PREVALENCE AND RISK FACTORS ASSOCIATED WITH BOVINE CAMPYLOBACTERIOSIS AND TRICHOMONOSIS IN SELECTED AREAS OF NAMIBIA

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ABSTRACT

Livestock production in Southern Africa, is faced with low fertility rate partly due to trichomonosis and campylobacteriosis infections. The purpose of this research was to investigate the prevalence of these two pathogens and associated risk factors in selected areas of Namibia. Three areas were selected for the research based on their production systems, Ovitoto communal area in Otjozondjupa region, Okakarara semi-commercial area in Otjozondjupa and Dordabis-Nina commercial area. A total of 172 bull were sampled, sheath scrapings were collected and transported in Steve's transport media®. Culture and PCR analysis of samples was done for *T. foetus* testing, whilst for *C. foetus* only PCR was done at Central Veterinary Laboratory, Windhoek (CVL). A questionnaire guided interview was conducted with the owners/managers of the bulls prior to sample collection to determine the associated risk factors of both diseases.

The findings were analysed in Microsoft Excel and the associated risk factors and the prevalence of the two pathogens were assessed. Pearson's chi-square, Z- score and multiple regression model statistical analysis were used to determine the significant association of the risk factors to the prevalence of the two pathogens.

The overall observed true prevalence of the herd was 19.4% for *T. foetus* and 10.7% for *C. fetus* out of 172 bulls. The prevalence of *T. foetus* was the highest in Okakarara semi-commercial areas (36%), but with no significant difference between Ovitoto communal area (7.5%) and Dordabis/Nina commercial farms (14.68%). Furthermore, the prevalence for *C. fetus* was 12% for Ovitoto communal areas showing no significant difference with that of Okakarara semi-commercial areas at 20%, and no positive cases were reported for Dordabis-Nina commercial farms.

There was a significant correlation between the two pathogens and research areas as well as between the prevalence of *C. fetus* and duration of keeping bulls. The age group of bulls with the highest positivity were found between the age of 4 to 6 years.

This research can form a basis for the methodology to determine prevalence and risk factors associated with trichomonosis and campylobacteriosis in beef herds in Namibia. However, further work on more farms is needed to influence and put strategic measures in place to curb the presence of both diseases in the country.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS OR ACRONYMS

AI: Artificial insemination

AP: Apparent Prevalence

ARC-OVI: Agricultural Research Council – Onderstepoort Veterinary Institute

PBS: Phosphate buffered saline

Cfv: *Campylobacter foetus* subspecies *venerealis*

CI: Confidence interval

CVL: Central Veterinary Laboratory, Windhoek.

DVS: Directorate of Veterinary Services

ELISA: Enzyme-linked immunosorbent assay

FAO: Food and Agricultural Organisation

GDP: Gross domestic product

GPS: Global positioning system

IFAT: Indirect fluorescent antibody test

IM: Intra-muscular

IV: Intravenous

MAWF: Ministry of Agriculture, Water and Forestry

MAWLR: Ministry of Agriculture, Water and Land Reform

NIP: Namibian Institute of Pathology

PCR: Polymerase chain reaction

Se: Sensitivity

Sp: Specificity

SS5A: Trichomonas medium and ascorbic acid

TSM: Trichomonas serum medium

TP: True Prevalence

UNAM: University of Namibia

WOAH: World Organization for Animal Health

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Thanks a million! May God Bless you all!

DEDICATION

This study is dedicated to my wife Fabiola and my children Meundju, Kuamberako, Kavejozikua, Naomi, Meunae and Uvatera and to my uncle Mr O.F Kaatura.

"The ability of leaders to communicate organisational goals clearly and guide employees to focus their attention on achieving these goals is crucial to success", Henry (2007: 338)

DECLARATION

I, Israel Kaatura, do hereby declare that the study of the prevalence and risk factors associated with bovine campylobacteriosis and trichomonosis in selected areas of Namibia, is a true reflection of my own study and that it has not been submitted for any degree at other universities. I do grant the University of Namibia the right to produce this thesis in whole or in part in any manner or format deemed fit. No part of this work may be reproduced, stored in any retrieval system or transmitted in any form or means without permission of the author or the University of Namibia on behalf of the author.

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Israel Kaatura **Date**

CHAPTER 1

INTRODUCTION

1.1. Background of the study

It is estimated that 60 % of people depend on livestock production especially in developing countries (Pica-Ciamarra, et al, 2014). Livestock production contributes towards the gross domestic product (GDP) in most countries. In Namibia, the livestock industry contributes 75 % to the agricultural GDP and most revenue is derived from export of beef to lucrative markets (MAWF, 2016). In 2020, a total of 159 414 cattle was exported mainly to neighbouring countries and 50 442 cattle were slaughtered at export abattoirs in Namibia (Meat Board of Namibia, 2022).

Despite the success of livestock production in Namibia, the industry is faced with reproductive performance challenges with low fertility rate being the highest especially during drought periods (Samkange, et al. 2019). One factor that contributes to the low reproductive performance in cattle is venereal disease infection. The most common venereal diseases in Southern Africa are campylobacteriosis and trichomonosis (Schmidt et al., 2010). The two diseases are known to cause embryonic death or abortion and irregular oestrus cycles in cows (WOAH, 2008; Irons et al, 2004).

Bulls infected with *Campylobacter fetus subspecies venerealis* (Cfv) and *Tritrichomonas foetus* (*T. foetus*) do not show any or very little clinical signs and tend to be carriers of the two diseases (Mai et al, 2013). Therefore, it is important to ensure accurate detection and diagnosis of bulls that are infected in order to stop the spread of these diseases. Sample collection for diagnostic purposes is done by sheath wash or sheath scraping in bulls (Madoroba et al, 2011). The WOAH testing protocol for bovine campylobacteriosis includes bacterial culture and fluorescent antibody testing of the preputial sheath washing sample, an enzyme linked immuno-sorbent assays (ELISA) and an agglutination test on vagina mucous (WOAH, 2018). The testing protocol for bovine trichomonosis include culture of the protozoa as well as polymerase chain reaction (PCR) amplification (WOAH, 2018). During sample collection, care should be taken to avoid sample contamination with intestinal trichomonad protozoa which might be mistaken for *T. foetus* (Irons et al, 2004).

In Namibia, the prevalence of bovine diseases is expected to be relatively higher in farming areas with no boundary fences (such as in most communal areas), with cattle sharing the same grazing areas and breeding bulls (Mendelson, 2008). Thus, buying bulls into these areas with no previous testing of venereal diseases could increase the chances of infection and thereby introducing the disease into previously uninfected herds. Similarly, a 20.7% prevalence for *T. foetus* was found in Spain beef herds and the associated risk factors were those herds with no control grazing management- or grazing in areas with no fences, in addition to bulls older than 4 years old and herds with reproductive complications (Collantes-Fernandez, 2019). The mean within-herd prevalence for *C. fetus* was 7.7% in Spain (Pena-Fernandez, 2021), whilst in Brazil 50.8% of herds (95% CI 41.6% - 60.1%) were infected with *Cfv* with a mean within-herd prevalence of 19.7% (95% CI 13.3% - 25.1%) (Haas, et al 2020). Studies done in Argentina showed a prevalence rate of 3.03% for *T. foetus* and 4.12% for *C. fetus* (Molina, 2018).

A study done in three states in Nigeria showed a prevalence of 25.5% for *C. fetus* and no positive cases of *T. foetus* reported (Mai H.M et al, 2013). A retrospective study done by Madoroba, 2011, on sample submitted at Onderstepoort Biological Institute found 4.5% prevalence of *T. foetus* in South Africa, 4.1% from Namibia, 3.33% from Botswana and no detection from samples from Swaziland and Zambia. Furthermore, the prevalence of *C. fetus* was found to be 2.44% from Zambia, whilst Namibia had 2.08% and South Africa had a prevalence of 1.78%. These international and regional figures show the variability in the occurrence of these venereal diseases across countries, which encourage participation in awareness and testing to confirm the diseases.

The purpose of this research is to estimate the prevalence of bovine campylobacteriosis and trichomonosis and to investigate potential associated risk factors in three selected areas representing typical production systems in Namibia.

1.2. Statement of the problem

In Namibia, bulls registered for stud breeding requires testing for trichomonosis and campylobacteriosis and have to be issued with a breeding soundness certificate (annexure A). However, most herd bulls are not issued with a breeding soundness certificate and therefore their reproductive health status is unknown. Farmers who buy bulls with unknown disease status increase the risk for their herd to be infected with venereal diseases. In most communal areas, there are no controlled breeding systems and bulls tend to mate with any cows that are on heat. In these areas, there are no fences and animals share a common grazing area and water points, resulting in an increased risk for trichomonosis and campylobacteriosis infection and spread between herds. In commercial areas, each farm is surrounded by a fence, thereby restricting free movement of bulls from one farm to another and it is expected that of the risk for venereal disease infection should be less. Stud farmers that sell bulls commercially have their bulls tested frequently prior to selling, therefore, the risk of infection with these diseases is expected to be lower in these farms.

Inadequate knowledge of farmers about the importance of having bulls regularly tested contributes to the spread of the two diseases. Coupled to not having their bulls tested, there are only a few farmers who get their cows tested for pregnancy. Cases of repeat breeding have been reported in most areas, especially in communal areas. Repeat breeding results in prolonged calving periods which contributes towards financial losses. The study by Madoroba et al (2011) titled "Prevalence of *Campylobacter foetus* and *Trichomonas foetus* among cattle from Southern Africa" found that 45 (3.75%) out of 1201 samples submitted to the bacterial PCR laboratory of the ARC-OVI were positive for *T. foetus* and 60 (1.9%) samples tested positive for *C. foetus* out of the 3161 submitted. Shortfalls found in this paper are that the samples collected do not indicate the place of origin, nor do they indicate the date of samples collected as well as indicate samples voluntarily sent to the laboratory for routine examination and certification of bulls for sale. Therefore, this cannot be a true reflection of the prevalence of the two pathogens in Namibia as the test was performed to confirm negative status rather than as a survey of the disease prevalence. Furthermore, the study done by Casteriano, et al (2016), titled "Novel genotype of *Tritrichomonas foetus* from cattle in Southern Africa", indicated that the Central Veterinary Laboratory in Windhoek (CVL) received a total of 4000 sheath wash samples all over Namibia between 2014 and 2015 of which 36 (0.9%) tested positive for *T. foetus*. However, the research did not focus on the prevalence and relied on samples submitted at the CVL of which most of them are from stud breeders who test their bulls annually.

