y**	N
21	50	38	0	0.0	N	N
22	60	39	0	0.0	N	N
23	18	17	0	0.0	N	**Y**
24	84	84	2	2.4	N	N
Totals	1960	1076	86	8%	5/24	12/24

Key: N = no; Y= yes

Prevalence levels

The individual apparent seroprevalences of *C. abortus* per farm ranged from 0 % (18 farms) to

54 %. The seroprevalence on the six seropositive farms ranged from 2.4 % to 54 % (TABLE 1).

Six of the 24 farms that were tested had at least one positive breeding animal. This gave an apparent farm prevalence of 25 %. Eighty-six of the 1076 animals tested positive, giving an apparent individual prevalence level of 8 %.

Questionnaire results

Structure of the goat population

The 24 farms that were included in this study had an average number of 6 bucks and 75 does per farm (TABLE 2).

TABLE 2 The structure of the goat populations of the farms that were included in the study as gathered from the questionnaires. The table shows the total goat population on each farm sampled, the number of bucks and does, and the doe to buck ratios.

Farm number	Total goat pop.	Bucks	Does	Total: does & bucks	Buck / Doe ratio
1	471	5	262	267	1 : 52.4
2	166	4	96	100	1 : 24.0
3	278	7	194	201	1 : 27.7
4	102	4	61	65	1 : 15.3
5	145	10	117	127	1 : 11.7
6	200	24	105	129	1 : 4.4
7	120	6	88	94	1 : 14.7
8	113	13	40	53	1 : 3.1
9	150	8	72	80	1 : 9.0
10	260	12	138	150	1 : 11.5
11	114	4	56	60	1 : 14.0
12	45	2	24	26	1 : 12.0
13	34	2	23	25	1 : 11.5
14	66	1	45	46	1 : 45.0
15	50	3	35	38	1 : 11.7
16	347	2	98	100	1 : 49.0
17	72	2	70	72	1 : 35.0
18	18	2	10	12	1 : 5.0
19	93	12	44	56	1 : 3.7
20	68	1	46	47	1 : 46.0
21	50	7	43	50	1 : 6.1
22	60	6	54	60	1 : 9.0
23	18	5	13	18	1 : 2.6
24	205	7	77	84	1 : 11.0
Totals	3245	149	1811	1960	
Average	135	6	75	82	12.5

The number of bucks ranged from 1 to 24 whilst the does ranged from 10 to 262. The total average goat population per farm was 135. The buck/doe ratio averaged 1:12.5 and ranged from 1:3.1 to 1:52.4.

Clinical conditions present on the farms

Section C of the questionnaire dealt with the clinical conditions observed on the farm over the previous 12 months.

TABLE 3 A summary of the clinical conditions as gathered from the questionnaires. The score "1" means that the clinical condition was reported and "0" means that it was not reported. The total score reflects the total number of clinical conditions reported by the farmer out of a total of the 8 listed conditions. The table also indicates whether or not there were significant levels (≥5 %) of abortions on each farm.

Farm name	Abortions	Weak kids	Stillbirths	Retained placentas	Eyes	Arthritis	Cough	Orchitis	Total scores	Signifiant abortions
1	1	1	1	1	1	0	0	0	5	Y
2	1	1	1	1	1	0	0	0	5	Y
3	1	0	0	1	1	0	1	0	4	Y
4	1	1	1	1	0	0	0	0	4	Y
5	1	0	0	1	0	1	0	0	3	N
6	1	0	0	1	1	1	1	0	5	Y
7	1	1	0	1	1	1	1	1	7	Y
8	1	1	0	0	0	0	0	0	2	N
9	1	1	0	0	0	0	0	0	2	Y
10	1	1	1	0	0	0	0	0	3	Y
11	1	1	0	0	1	0	0	0	3	N
12	1	0	1	1	0	0	0	0	3	N
13	1	1	1	1	1	1	1	0	7	Y
14	1	1	0	1	0	0	0	0	3	Y
15	1	1	0	1	0	0	0	0	3	Y
16	1	1	1	0	1	1	1	0	6	N
17	1	1	0	1	1	0	1	0	5	N
18	1	0	0	0	0	0	0	0	1	N
19	0	0	0	0	0	0	0	0	0	N
20	1	0	0	0	1	0	0	0	2	N
21	1	1	0	0	0	0	0	0	2	N
22	0	0	0	0	0	0	0	0	0	N
23	1	1	0	0	0	0	0	1	3	Y
24	0	1	0	0	0	0	1	0	2	N
Totals	21	16	7	12	10	5	7	2	80	12/24
Percentage	88	67	29	50	42	21	29	8		50

Key: 1 = present; 0 = absent

A total of 22 farms (92%) reported at least one out of the eight listed clinical conditions

suggestive of chlamydophilosis (TABLE 3). All 12 farms that had significant levels of abortions reported at least three of these clinical conditions and they had a higher average score of 4.25 compared to an average score of only 2.4 for the 12 remaining farms with insignificant levels of abortions.