Furthermore, according to the draft South African import permit of livestock, testing of trichomonosis and campylobacteriosis are not requested for importation and a farmer can import a bull from the neighbouring country with an unknown venereal disease status into South Africa. However, permits found for importation of bulls from South Africa into Namibia and Botswana request at least one sample test result.

No published study has been carried out to determine the prevalence of the two pathogens in different regions of Namibia. Nor has there been any study done to determine the impact the associated risk factors contribute to the prevalence of the two diseases. Knowing the prevalence and the risk factors will assist farmers and competent authority in understanding and applying mitigating measures in the control of the two diseases.

1.3. Objectives of the study

1.3.1. **Main objective**

The main objective is to determine the epidemiology of bovine campylobacteriosis and trichomonosis in selected areas of Namibia.

1.3.2. **Specific objectives**

The specific objectives are as follows:

i. To determine the prevalence of *Campylobacter fetus* and *Tritrichomonas foetus* in the herd in selected communities
ii. To compare the prevalence of these two pathogens as well as animal level prevalence between different identified areas.
iii. To determine the risk factors associated with these two pathogens in Namibia.

1.4. Significance of the study

The findings of the study will contribute towards the understanding of the epidemiology of the two pathogens in selected areas of Namibia. This should help in identifying the distribution and risk factors which will aid in providing diagnostic strategies and application of remedial control measures of the two diseases.

1.5. Limitation of the study

The research investigated the prevalence of bovine campylobacteriosis and trichomonosis in bulls of which sampling requires proper handling facilities. However, some homestead had inadequate handling facilities but through collaboration with neighbouring farmers, those bulls were moved to homestead with better handling facility. Farmers were responsible for paying for the test results, however, some were not willing while others were financially unable to participate. Farmers associations responsible for that area were approached to assist farmers in this regard. Farmers migrating to other places due to drought affected the sampling population. The target was to sample 361 bulls and only 172 bulls were presented in the study area. This resulted in collecting samples from all presented bulls, hence, the sampling population was drastically reduced.

CHAPTER 2

LITERATURE REVIEW

2.1 Bovine trichomonosis

Commonly known as trichomoniasis (WOAH, 2018), bovine trichomoniasis or just trich (Buller & Corney, 2013), is a protozoan disease found in bulls showing no clinical signs and transmitted to cows during mating. The disease causes infertility and abortions in cows (WOAH, 2018; Calvani et al, 2021). Risk factors relevant to the presence of the disease previously described include bulls older than 3 years and the presence of reproductive disorders within a herd (Collantes-Fernandez, 2019).

2.1.1 Aetiology

Bovine trichomonosis is caused by a protozoan parasite *Tritrichomonas foetus* which belongs in the family of Trichomonadidae (WOAH, 2018; Polo et al, 2022). Morphologically, the protozoa *Tritrichomonas foetus* is 8 to 18 micrometer long, 4 to 9 micrometer wide (Yao & Koster, 2015), with one posterior flagellum and three anterior flagellae and an undulating membrane (World Health Organization for Animal Health, 2018). The morphological structure explained above result in the organism to move with jerky and rolling motion when seen under a light microscope. This form part of the diagnostic approach of this protozoon (Polo et al, 2022). *Tritrichomonas foetus* has three different serotypes based on agglutination, namely the Manley strain, Brisbane strain and Belfast strain (Skirrow & BonDurrant, 1988; Gregory et al,1990; Karli, 2020).

2.1.2 Geographical Distribution

Bovine trichomonosis was first diagnosed in France in 1888 (Buller & Corney, 2013). The disease is now found throughout the world (WOAH, 2018) with an infection rate in bulls that vary from 2 to 40% (Coetzee, Thomson and Tustin, 2004). The disease is common in herds that are breeding naturally especially in developing countries (Mitchi et al, 2016). However, studies have reported some developed countries with positive herds such as Argentina (Oyhenart, 2024), United States of America (Szonvi et al, 2012) and Spain (Pena-Fernandez, 2021). In Namibia the disease was first reported in 1972 in Windhoek district (Schneider, 1994).

2.1.3 Pathogenesis

A bull gets infected after mating with an infected cow, the organism then lodges in the preputial folds of the epithelial lining as well as the distal portion of the penis (glans penis) and in the urethra (Rae and Crews, 2006). The infection is more exterior resulting poor immune response and pathogenic lesions (Najera, 2022). Younger bulls tend to get rid of the disease easier whilst in older bulls where the preputial folds are enlarged with deep crypts result in *T. foetus* to lodge inside the crypts and the bulls to become persistently infected (Gharban, 2023; WOAH, 2018).

Cows get infected through the process of mating or by artificial insemination using infected semen (Alavi-Shoushtari, 2014; Janzen, 2020). Upon introduction of the organism into the female reproductive tract, it lodges in the cervix, but the infection does not affect fertilization (Parsonson et al, 1976; Bielanski et al, 2004). However, an infection with *T. foetus* results in an embryonic death due to endometritis, and repeat breeders follow as seen with prolonged inter-calving periods. Infected cows eventually clear themselves within oestrus cycles (Hairgrove and Gill, 2009; Collantes-Fernandez et al, 2017; Martin et al, 2021). During the oestrus cycle, an increased concentration of oestrogen during the follicular phase stimulate the production of local immunity as well as the opening of the cervix, which facilitate the clearing of the uterine fluid post luteal phase (Adeyeye et al, 2012; Alavi-Shoushtari et al, 2014; Zangure, 2019). Cows are not persistently infected with *T. foetus* and *C. fetus* as they are able to produce IgG antibodies whilst bull's antibodies concentration is low and unable to clear the infection (Cobo et al, 2011). However, there have been reports of cows to carry the infection during pregnancy up until 2 months post-partum and hence can re-infect bulls during coitus (Martin et al, 2020)

T. foetus is also a zoonotic disease and has been reported to cause peritonitis as well as meningoencephalitis in humans especially those that are immunocompromised and immunosuppressed (Yao, 2012). Other animals reported to be affected by *T. foetus* are horses and domestic cats (Tolbert, 2021).

Differential diagnosis includes diseases such as leptospirosis, infectious bovine rhinotracheitis, campylobacteriosis, bovine viral diarrhoea, neosporosis, brucellosis, anaplasmosis and chlamydiosis (Coetzee et al, 2004; Rae and Crews, 2006).

2.1.4 Clinical Signs

Preputial discharges with small nodules have been reported in bulls initially infected with *T. foetus*. However chronically infected bulls do not show any clinical signs (BonDurant, 1997). Cows infected result in vaginitis with invasion of the organism into the cervix and uterus. If the cow is pregnant, endometritis with complication of placentitis leading to early abortion (commonly seen is repeat breeding) coupled with uterine discharges and or pyometra (Skirrow and BonDurrant, 1988; BonDurant, 1997). Cows clear themselves within 90 days with immune response lasting for at least 1 year (Funnel 2022; Cobo et al, 2011).

2.1.5 Diagnosis

There are three methods of collecting samples from the preputial cavity of the bull and vagina of the cow; namely swabbing, washing and scraping (Coetzee et al, 2004; Rae and Crews, 2006). Studies done by Mukhufhi et al, (2003), did not find the differences in the sensitivity between the sampling methods on gel electrophoresis PCR. In order to increase the sensitivity of the test, it is recommended to sample the bulls three times at 7 to 10 days apart (Kimsey et al, 1980; Yao, 2013). Due to the low sensitivity in sample collection and that the protozoan is not always found in the preputial cavity (Yao, 2013), in order to obtain at least a 95% or higher sensitivity, bulls have to be sampled three times with sexual rest during sampling.

Sheath scraping is achieved by inserting an artificial insemination pipette attached to a 20ml syringe into the bull prepuce and scraping back and forth 20 times whilst firmly holding on the prepuce filing the pipette with negative pressure for suction of smegma (Mendoza-Ibarra et al, 2012). The negative pressure on the syringe is then released during the withdrawal of the pipette to avoid sucking in urine. The sucked content is washed with PBS and 2ml of the mixture is transferred into transport media. Quality control of samples is pertinent to obtain reliable test results. Some of the quality factors to be observed include the method used for sample collection, the transport media used, the manner in which the sample was stored, temperature control, and the duration the sample takes to reach the laboratory, as well as the testing methods used (Yao, 2013).

Protozoa culture is the main standard for the diagnosis, however, other testing methods such direct examination using a microscope has also been done (Irons, 2002; Coetzee et al, 2004). Furthermore, Chapter 3.4.15 of WOAH (2018), gives a list of different diagnostic procedures available such conventional PCR on clinical samples, a combination of culture and conventional PCR and Real Time PCR. During sample collection, it is important to ensure that the prepuce is clean by wiping off dirt and using a sterile pipette. Hygienic conditions guarantee minimal contamination with the outside prepuce area. Other trichomonads such as *T. enteris, T. buttreyi, T. pavlova, Pentatrichomonas hominis* and Pseudotrichomonas species may cross-contaminate and result in false positive test results. False positivity resulting from low sensitivity is particularly high when culture and/or direct microscope are used as the only laboratory test, thereby resulting in culling of false positive bulls (Taylor et al, 1994; Adeyeye et al, 2012; Michi et al, 2016). In sampling 2832 bulls, Perez et al (2006) study found a sensitivity and specificity of 72% (range of 59 to 87%) and 95.4% (range of 94 – 96%) respectively. Studies done by Mukhuthi et al (2003) found 83% positive cases after infecting 29 bulls and sampling them after 2 to 4 days. The diagnosis of *T. foetus* by culture alone has a sensitivity range of 84% to 96%, however due to contamination, culture lacks specificity (Michi et al, 2016). Whereas, the sensitivity and specificity of a single sample using PCR alone is at 65.8% and 90% respectively (Cobo et al 2007). Therefore, the best diagnostic approach is combined culture with PCR with three weekly sampling that have a sensitivity and specificity of 87.5% and 95.6% respectively (Cobo et al 2007). This result coincides with Zangure (2019) who found a sensitivity of 88.7% and specificity of 100% with combined diagnostic approach of culture and PCR.

2.1.6 Treatment

There is currently no successful treatment registered for bovine trichomonosis (Michi et al, 2016; Funnel, 2022). Nitroimidazole class of drugs such as dimitridazole (50mg PO) and ipomidazole (60g IM) have been used successfully used in treatment of *T. foetus*, however, the two drugs have been prohibited from use in food producing animals due to their carcinogenic properties (Love et al, 2017). Metronidazole (75mg IV) is given for three days, however, the drug is known to cause side effects such as inappetence, rumen stasis and thiamine deficiency due to the effect on rumen microbes (Love et al, 2017).

2.1.7 Prevention and control

Good farming practices is the golden standard to prevent the introduction of the pathogen in your herd (Rae and Crews, 2006). The use of bulls that have been tested, removing positive bulls and use of artificial insemination are some of the control measures to prevent the presence of this disease in a herd (WOAH, 2018; Funnel, 2022). A live vaccine that produces both the innate and adaptive immune response that are short – term is available (WOAH, 2018). Polyvalent vaccine containing Tritrichomonas, Campylobacter and Leptospira species such as

Trichguard® can also be used in the herd to improve herd immunity (Corbeil,1994). Vaccines against trichomonosis are only registered with a claim to decrease the financial or reproductive losses associated with the disease, and not as a sole means of controlling the disease (Ortega-Mora et al, 2022). A new inactivated *Tritrichomonas foetus* vaccine known as Trichobovis® that improves genital clearance of the infection and calving intervals in cattle has been developed, but does not reduce the transmission of the pathogen (Ortega-Mora et al, 2022). Therefore, testing and culling remains the recognised method to control the disease (Rae & Crews, 2006; Ondrak JD, 2016; Funnel, 2022).

2.1.8 Legislation

WOAH requires member states to report listed diseases of animals. There are currently 117 animal diseases that are required to be reported to WOAH and the list includes trichomonosis (https://www.animalhealthsurveillance.agriculture.gov.ie/oielisteddiseases/).Contrary, the Animal Health Act 1 of 2011 in Namibia has not listed trichomonosis as notifiable disease and neither its regulations. Circular No. 6 of 2017 of the Directorate of Veterinary Services (DVS) in Namibia has listed notifiable diseases but also excluded trichomonosis. However, the disease has been listed as one of the mandatory testing requirements for importing bulls from neighbouring country but not in cows as seen in the livestock import permit of Namibia.

2.2 Bovine Campylobacter

Commonly known as bovine genital campylobacteriosis, or bovine venereal campylobacteriosis, it was also formerly known as Vibriosis (Coetzee et al, 2004). The infection causes infertility and abortions in cows (Seid, 2019; WOAH Terrestrial Manual, 2021).

2.2.1 Aetiology

Bovine campylobacteriosis is a bacterial disease caused by *Campylobacter fetus subspecies venerealis* (previously known as *Vibrio fetus venerealis*) which is generally found in the reproductive tract of cattle (Coetzee et al., 2004; WOAH Terrestrial Manual, 2021). Morphologically, *C. fetus* is a small, curved to spiral gram-negative rod with single polar flagellum at both ends that aid in a corkscrew-like movement (da Silva, 2021). The bacteria is oxidase positive (van Bergen et al, 2005), grow well in 15%- oxygen concentration and 5%

carbon dioxide concentration and is commonly found in the reproductive tract, being transmitted from one animal to another through coitus (Hum & Quinn, 1994). *Campylobacter fetus subspecies fetus* found in the gastrointestinal tract but occasionally infect the reproductive organs through contamination causing infertility in cattle (Thompson & Blaser, 2000).

2.2.2 Geographic Distribution

C. fetus subspecies venerealis (Cfv) is found throughout the world. Coetzee et al. (2004), reported that the disease is endemic in Southern Africa while Hum et al. (2009), indicated that the regional veterinary laboratories in South Africa and Namibia have reported a prevalence rate between 0 to 12%. Hum et al 2009 further stated that in one particular study conducted on bulls which were communally grazed had a substantially higher prevalence of 29%. Studies conducted in South Africa have reported the prevalence of *C. fetus* as 1.9% (60/3161) and *T. foetus* 4.1% (142/3161) (Madoroba et al, 2011). In Namibia, bovine *C. foetus* was first diagnosed in 1965 in the Gobabis district and then a year later it was detected in Omaruru district (Schneider, 1994).

2.2.3 Pathogenesis

The bull gets infected after mating with an infected cow, the organism then lodges in the preputial folds (crypts) of the epithelial lining as well as the distal portion of the penis (glans penis) and the urethra (Noakes et al, 2001; Sahin et al, 2017). Older bulls that have well developed crypts tend to maintain the bacteria as it provides good environment with low oxygen concentration (Sahin et al, 2017). Studies done by Cagnoli et al (2020) found that Cfv can attach to spermatozoa thereby affecting its viability. Cows get infected during mating or through artificial insemination and the organism is found in the vagina and cervix. By day 14 it ascends into the uterus and eventually into the oviducts causing endometritis and salpingitis (Michi et al, 2016; Cagnoli et al, 2020). Conception takes place, however, due to the inflammatory response embryonic death occurs resulting in abortion or embryonic resorption within the first trimester of gestation period (Balzan et al, 2020).

2.2.4 Clinical Signs

Preputial discharges with small nodules have been reported in bulls initially infected with *C. foetus*. However chronically infected bulls do not show any clinical signs whilst cows show signs of returning to oestrus, repeat breeding and low conception rate (Schmidt 2008; Hum et al, 2009; Sahin et al, 2017). The immune response is the same as seen in *T. foetus* infected cows and once the cow is infected, they develop a temporary immune response lasting for at least 1 year whilst bulls that are older than 4 years are persistently infected (BonDurant, 1997; Adeyeye et al, 2012).

2.2.5 Diagnosis

Similar to the *T. foetus,* there are three methods of collecting *C. fetus* samples from the preputial cavity of the bull and vagina of the cow namely; swabbing, washing and scraping (Coetzee et al., 2004; Rae and Crews, 2006; Martin et al 2021). Culture and subspecies identification are the main standard for the diagnosis, however, other testing methods such as IFAT and Mab-based ELISA have also been recommended (WOAH Terrestrial Manual, 2021). Although culturing the bacteria coupled with phenotypic identification is the recommended method for *Cfv* (WOAH, 2021), bacterial culture is faced with challenge of sample contamination resulting in bacterial overgrowth (Guerra et al. 2014; Michi et al. 2016). Diagnostic procedure using PCR is widely acceptable in most laboratories as it is faster and less laborious (Hum et al 1997; Chaban et al. 2012).

Both conventional and real-time Polymerase chain reaction (PCR) assay targeting nahE, multilocus sequence typing (MLST) and amplified fragment length polymorphism (AFLP) are able to identify *C. fetus* up to species level (Abril et al, 2007; van der Graaf-van Bloois et al,. 2013), but none of them is able to identify the *C. fetus* isolates reliably to subspecies level (WOAH Terrestrial Manual, 2021). The Namibian CVL diagnostic for *C. fetus* is only up to species level. Abril et al (2007) found 100% sensitivity and 100% specificity of PCR assay at species. Studies done by Guerra et al, 2014, for real time PCR found specificity and sensitivity of the test to be 85% (95% CI, 80.5% - 88.6%) and 85.4% (95% CI, 80.6 to 89.2%) which are similar to the testing protocol done by CVL in Windhoek. Earlier studies done by McGoldrick et al (2013), showed a specificity and sensitivity test of 99.8% and 98.7% respectively using real time PCR assays targeting nahE gene and ISCfe.

Differential diagnosis for *C. fetus* are similar to those of *T foetus,* and include disease such as leptospirosis, infectious bovine rhinotracheitis, trichomonosis, bovine viral diarrhoea, neosporosis, brucellosis, anaplasmosis and chlamydiosis (Coetzee et al, 2004; Rae and Crews, 2006).

2.2.6 Treatment

Studies done by Erickson (2017) and Truyers (2014), using long-acting oxytetracycline and monovalent vaccine two weeks apart in one group bulls whilst the other group were not treated found no significant differences between the two groups as it did not stop the spreading of the pathogen. However, studies done by Cobo (2003), found higher pregnancy rate and systemic immunity response in vaccinated heifers comparing to the control group. WOAH has not recommended an international standard for these products (WOAH Terrestrial Manual, 2021).

2.2.7 Prevention and control

Eliminating persistently infected bulls is recommended in herd diagnosed with bovine campylobacteriosis (Fascolo et al. 2018; WOAH Terrestrial Manual, 2021). Bulls with negative results or virgin bulls can be bred with heifers or use artificial insemination to prevent the introduction of the disease in your herd (Noakes et al. 2001; Fascolo et al. 2018). Cows infected with the disease can clear themselves after three oestrus cycles (Balzan et al. 2020). Cows originating from an unknown disease status introduced in the herd must first be isolated for three months (Balzan et al. 2020).

2.2.8 Legislation

The statement indicated under trichomonosis legislation also applies to venereal campylobacteriosis.

CHAPTER 3

MATERIALS AND METHODS

3.1.0 Research Design

A mixed research method was used, including a descriptive data collection, cross-sectional and quantitative methods to assess the prevalence and risk factors of trichomonosis and campylobacteriosis. The quantitative study consisted of a questionnaire guided interview to obtain the history of the herd in order to identify the possible risk factors associated with the two diseases (see annexure B). The cross -sectional study was used to collect sheath scraping samples in bulls to determine the prevalence of the two diseases. Data were transcribed on MS Excel® Windows 11 version 23H2 for x64-based system (KB5034848) and online social science statistic calculator and analysed.