Abortions were the most common problem reported by the interviewed farmers (88%), followed by the birth of weak kids (67%), retained placentas (50%), eye problems (42%), stillbirths and coughing (both 29%), arthritis (5%), and finally orchitis which was reported by only 2% of the interviewed farmers.

Other information

Out of a total of 24 farmers that were interviewed, only 5 (or 20.8 %) of these were aware of chlamydophilosis and its zoonotic dangers. None of the selected farms practised any form of vaccination against chlamydophilosis.

Of the 24 farms that were selected, coincidentally, exactly half of these had significant levels of abortions (≥5 %) and the other half had insignificant levels (<5 %) of abortions (TABLE 4). Only 2 out of the 12 farmers who had experienced significant levels of abortions in their does were aware of chlamydophilosis and its zoonotic dangers.

TABLE 4 Stratified summary of the level of awareness of chlamydophilosis and its zoonotic dangers by farmers in Otavi Veterinary district. The table shows that there was a higher number of seropositive farms (41.7 %) in the category with significant abortion levels compared to the category with insignificant abortion levels (8.3 %).

	Number of farms	Number of farmers aware of chlamydophilosis	Percentage awareness	Number seropositive	Percentage seropositive
Farms with significant (≥5%) abortion levels	12	2	16.7 %	5	41.7 %
Farms with insignificant (<5%) abortion levels	12	3	25 %	1	8.3 %
Totals	24	5	21%	6	25 %

A total of 5 (83.3 %) out of the 6 seropositive farms had indicated significant levels (≥5 %) of abortions on their farms. The seroprevalence levels at these 5 farms ranged from 16.1 % to 54 %. The remaining one seropositive farm had an insignificant level of abortions (<5 %) and the lowest seroprevalence level of only 2.4 %. The odds of a farm with significant levels of abortions being seropositive were calculated to be 7.86 times greater than for a farm with insignificant levels of abortions. Nonetheless, Chi-square analysis indicated that there was no statistically significant difference between these two categories of farms (P=0.059). At individual animal level, this was not the case.

At individual animal level, the odds of an animal on a farm with significant levels of abortions being seropositive were calculated to be 113.7 times greater than that of an animal on a farm with insignificant levels of abortions being seropositive. Chi-square statistical analysis indicated that this was a highly statistically significant association (P=0.0000).

Chapter 5

Discussion

Studies carried out under field conditions usually present unique challenges. In this study, the actual number of serum samples collected did not always correlate with the table given in Annexure 2. This was because of the difficulties that are usually encountered in the field. For instance, not all animals can be rounded up at any one time. However, the sample size formula by Martin *et al*. (1987), which was used in this study, results in a large sample size when applied at flock level. When the same formula is applied to all farms pooled together, a much lower sample size of no more than 135 sera would have been required. This is far less than the 1076 sera that were collected in this study. Therefore the variation in the number of samples collected was accounted for by the application of the formula at flock level, which resulted in a larger sample size.

This study was undertaken in order to provide information pertaining to the role of *Chlamydophila abortus* in the health status of breeding goats in OVD, as outlined under the objectives. The farm level and individual levels of apparent prevalence were 25 % and 8 %, respectively. These figures were much lower than the results of the previous study, sixteen years earlier, by Apel *et al.* (1989), which determined farm level and individual apparent prevalence levels of 86 % and 50 %, respectively. However, such differences were not altogether unexpected, especially when one considers that there was a sixteen-year period between the two studies, and that Namibia received her independence less than a year after the results of the first study were published.

Post-independence Namibia saw a lot of changes in the livestock population dynamics. This included the movements of small stock, especially goats, from communal areas in the extreme

north of the country, where no *Chlamydophila* seroprevalence studies had ever been done, into the commercial areas where the first study had been done. The post-independence era also saw a major shift in farm ownership with more black farmers now owning farms formerly occupied by white farmers. A change of farm ownership is almost always followed by livestock movements and therefore changes in animal population structure. This may partly account for the differences in seroprevalence of chlamydophilosis.