An awareness campaign was made through the radio over the importance of testing bulls against the two pathogens and informing farmers about the research that will take place, resulting in a high farmer's response in Okakarara and Ovitoto but Dordabis-Nina farmers were reluctant to participate in the research. Another communication was also made through Kambekura Farmers Association in Ovitoto and the Okakarara Farmers Association to their responsible farmers.

3.1.1 Study Area

The research took place in 3 different places in Namibia as shown in figure 1. Each area has their own unique farming practices with potentially different risk factors of the two diseases. Research areas were conveniently selected based on their production systems mainly commercial, communal and semi-commercial.: In summary, Khomas region, the Dordabis – Nina farms (GPS coordinate of 22.9482°S, 17.6615°E) consisting of 30 commercial farms with an average of 10 bulls per farm adding up to a target population of 300 bulls. Dordabis-Nina farms is located about 120km south-east of Windhoek. The commercial farms have border fences preventing mixing of livestock from one farm to another. In Otjozondjupa region, two areas with different production systems areas were selected, namely, Okakarara (GPS coordinate of 20.5907°S, 17.4588°E) and Ovitoto (GPS coordinate of 22.57887°S, 17.04424°E). Ovitoto communal had 23 villages, each with 10 household and 7 bulls per village (total population of 161 bulls). There were no fences between villages and livestock sharing grazing areas. In Okakarara semi-commercial areas (from Okakarara turn off to Ohorongombaranga) the 13 selected villages run along the Namwater pipeline, and all the villages were each fenced off (in a form of units of about 3km by 7km) with 5 households and 4 bull per village (hence a total population of 52 bulls).

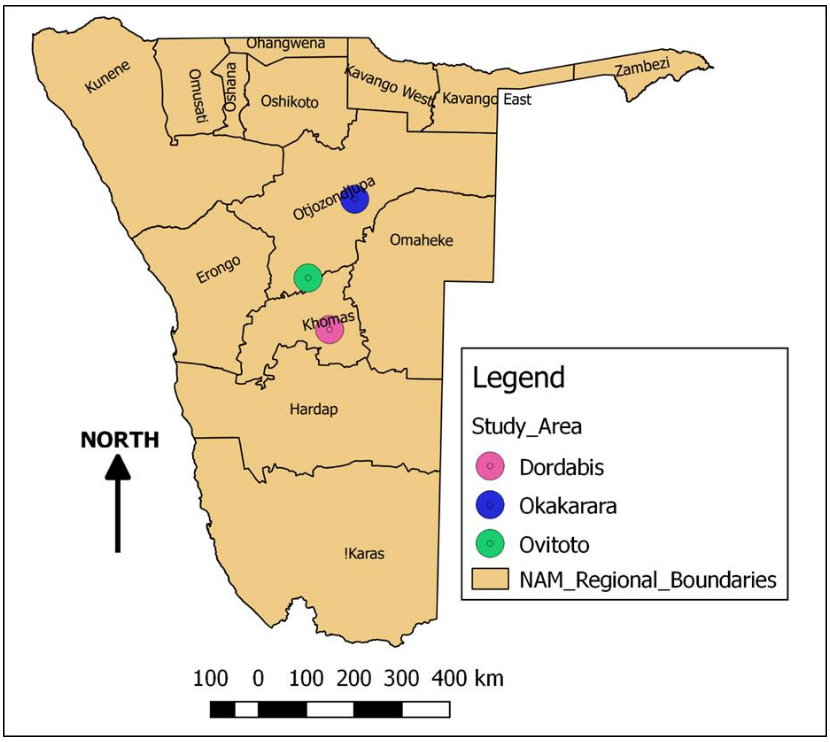

Figure 1. Namibian map showing research areas (**map designed by Mrs H. Nambahu, 2022)**

3.1.2 Sample Size

In order to calculate the sample size, the formulae adopted by Thrusfield (2018), were used as follows:

$$n = \frac{(1.96)^2}{(d)} \ X \ \frac{[(Se \times P_{exp}) + (1 - Sp)][(1 - Se \times P_{exp}) - (1 - Sp)(1 - P_{exp})]}{(Se + Sp - 1)^2}$$

Where:

n = sample size

P_{exp} = expected prevalence

d = desired absolute precision

Se = Sensitivity

Sp = Specificity

The sample size was calculated as indicated in Table 3 and randomized sampling technique was used in which individual names of villages and farms were written in small pieces of paper and placed in a bucket and randomly picked up to the number as identified as herd size (Annexure C). Out of 23 villages in Ovitoto, 15 villages were randomly selected were as in Okakarara and Dordabis-Nina all identified places were targeted for sample collection.

Based on Table 1, a total population of the research was 548 bulls of which 361 was the sample size.

Table 1: Calculation of sample size

Description	**Dordabis-Nina Farms**	**Ovitoto Communal Villages**	**Okakarara Communal Villages**
Expected Prevalence of Trichomonas and Campylobacteriosis is estimated at 30%			
Sample size	300	196	52
Desired Precision: 0.05 Confidence level: 0.95 Specificity of the test: 0.998 (McGoldrick, 2013) Sensitivity of the test: 0.987 (McGoldrick, 2013)	$n= \frac{(1.96)^2}{(0.05)} \text{ X } \frac{[(0.987 \text{ x } 0.3) + (1 - 0.998)][(1- 0.987 \text{ X } 0.3) - (1 - 0.998)(1 - 0.3)]}{(0.95 + 0.9 - 1)^2}$ $n= \frac{(1.96)^2}{(0.05)} \text{ X } \frac{[(0.95 \text{ x } 0.3) + (1 - 0.9)][(1- 0.95 \text{ X } 0.3) - (1 - 0.9)(1 - 0.3)]}{(0.987 + 0.998 - 1)^2}$		
Number of bulls to be sampled	530 bulls this figure is larger than the total available sample size (300)		
The sample size obtained from this formula is relatively larger than the target population and hence another adjusted formula that will in cooperate the above sample size has been suggested by Thrusfeld, 2008, as follows: $nadj = \frac{N \text{ x } n}{N + n}$ Where nadj = sample size adjusted N = target population n = calculated sample size from the original formulae	$nadj = \frac{300 \text{ X } 530}{300 + 530}$ nadj = 191 bulls to be sampled	$nadj = \frac{161 \text{ X } 530}{161 + 530}$ nadj = 123 bulls to be sampled	$nadj = \frac{52 \text{ X } 530}{52 + 530}$ nadj = 47 bulls to be sampled
Number of farms / villages	10 bulls per farm to get the number of farms to be sampled which was 19 farms (191 bulls / 10 bulls per farm = 19)	7 bulls per village to get the number of villages to be sampled which was 17 villages (123 bulls / 7 bulls per farm = 17)	4 bulls per village to get the number of villages to be sampled which was 12 farms (47 bulls / 4 bulls per farm = 12)

3.2 Research Procedure

3.2.1 Data Collection

Sheath scraping in bulls from selected farming areas of Namibia was done from 11th June 2022 to the 8th of July 2022. A total of 172 bulls were sampled within the research areas, the target estimate was to sample 361 bulls. The reason for the low sampling population was that out of the 19 farms targeted to participate in the research from Dordabis and Nina, only 7 farmers (with total number of 39 bulls) indicated the willingness to participate. Our assumption was that each farm has 10 bulls giving us a total of 190 bulls in commercial farms but since only 7 participated, this reduces our estimated sampling to 70 bulls. Most communal farmers experienced drought that killed most of their animals, thereby reducing livestock population. During the population estimation this factor was not taken into consideration. Decision was then made to sample bulls in all the villages and not randomly selecting them. The research team and participating farmers were all blinded to the results of the first round of sampling until the second round of samples received. The research was an active participatory and each farmer paid for their test results. The test results were given to the farmers and an information day per region was organised by farmers for feedback and way forward.

3.2.2 Sheath Scraping

Bulls were restrained in crush pens and immobilized using El Toro® 3 electro – ejaculator with a component of an immobiliser. The hair around the prepuce was cut off with a scissor, cleaned with F10® antiseptic solution and wiped off with paper towel. A pipette attached to a 10ml syringe was inserted into the prepuce and penile mucosa directed towards the spermatid cord and negative pressure was applied on the syringe. Sheath scrapping was then performed by forward and backward movement of the pipette for about 30 seconds whilst maintaining 10ml of negative pressure on the pipette for aspiration of the smegma obtained by the scraping action. After 30 seconds the negative pressure on the syringe was released to avoid urine aspiration and the suctioned smegma was transferred into a test tube with 5ml phosphate buffer solution and the smegma was flushed out using the PBS as shown in figure 2.

Thereafter, 2ml of the mixture was transferred into Steve's transport medium using a syringe and ensuring no air is pushed into the transport media by filling up the sample bottle with PBS. Samples were marked and placed in a cooler box and transported on the 3rd day to CVL in Windhoek. Bulls were sampled twice at 5 days apart. At CVL, the following tests were performed by the personnel of the laboratory whilst the researcher was allowed to observe the diagnostic processes: *Tritrichomonas foetus* spp. culture detection (test procedure CLM SOP4), *Tritrichomonas foetus* spp. identification (real-time) (test procedure BIO SOP 31) and *Campylobacter fetus* spp. identification (test procedure BIO SOP 28). The summarised version of the testing procedure is explained in the headings below.

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Figure 2: Mixing of smegma with PBS

3.2.3 Testing Methods for *C. fetus* and *T. foetus*

Prior to sample collection, the laboratory was informed in advance to prepare culture media. Samples received by the laboratory were subjected to quality control processes such as temperature recording, verification of identification numbers, labelling with the sample submission numbers and then all data were transferred to the Laboratory Management System (SILAB) as per CVL protocol. Due to the large number of the samples, data capturing took most of the time especially the morning hours and sample processing was done in the afternoon.