There is also a precedent for disparities in seroprevalence levels when it comes to serosurveys like this one. For instance, in serosurveys conducted in Slovakia for five consecutive years by Tràvnicek, Kovacova, Zubricky & Cislakova (2001) during 1996, 1997, 1998, 1999 and 2000, the seroprevalences for chlamydophilosis in goats were 3.94 %, 10.02 %, 2.96 %, 3.96 % and 6.08 %, respectively. It is therefore not uncommon in cross-sectional studies to come up with different prevalence rates. This is because, unlike cohort studies that provide incidence data, prevalence studies cannot do so since they focus on a point in time. This makes the results of prevalence studies more prone to variation. Therefore, for the prevalence results to be comparable they have to be conducted at similar times and in the same population, which is often challenging under field conditions.

At farm level, the study indicated that there was an absolute difference in *C. abortus* seroprevalence levels between the two categories of farms studied. The 12 farms with significant levels of abortions (≥5 %) in breeding does had a higher farm prevalence level of 41.7% compared to 8.3% for the other 12 farms with insignificant levels of abortions (<5 %), even though the difference was not statistically significant (P=0.059). Therefore, the number of farms having significant levels of abortions and being seropositive at the same time were higher than those having insignificant levels of abortions and being seronegative. However, although the farm level difference was not statistically significant, the animal level difference was statistically

very significant (P=0.0000).

The farms with significant abortion levels also had a higher average score of 4.25 compared to 2.4 for farms with insignificant abortion levels for clinical conditions suggestive of caprine chlamydophilosis, especially abortions, birth of weak kids, retained placentas and eye problems. This showed that there was an association between clinical conditions suggestive of chlamydophilosis and the seropositivity of the farms.

These findings were in agreement with a previous study by Apel *et al.* (1989), which found that high *Chlamydophila* seroprevalences correlated well with clinical signs suggestive of *Chlamydophila* infections, especially abortions and keratoconjunctivitis. Other workers even found similar trends in cattle (Cavirani, Cabassi, Donofrio, De Iaco Taddei & Flammini 2001).

Only 5 out of the 24 farmers interviewed were aware of chlamydophilosis and its zoonotic implications, which represent about 21 % of the interviewed farmers. The previous study by Apel *et al.* (1989) did not look at the aspect of farmer awareness to chlamydophilosis. The fairly low level of awareness detected in this study could be partly explained by the fact that over the past couple of years there has been a steady increase in the number of new black farmers buying commercial farms. These new farmers are inexperienced and are still learning about modern animal husbandry practises and common animal diseases. However, another study with a much larger sample size of interviewed farmers, and covering a much wider area, would give a better picture of the real situation, not only in the OVD but also in the country at large.

None of the 24 farmers interviewed practised any vaccination against chlamydophilosis during the last 7 years. This is unfortunate, especially when one considers that seroprevalence levels detected on farms with significant levels of abortions ranged from 16.1% to 54 %. There is clearly a need for more farmer education and awareness about chlamydophilosis. However, the

relatively small farmer sample size of 24 farmers requires caution on the extrapolation of these results to the rest of the country's farmers. A much bigger countrywide study involving farmers in all the districts is therefore needed in order to gauge the true awareness level and the use of vaccination in the control of chlamydophilosis in the country as a whole.

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Annexure 1

QUESTIONNAIRE

Chlamydophila abortus prevalence in goats

SECTION A

GENERAL FARM INFORMATION

SV district	
Farm name	
Farm number	
Magisterial district	
Owner	
Owner address & tel.	
Date of sampling	

SECTION B

STRUCTURE OF GOAT POPULATION

Total number of goats on the farm	
Number of breeding does	
Number of bucks	
Number of blood samples collected	

SECTION C

HISTORY OF CLINICAL CONDITIONS SUGGESTIVE OF CHLAMYDIOSIS

Have any of the following conditions been experienced in this goat flock over the past year?

CONDITION	YES	NO	DON'T KNOW	NUMBER OF CASES
Abortions				
Kids born weak				
Stillbirths				
Retained placentas				
Eyes problems				
Arthritis				
Chronic cough				
Orchitis/epididymitis				

SECTION D

VACCINATION PRACTISES ON THE FARM

Is routine vaccination for chlamydiosis practised?	
Vaccine(s) used?	
When was the last vaccination?	

SECTION E

OTHER INFORMATION

Have you ever heard of the disease called chlamydiosis (enzootic abortion)?	Yes	No
Are you aware that chlamydiosis can affect people?	Yes	No

Annexure 2

SAMPLE SIZE CALCULATIONS

Illustration of the numbers of breeding goats (does and bucks) that were collected at each selected farm, depending on the size of the breeding goat population

BREEDING GOAT POPULATION	SAMPLES COLLECTED
10	9
20	18
40	31
50	37
80	51
100	58
120	65
150	72
180	78
200	82
250	89
300	95
350	99
400	103
500	109
600	113
800	118
1000	122
5000	135