3.2.3.1 Microbiological Section

The process of culturing *T. foetus* was based on the protocol (CLM SOP 04) designed by CVL and is available at CVL on request. In summary two different culture media namely, Trichomonas Medium with Ascorbic Acid (known as SS5A), and Trichomonas Serum Medium (known as TSM) were prepared in advance. One ml of the sheath scrape sample was transferred to 15ml of each culture media and 1ml of penicillin (10,000 units/ml) – streptomycin (10,000 µg/ml) combination was also added to prevent bacterial growth. The mixture was then placed in rags as indicated in figure 3, together with a positive control and incubated at 37°C for 5 days. A random sample from the incubator together with the positive control were read daily. Positive control was read daily to ensure that the growth media was properly prepared. On day 5, the final readings were made starting with the positive control and continuing with the test sample by placing a drop of the sample on a glass slide and inspected under a light microscope. A suspicious sample was the one showing movement and the shape of the organism. All suspicious samples were then taken to the molecular section for confirmation.

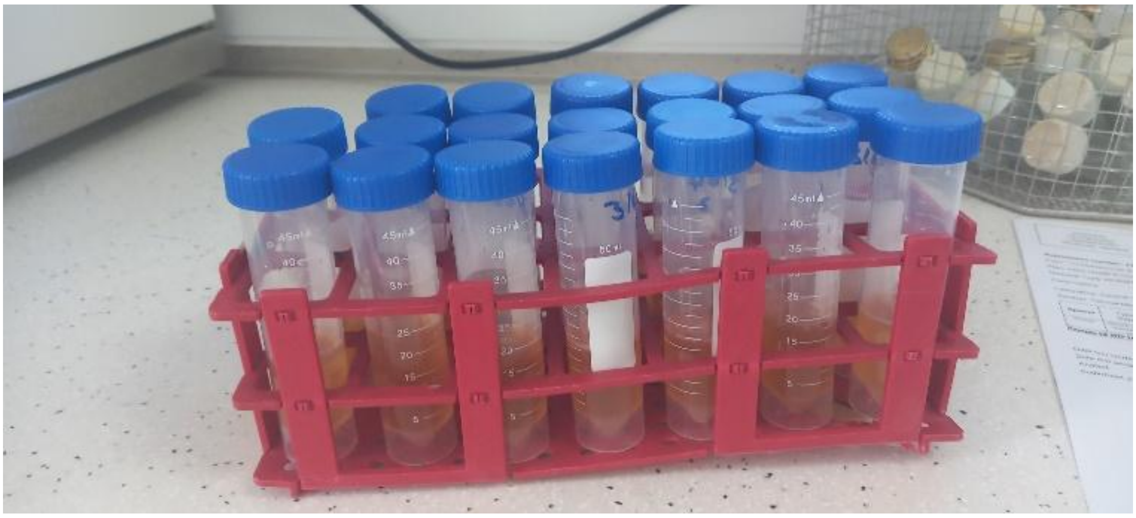


Figure 3: A sample rag with the SS5A and TSM Culture Media for *T. foetus*

3.2.3.2 Molecular Section

3.2.3.2.1 Tritrichomonas foetus

The molecular section is responsible for confirmation test of *Tritrichomonas foetus* by real time PCR using CVL protocol number BIO SOP 31 that uses Kapa Sybr® Fast qPCR Master Mix on CFX 96 Touch™ RT-PCR detection system with corresponding CFX manager 3.1 software. In summary, samples that were positive suspect on culture would then be confirmed with real-time RT-PCR.

DNA extraction was done following the Qiagen Dneasy Blood and Tissue Kit extraction protocol (http://www.bea.ki.se/documents/EN-DNeasy%20handbook.pdf). In summary, the suspected cultured sample was centrifuged at 13000 rpm for about 5 minutes to concentrate the pathogen and remove contaminants. The pellet was thereafter suspended in 200µl of Phosphate Buffer Solution (PBS), 20µl Protease K and 200µl of Buffer AL whilst placed in 96-well plate with proper identification of each sample. The microtubes were sealed with caps and placed on racks and thereafter vigorously shake for about 15 seconds followed by centrifugation at 3000 rpm. The mixture was then incubated at 56°C for 10 minutes with mixing done occasionally.

A volume of 900µl of lysed sample per well was transferred into the DNeasy 96 plates and thereafter the plates were sealed with AirPore Tape Sheet. This was followed by centrifuge of the plates at 6000 rpm for 4 minutes. The tape was then removed and a volume of 500µl of Buffer AW1 was added to each sample and thereafter resealed again for centrifugation at 6000 rpm for 2 minutes before adding 500µl of Buffer AW2. This was followed by another centrifugation at 6000 rpm for 2 minutes. The DNA was eluted by adding 200µl Buffer AE to each sample and resealed again followed by centrifugation at 6000 rpm for 4 minutes.

PCR amplification was conducted on the extracted DNA using *Trichomonas foetus* DNA Test Kit known as Gold Trich Detection kit (VetMax™) according to the manufacturer's instructions and all PCR products were visualized under UV after electrophoresis on 1.5% agarose gel. The cyclic conditions applied were consistent with those previously used (Gookin et al, 2002) Details of the PCR primer pairs, the anticipated amplicon size in base pairs (bp), and the annealing temperatures are provided in Supplementary Table 2. As a control, a negative sample of ultra-pure water was used whilst the positive control obtained from commercial kit was used (Macrogen Ltd: Seoul, Korea http://dna.macrogen.com/).

Table 2: Shows details of PCR primers, amplicon sizes in the base pairs for *T. foetus* PCR diagnosis

Target Primer	Primer Sequence	Annealing Temperatures (°C)	Expected amplicon length (bp)	Ref
TFR3	5'CGGGTCTTCCTATATGAGACAGAACC3'	61.2	347 bp	Gookin et al, (2002)
TFR4	5'CCTGCCGCCGTTGGATCAGTTTCGTTAA3A'	59.7		

3.2.3.2.2 Campylobacter. foetus

No culture was done for *C. foetus* except the screening test using conventional PCR as indicated in protocol number BIO SOP 28 also available on request at CVL. DNA extraction was done following the Qiagen Dneasy Blood and Tissue Kit extraction protocol as described above for *T. foetus*.

Primers MG3F and MG4R were used to amplify a 764 bp amplicon fragment of the carbon starvation gene from the extracted DNA. 4µL of sample was added to make a total reaction volume of 25µL containing 1 Unit of amplitaq DNA polymerase whereas the final concentration for primers, DNTPs and magnesium were 0.4µ, 200µM and 2mM respectively. The PCR amplification profile comprised of an initial denaturation at 95°C for 30 seconds, annealing at 54°C for 30 seconds, elongation at 72°C for 60 seconds and a final elongation step at 72°C for 7 minutes (Schmidt et al. (2010).

Agarose gel electrophoresis was used to determine the amplicons size. In this method, DNA is forced to migrate through a highly cross-linked agarose matrix containing ethidium bromide in response to an electric current for a set time. In solution, the phosphates on the DNA are negatively charged, and the DNA molecule will therefore migrate to the positive (red) pole. The agarose gel was the viewed under ultraviolet radiation (UV) for the presence of DNA bands at the expected positions. The validity of the run was based on the presence of a 764 bp band in the positive control and its negative control. The animal was considered infected with *C. foetus* species if its sample generated an amplicon band size of 764 bp on the agarose gel.

3.3 Statistical Analysis

3.3.1 Calculation of the Prevalence of *T. foetus* and *C. fetus*

Results obtained from the samples submitted was analysed using the formula on determining prevalence (Thrusfield, 2018):

$$P = \frac{\text{Number of animals with the disease at a specified time}}{\text{Number of animals in the population at risk at the specified time}}$$

In order to account for sensitivity and specificity of the test, the true prevalence was calculated as follows (Habibzadeh et al. 2022):

$$\text{True prevalence} = \frac{(\text{Apparent prevalence} + \text{specificity} - 1)}{(\text{Sensitivity} + \text{specificity} - 1)}$$

For this research the sensitivity and specificity test adopted for *T. foetus* were from Zangure (2019) which is 88.7% Se and 100% Sp, whilst that of *C. fetus* at species level was from Abril et al. (2014) with Se of 100% and Sp of 100%.

3.3.2 Risk Factors Association with *T. foetus* and *C. fetus*

Data analysis was done using Microsoft Excel 2019 spreadsheet using the Pearson's Chi square test with adjusted residual post hoc analysis in order to determine the associations between the positive test results and risk factors. The significant associations of individual risk factors were calculated using Bonferroni test and the multiple regression model was used to determine if the identified risk factors were confounded by another factor or was independent.

3.4 Research Ethics

3.3.1 Permission Letters

Ethical clearance certificate and research permission letter were obtained from the University of Namibia (UNAM) Research Committee (see annexures D and E). Furthermore, permission letter from the Directorate of Veterinary Services under the Ministry of Agriculture, Water and Land Reform (MAWLR) was obtained to allow the researcher to make use of the Central Veterinary Laboratory (CVL) and use the results for his thesis (see annexure H).

3.3.2 Confidentiality and Anonymity

A questionnaire guided interview required owners of the bulls to write their names as well as the stock brand and individual ear tag of the bulls for sample recording at the laboratory. Each farmer was given back the test results from the laboratory on the form, hence the need to have their personal information. Their anonymity as well as their freedom of participation in this important trial were reiterated. Documents obtained from farmers used in the research have been edited in a manner that personal details were absent.

4. CHAPTER 4

RESULTS

4.1 PREVALENCE

A snapshot format of test results of the sheath scrap samples submitted to the laboratory is indicated in Figure 4 (see annexure F for the full details)

Sample: Sheath scraping in Steves transport medium - Species: Bovine

Samples	Sample Id	Test	Test Result	Method
1		Campylobacter fetus spp: Identification	Positive	PCR
1		Tri-trichomonas fetus spp: Detection	TF detected.	Isolation

Sample: Trichomonas culture - Species: Bovine

Samples	Sample Id	Test	Test Result	Method
1		Tritrichomonas foetus spp. identification (Real-time)	Positive	Real-Time PCR

Test	Method	Test procedure	Testing Laboratory
Tri-trichomonas fetus spp: Detection	Isolation	CLM SOP 04	Central Veterinary Laboratory
Tritrichomonas foetus spp. identification (Real-time)	Real-Time PCR	BIO SOP 31	Central Veterinary Laboratory
Campylobacter fetus spp: Identification	PCR	BIO SOP 28	Central Veterinary Laboratory

Figure 4: A snap shot of a bull positive test result with both *T. foetus* and *C. fetus* in communal area of Namibia

A number of bulls tested positive for both pathogens as shown in Table 3 and also seen in Figure 4.

Table 3: Indicates the test results of bulls that tested positive for both pathogens as well as either pathogen

	Ovitoto	Okakarara	Dordabis-Nina
Total number of bulls sampled	89	44	39
Bull positive with both pathogens	3	7	0
Positive for *T. foetus* only	3	7	5
Positive for *C. fetus* only	8	2	0
Total positive for *T. foetus*	**6**	**14**	**5**
Total positive for *C. fetus*	**11**	**9**	**0**

The bulls were sampled twice at an interval of five to six days. Initial test results from Ovitoto showed that 4 bulls tested positive for *T. foetus* and on the second round of sampling, an additional 2 bulls tested positive giving us a total positivity of 6 bulls. The differences could be due to the sensitivity and specificity of the test. Some of the bulls were positive for both pathogens as seen in annexure F. Sample test results for both pathogens in the other research area remained the same during the second sampling.

The true prevalence of *T. foetus* in the sub-areas was 7.5% for Ovitoto, 36% for Okakarara and 14.68% for Dordabis- Nina area as seen in Table 4. The overall prevalence of the three study areas was 19.4% for *T. foetus*. Comparison of prevalence between research areas was done using Z-score population proportion test with the following results; The proportion between Okakarara area and Ovitoto area was -4.1029 with a $p < 0.00001$ on z score test and hence the result indicates that there is a significant difference between the two areas at $p < 0.05$. Therefore, Okakarara had a higher prevalence than Ovitoto. Whilst between Okakarara area and Dordabis – Nina area indicates z score of 2.2199 with a $p < 0.02642$ with a significant difference between the two areas at $p < 0.05$. Therefore, Okakarara had a higher prevalence than Dordabis-Nina. However, no significant difference was seen between Ovitoto and Dordabis - Nina area was z score is 1.2672 with a $p > 0.20408$, The 95% CI in Table 4 indicate that there is a significant difference across the three research areas at 5% level of significance as the error bars do not overlap ($8.44<13<34.5$).

For *C. fetus* the true prevalence was the same as for apparent prevalence as the test used at CVL only test up to species level and not at subspecies level. The test had a sensitivity and specificity of 100%. The overall prevalence of the three areas was 10.7% as seen in Table 5. The proportion between Okakarara and Ovitoto areas had a z- score of 1.2277 with a p value of 0.2187 which was greater than 0.05 and therefore there was no significant difference. The prevalence between the two research areas was the same for *C. fetus* whilst no positive was found in Dordabis- Nina area. The 95% CI in Table 5 indicate that there is a significant difference across the three research areas at 5% level of significance as the error bars do not overlap ($0<11<18.5$).

For this research the formulae used was adjusted as follows:

$$P= \frac{\text{Number of bulls that tested positive}}{\text{Total number of bulls sampled}} \times 100$$

Table 4: Calculation of prevalence and odds ratio of *T. foetus* in sub-area

Prevalence formulae	Research Area	Number of bulls sampled	Bulls tested positive	Apparent Prevalence (AP) in %	True Prevalence (TP) TP- = (AP + Sp – 1) / (Se + Sp – 1) Sp= 100% and Se = 88.7%	95% Confidence Interval of prevalence Confidence interval = p +/- z*{sq. root[(p(1-p)/n]}	Odds ratio	p-value
$P = \frac{\text{Bulls tested positive}}{\text{Bulls sampled}} X 100$	Ovitoto	89	6	6.7	7.5	6.36 to 8.44	3.4	0.035
	Okakarara	44	14	32	36	34.5 to 37.5	0.498	0.275
	Dordabis/Nina	39	5	13	14.6	13 to 16.2	--------	

Where: p is prevalence, z is 1.96 (for 95%), n is sample size

Table 5: Calculation of prevalence and odds ratio of *C. foetus* in sub-area

Prevalence formulae	Research Area	Number of bulls sampled	Bulls tested positive	Apparent Prevalence (AP) in %	True Prevalence (TP) TP- = (AP + Sp – 1) / (Se + Sp – 1) Sp= 100% and Se = 100%	95% Confidence Interval of prevalence Confidence interval = p +/- z*{sq. root[(p(1-p)/n]}	Odds ratio	p-value
$P \frac{\text{Bulls tested positive}}{\text{Bulls sampled}}$ X 100	Ovitoto	89	11	12	12	11 to 13	---------	
	Okakarara	44	9	20	20	18.5 to 21.5	1.934	0.183
	Dordabis/Nina	39	0	0	0	0	0.0000964	0.831

4.2 COMPARISON OF THE PREVALENCE OF THE TWO DISEASES

In order to address the second objective which, stipulate "To compare the prevalence of these two pathogens between different research areas", the overall prevalence between the two pathogens were calculated using the Z- score population proportion test and the results indicate a statistical significant difference of Z score value of 2.2564 with $p < 0.02382$. *T. foetus* had a highest prevalence of 19.4% at 95% CI = 19 to 20.4 whilst *C. fetus* prevalence was 10.7% at 95% CI = 9.95 to 11.4.

4.3 ASSOCIATED RISK FACTORS

In order to determine the risk factors associated with Campylobacter and Trichomonas species in Namibia as the third objective, the following information was obtained using a questionnaire guided interview as indicated in Table 6.

Bulls between the age group of 5 – 7 years old was found to have the highest population between the research areas whilst within the research area, in Okakarara the age group between 5 – 7 years more than the other two groups similar in Ovitoto. However, in Dordabis-Nina area, the highest age group was between 2 – 4 years old as seen in Table 6. Very few farmers keep bull longer than 7 years and above both between the research areas and within the different research areas. This means that there is constantly introduction of new bulls especially in Okakarara and Ovitoto a seen in origin bulls in the table below. The highest positivity of *T. foetus* and *C. fetus* was found in the age group between 5 to 7 and this is possibly due to the highest population age group. Bull previously not tested were more than the bulls tested and hence farmers keep bulls with unknown disease status.

Table 6: Shows the Different risk factors and odds ratio in different research areas

Variable	Category	Ovitoto		Okakarara		Dordabis-Nina		p - value
		n	%	n	%	N	%	
Age of bull (years)	2 - 4	21	23.5	14	31.8	18	46.2	0.3507
	5 - 7	45	50.6	19	43.2	14	35.9	<0.0001
	8 above	23	25.8	11	25	7	17.9	0.0011
	Total	89	100	44	100	39	100	
	p - value	0.002		0.326		0.089		
Duration of keeping	1 – 3	40	44.9	26	59	31	79.4	0.0232
	4 – 6	29	32.5	14	32	5	12.8	<0.0001

bull (years)	7 above	20	22.4	4	9	3	7.7	<0.0001
	Total	89	100	44	100	39	100	
	p-value	0.033		0.0002		<0.0001		
Age distribution (years) with highest positivity of *T. foetus*	2 - 4	0	0	2	14.3	0	0	
	5 - 7	5	83.3	7	50	2	40	.42952
	8 above	1	16.7	5	35.7	3	60	.31732
	Total	6		14		5		
	p-value	.02088		.44726		.5287		
Age distribution (years) with highest positivity of *C. fetus*	2 - 4	1	9.1	1	11	0	0	1
	5 - 7	5	45.5	5	55.6	0	0	1
	8 above	5	45.5	3	33.4	0	0	.31732
	Total	11		9		0		
	p-value	.02088		.34212				
Origin of bull	Bought	59	66.3	34	77.3	18	46.2	0.0003
	Bred	30	33.7	10	22.7	21	53.8	<0.0001
	Total	89	100	44	100	39	100	
	p-value	0.001		<0.0001		0.634		
Bulls previously tested	Bulls tested	6	6.7	4	9.1	19	48.7	< .00001
	Bulls not Tested	83	93.3	40	90.9	20	51.3	< .00001
	Total	89	100	44	100	39	100	
	p-value	< .00001		< .00001		< .00001		

Most farmers sourced bulls locally within the research area or locally breed their bulls as seen in Table 11. In Ovitoto, apart from bulls being sourced locally, it was found that 22.4% of bull were bought in Hochfeldt and 14% from Midgart area. Both these areas are closer to Ovitoto. Okakarara communal area recorded the highest number of bulls 80% to have been bought. Okakarara farmers also do sourced their bulls from Hochfeldt followed by Otjiwarongo with

13.6% as seen in Table 7. Farmers in Dordabis – Nina area locally breed their own bulls with a total of 21 (54%) bulls out of 39 bulls.

Table 7: Places where the bulls originated from the three study sites

OVITOTO				OKAKARARA			DORDABIS-NINA		
Places where the bulls were bought	n	%	Distance (Km) from research area	n	%	Distance (Km) from research area	n	%	Distance (Km) from research area
Locally bred (with research area)	31	34.8	30km radius	15	34.1	50km radius	27	69	30km radius
Hochfeldt	20	22.4	100	10	22.7	140	0	0	170
Midgart	13	14	68	0	0	290	1	2.6	200
Okahandja	5	5.6	40	1	2.3	300	0	0	180
Seeis	5	5.6	140	0	0	350	0	0	90
Outjo	3	3.4	350	1	2.3	170	0	0	420
Gobabis	2	2.2	230	1	2.3	330	0	0	200
Mariental	2	2.2	280	0	0	562	0	0	370
Otavi	2	2.2	370	0	0	224	0	0	470
Windhoek	2	2.2	100	1	2.3	300	3	7.7	100
Okakarara	1	1.1	294	0	0	0	0	0	400
Okamatapati	1	1.1	350	0	0	100	0	0	450
Okondjatu	1	1.1	230	2	4.5	100	0	0	380
Otjiwarongo	1	1.1	250	6	13.6	120	6	15.4	400
Grootfontein	0	0	460	4	9.1	200	0	0	400

Dordabis	0	0	200	1	2.3	380	0	0	0
Omaruru	0	0	208	1	2.3	240	0	0	310
Rehoboth	0	0	170	1	2.3	370	0	0	170
Aranos	0	0	350	0	0	400	2	5.1	200
Total	**89**	**100**		**44**	**100**		**39**	**100**	

There is a wide distribution of areas were farmers in Ovitoto and Okakarara sourced their bulls as compared to Dordabis-Nina. About 69% of farmers in Dordabis-Nina do produced their own bulls followed by Otjiwarongo with 15.4%.

4.3.1 Correlation between the prevalence and the risk factors

A chi-square statistical analysis tool was used to determine the significance of the associated risk with the prevalence of the two diseases. The identified associated risk factors were as follows:

- Research area, fenced and not fenced area
- Bulls bought or locally bred
- Bulls previously tested or not
- Age group of the bulls
- Duration of keeping the bulls
- Bull to cow ratio
- Bulls with both pathogens

Table 8: Summary of risk factors associated with the prevalence of *T. foetus*

Risk Factor Identified	**Chi-Square Results**
Research Area	Chi-square test for 3x2 contingency table [X^2 (2, N= 172) = 15.0242, ***p*– value is 0.000546** which is significant at $p < 0.05$]
Bulls with both pathogens	Chi-square test for 2x2 contingency table [X^2 (1, N = 133) = 6.65682, ***p* – value is 0.03585** which is significant at $p < 0.05$]
Bull to cow ratio	Chi-square test for 3x2 contingency table [X^2 (2, N = 6977) = 3.21469, ***p* – value is 0.043942** which is significant at $p < 0.05$]
Age group distribution-Dordabis/Nina	Chi-square test for 3x2 contingency table [X^2 (2, N = 39) = 6.88645, ***p* – value is 0.031961** which is significant at $p < 0.05$]
Dordabis/Nina – Bulls previously tested	Chi-square test for 2x2 contingency table [X^2 (1, N = 39) = 5.448529, ***p* – value is 0.019585** which is significant at $p < 0.05$]
Dordabis/Nina – Origin of bulls	Chi-square test for 2x2 contingency table [X^2 (1, N = 39) = 6.691176, ***p* – value is 0.035239** which is significant at $p < 0.05$]

Table 9: Summary of risk factors associated with the prevalence of *C. fetus*

Risk Factor Identified	**Chi-Square Results**
Research Area	Chi-square test for 3x2 contingency table [X^2 (2, N = 172) = 12.987, ***p* – value is 0.001513** which is significant at $p < 0.05$]
Bulls with both pathogens	Chi-square test for 2x2 contingency table [X^2 (1, N = 133) = 6.65682, *p* – value is 0.03585 which is significant at $p < 0.05$]
Bull to cow ratio	Chi-square test for 3x2 contingency table [X^2 (2, N = 6977) = 3.21469, *p* – value is 0.043942 which is significant at $p < 0.05$]
Ovitoto – Duration of Keeping the bulls	Chi-square test for 3x2 contingency table [X^2 (2, N = 89) = 6.1031, ***p* – value is 0.047285** which is significant at $p < 0.05$]

4.2.4 Multiple Regression Model of Associated Risk Factors

The data was analysed in the R-studio software where several packages were utilised for data manipulation (e.g dplyr, tidyyerse, car), data exploration and inference. The Z- score test for proportions was used to test for the equality of two proportions while the Chi-square goodness of fit test was used to test for the equality of three (or more) proportions. The chi-square test for independence was used to test for significant association between each individual categorical risk factor and the outcome. The multiple variable binary logistic regression model fitted using the glm (binomial family) function was used to assess the combined effects of all the risk factors on the outcome. The odds ratios were computed from the single factor binary logistics model and the multifactor binary logistic regression model to compare the odds that an outcome will occur given a particular exposure category, compared to the odds of the outcome of the reference category.

For *T. foetus*, odds ratio in the research area showed that the risk of animals being infected is higher in Okakarara area than in Ovitoto and Dordabis-Nina areas. No significant difference was seen between Ovitoto and Dordabis-Nina areas as seen in Table 10. The age group of bull between the age of 5 – 7 have a higher chance of being infected than those between 2 – 4 years followed by bulls older than 8 years. Further-more, the odds ratio calculation showed that the proportion of bulls kept longer than 7 years have a higher chance of being infected than those kept between 1 to 3 years. Although there was no significant difference between the bull bought and those locally bred, the odds ratio showed that bulls have a greater risk of introducing the disease in the herd than those locally bred. Afrikaner breeder was shown to have a higher chance of being infected with *T. foetus* followed by Simmental and Simbra breeds. There was no significant difference between bulls previously tested with those not tested, however, the odds ratio indicates that bulls previously tested are more likely to be infected with *T. foetus* than those not previously tested as shown in Table 10

Table 10: Parameter estimates and odds ratio from the multinomial logistic regression model for *T. foetus*

Variable	Category	Coefficient	Standard error	Z-score test	p-value	Odds ratio
	(Intercept)	-2.931	0.851	-3.446	0	0.05
Research areas	Dordabis- Nina	-0.356	1.431	-0.249	0.804	0.70
	Okakarara	2.31	0.664	3.478	0.000	
	Ovitoto	*				
Age of bull (years)	2-4	-0.41	1.151	-0.356	0.722	0.66
	5-7	0.839	0.765	1.097	0.272	2.31
	8 above					
Duration of keeping bulls (years)	1-3	-1.639	1.115	-1.47	0.142	0.19
	4-6	-0.893	0.933	-0.957	0.338	0.41
	7 above	*				
Origin of bull	Bought	0.432	0.754	0.573	0.566	1.54
	Bred	*				
Breed	Simmental	1.853	0.936	1.979	0.048	6.38
	Afrikaner	5.15	1.704	3.022	0.002	172.41
	Beefmaster	-9.317	257.128	-0.036	0.972	0.00
	Bonsmara	0.475	0.942	0.504	0.614	1.61
	Other	-0.276	1.256	-0.22	0.826	0.76
	Simbra	0.916	0.994	0.922	0.356	2.50
	Brahman	*				
Previous testing of bulls	Tested	0.347	0.94	0.369	0.712	1.41
	Not tested	*				

The variable category with an * is the reference category

For *C. fetus*, there was no significant difference between the research areas although the odds ratio indicate that Okakarara has a higher case than the two areas followed by Ovitoto and less significant in Dordabis-Nina area. The duration of keeping bulls indicated that keeping bull more than 4 years have a higher chance being infected than those kept between 1 to 3 years as seen in Table 11 came out the same as in *T. foetus* Bull with unknown disease status (not previously tested) have a higher risk of *C. fetus* than those frequently tested.

Table 11: Parameter estimates and odds ratios from the multinomial logistic regression model for *C. fetus*

Variable	Category	Coefficient	Standard Error	p-value	Odds ratio
	(Intercept)	-.209	1.339	.876	
Research areas	Dordabis - Nina	-19.221	7419.580	.998	4.493E-9
	Okakarara	.563	.635	.375	1.756
	Ovitoto	*	.	.	.
Age of bull (years)	2-4	-1.564	1.166	.180	.209
	5-7	-.740	.755	.326	.477
	8 above	*	.	.	.
Duration of Keeping bulls (years)	1-3	-.544	1.126	.629	.580
	4-6	.243	.912	.789	1.276
	7 above	*	.	.	.
Origin	Bought	.312	.765	.683	1.366
	Bred	*	.	.	.
Breed	Simmental	-17.262	.000	.	3.186E-8
	Afrikaner	.481	12413.247	1.000	1.617
	Beefmaster	.107	1.322	.935	1.113
	Bonsmara	-.523	.990	.597	.592
	Other	-18.961	.000	.	5.823E-9
	Simbra	1.678	1.217	.168	5.353
	Brahman	*	.	.	.
Previous testing of bulls	Tested	-1.081	.872	.216	.339
	Not tested	*	.	.	.

The variable category with an * is the reference category

4.2.4 Clinical signs seen in cows per selected research area

A guided questionnaire was used in order to obtain clinical signs seen in cattle that are directly associated with the two diseases. Information obtain is as shown in Table 12.

Table 12: The percentage of clinical signs commonly seen that are linked to *T. foetus* and *C. fetus*

CLINICAL SIGNS	**OVITOTO**		**OKAKARARA**		**DORDABIS-NINA**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Retained Placenta	55	19.5%	25	8.9%	9	3.2%	**89**	**32%**
Abortions	27	9.6%	15	5.3%	6	2%	**48**	**17%**
Vaginal Discharge	1	0.3%	0	0	0	0	**1**	**0.4%**
Repeat Breeders	120	42.7%	3	1%	20	7%	**143**	**51%**
Total	203		43		35		**281**	

A cow infected with either pathogen will show one or more of the clinical signs shown above. Repeat breeding was seen to be the highest amongst the cases reported (which conforms to the presence of the two pathogens), followed by retained placenta.

CHAPTER 5

DISCUSSION

The purpose of this research was to investigate the prevalence of bovine campylobacteriosis and trichomonosis and associated risk factors in selected areas of Namibia. This discussion will attempt to answer the three specific objectives of the research namely; to estimate the prevalence of *C. fetus* and *T. foetus* in bulls in selected communities; to compare the prevalence of these two pathogens between different identified areas; to determine the risk factors associated with these pathogens in Namibia.

The study found *T. foetus* prevalence to be higher than *C. fetus* of 19.4% and 10.7% respectively. In the sub area, where the prevalence was calculated using the total number of bulls sampled per area, the highest prevalence for *T. foetus* was found in Okakarara whilst statistically there was not significant difference of *C. fetus* between Okakarara and Ovitoto. The research areas in Okakarara are surrounded by communal areas with unknown disease status and animals in communal areas share common grazing and water points, which could increase the chances of disease spreading out of the communal area. Whereas, Ovitoto research areas are surrounded by fenced commercial farms with strict movement of animals from one farm to another, thereby reducing chances of disease spreading. Similar findings in Argentina and Spain showed that using private allotments had a higher chance of disease spread than private fenced properties (Mardones et al, 2008; Jine et al, 2014). Therefore, there could be a constant greater risk of disease transmission in Okakarara than in Ovitoto.

On the other hand, the Dordabis-Nina commercial farms in the current study had an observed prevalence of 14.68% % for *T. foetus* but negative for *C. fetus* and no statistical difference between Dordabis – Nina farms and Ovitoto which had 7.5%. The prevalence of *T. foetus* in Dordabis-Nina area could be at par with unpublished data by Bruwer in 2014 that showed an 8.5% prevalence of *T. foetus* in 94 bulls in Dordabis farms, whilst *C. fetus* was higher at 18.08%. The high positivity in his study for *C. fetus* could be attributed to the widely distributed farms sampled in Dordabis – Nina area, in addition to targeting only those farmers that were not involved in stud breeding. Stud farmers usually test bulls regularly and know the status of their herds as is was the case in this study. Elsewhere during the same year, in Outjo commercial areas, Bruwer found a prevalence of 17.9% for *T. foetus* and 7.5% for *C. fetus* in

106 bulls, indicating that both diseases could be widely occurring and substantially spreading in Namibia.

For *C. fetus,* the true prevalence was the same as for apparent prevalence as the test used at CVL only test up to species level and not at subspecies level. In this regard two species might be detected namely *C. fetus subspecies venerealis* and *C. fetus subspecies fetus*. Even though the two subspecies have the same pathogenicity, *C. fetus subspecies fetus* is mainly due to contamination whilst *C fetus subspecies venerealis* is transmitted through coitus. The test had a sensitivity and specificity of 100% at species level for PCR. There was no significant difference in proportion between Okakarara and Ovitoto.

Out of the 89 bulls sampled in Ovitoto, two bulls were initially negative but tested positive on the second test results. Using the Z-score test, there was no significant differences between the 2 sampling results and it suggest that the testing approach done was satisfactory. However, these two bulls could have been certified to be negative despite them being positive and could introduce the disease in a herd.

Using the chi-square and multiple regression model computation, the research further found a significant correlation between the prevalence of both pathogens in bulls and research area. Chances could be high for a bull to be infected with either pathogens when moved to areas with high prevalence such as Okakarara and Ovitoto. Similar findings were found in studies done in Argentina by Mardones et al (2008), who found that livestock sharing the same grazing area are 5 times riskier to be infected with these pathogens.

The research further found that there was an association between bulls infected with both pathogens and research area Okakarara had the highest number of bulls having both pathogens (7 bulls compared to 3 in Ovitoto) resulting in bulls spreading both pathogens and therefore increasing the prevalence of both pathogens. A bull infected with both pathogens could result in spreading either pathogen to the cattle and hence increasing the prevalence of both pathogens. Whilst a bull infected with only one of the pathogens could only spread that specific pathogen and hence the area can only have an increased prevalence of that specific pathogen.

Furthermore, there was an association between the bull to cow ratio and research area. In Okakarara the bull to cow ratio was 1:42 whilst in Ovitoto was 1:33. This could increase the risk of bulls infected by either pathogen in Okakarara than in Ovitoto as chances are high for farmers to share bulls in areas with many cows thereby predisposing them being infected.

The research indicated that older bulls are more likely to be infected than younger ones, which coincides with other studies that bulls older than 3 years has a higher chance to be infected than young ones (Youngquist and Threlfael, 1997; Collantes-Fernández, 2019). The age groups of this study were divided into three categories which found bulls between the age of 5 to 7 years to have high positive cases followed by bulls of 8 years and older. The reason why this age group had the highest positivity could be due to the number of bulls in the study which based on the z – score test found the age group between 5 to 7 having the highest population. Older bulls are expected to have higher positivity due to their well -developed preputial crypts that provide favourable environment for the two pathogens to flourish (Youngquist and Threlfael, 1997). These bulls are known to be persistent carriers which directly correlate with a higher herd prevalence. The age group between 2 to 4 years had the lowest positivity rate for *T. foetus* with only 1 bull testing positive.

Communal farmers keep bulls longer than the commercial farmers. The research confirmed that most bulls in commercial farms have been bred on the hosting farms whilst in communal areas, most of the bulls were bought in from somewhere else with no breeding soundness certificates. Most of the bulls bought in communal farms were not previously tested increasing the risk of introducing the two pathogens in the herd and this supported by the study carried in Zimbabwe by Woldehiwet et al, (1989) and in Nigeria by Mshelia et al (2010). Ovitoto farmers bought most of their bulls in neighbouring farms mostly Midgard and Hochfeldt areas whilst farmers in Okakarara bought their bulls in from different regions elsewhere thereby increasing the risk of spreading the disease all over the country. This Okakarara communal area had the highest number of bulls bought with no previous testing done and this can explain why the two pathogens were reported to be high in this area.

Afrikaner breeds had the highest odds ratio for *T. foetus* and this is because only 3 farmers kept this type of breed and 5 bulls (out of 7) which tested positive came from one farmer.

The clinical sign reported to be common was repeat breeders followed by abortions which are both suspicious signs of the two pathogens. Although the clinical signs reported maybe reported in cattle with either pathogen, they are not pathognomonic of the two pathogens but rather of many reproductive diseases including the two pathogens as reported by Samkange, et al, 2019.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The research was conducted in communal and commercial and semi-commercial areas in central part of Namibia testing the presence of *T. foetus* and *C. fetus* in bulls. The research areas were conveniently selected based on their production systems and therefore the results can not represent the entire country. The overall observed true prevalence of the herd was 19.4% for *T. foetus* and 10.7% for *C. fetus* out of 172 bulls. The prevalence for *T. foetus* in the different production systems was 7.5% for Ovitoto communal areas, 36% Okakarara semi-commercial areas and 14.68% Dordabis-Nina commercial farms. Furthermore, the prevalence for *C. fetus* was 12% for Ovitoto communal areas, 20% Okakarara semi-commercial areas and positive cases reported for Dordabis-Nina commercial farms. The prevalence of *T. foetus* was the highest in Okakarara semi-commercial areas, but no significant difference between Ovitoto communal area and Dordabis/Nina commercial farms. No significant difference was seen for *C. fetus* between Ovitoto and Okakarara.

Literature review found that the two pathogens are not listed as notifiable diseases under the relevant legislations and hence measures of controlling them might not be a priority at national level. However, the Namibian livestock import permit for breeding requires at least one test of the two pathogens.

On multiple regression computation, the research area showed that the risk of animals being infected is higher in Okakarara area than in Ovitoto and Dordabis-Nina areas. Chances are very high for a bull to be infected with either pathogen if introduced in Okakarara or Ovitoto where there is communal grazing. There is a risk of introducing the disease when buying bulls of unknown disease status and bulls infected with both pathogens tend to increase the chances of spreading either one of the pathogens. The age group of bull between the age of 5 – 7 have a higher chance of being infected than those between 2 – 4 years followed by bulls older than 8 years. A significant difference was seen in the proportion of keeping bulls which indicate bulls kept longer than 7 years have a higher chance of being infected than those kept between 1 to 3 years. The odds ratio further showed that bulls have a greater risk of introducing the disease in the herd than those locally bred. Whilst Afrikaner breed showed to have a higher chance of being infected with *T. foetus* followed by Simmental and Simbra breeds, this was attributed by few numbers of Afrikaner breed of which 5 out 7 were positive with the pathogen and all coming from one farmer.

Cows infected with either pathogens will have a combination of clinical signs, however, repeat breeder was the main clinical sign commonly reported followed by retained placenta. Overall, the research indicates that the trichomonosis and campylobacteriosis diseases are present among cattle herds in Namibia. Hence, mitigating the risk factors of transmitting and spreading these pathogens is pertinent for improving reproductive performance of beef cattle in Namibia.

6.2 RECOMMENDATIONS

The research should be extended to other parts of the country and with proper awareness campaign farmers can cover most of the costs. Awareness campaign should be done so that farmers are made aware on what to look out for when purchasing bulls and know the importance of frequently testing bulls for venereal diseases. Systematic survey should be done in order to more accurately estimate the true prevalence, at herd level as well as at animal level. By doing this, one could follow trends over time to investigate the impact of certain control interventions.

Farmers in communal area can apply for 20 hectares as stipulated in regulation number 37 of 2003 under section 45 of the Communal Land Reform Act, 5 of 2002, and this land can be fenced off and be used as a camp for keeping bulls. The fences along the farming units must be upgraded to reduce movement of livestock from one village to another thereby reducing the risk of introducing diseases.

Decentralise diagnostic laboratories in regions is also important in order to reduce the workload currently experienced at CVL. This can be done by capacitating other regional laboratories such as Pathcare, Namibia Institute of Pathology and UNAM School of Veterinary Medicine Laboratory. Improve molecular analysis to diagnosed the pathogens up to subspecies level. Further research should be done to understand the natural immune response of cows in the affected areas where the disease had persisted for years.

The Directorate of Veterinary Services (DVS) should consider adding these two diseases as notifiable diseases as recommended by WOAH. Regulations of the Animal Health Act 1 of 2011 must be reviewed to include testing of bulls prior to sale or movement from one farm to another. There must be enforcement of more artificial insemination centres and only semen from bulls that have been tested should be used thereby drastically reducing the prevalence of these two pathogens. Bulls sold at any auction for breeding purposed must have a breeding soundness certificate with negative test results for both pathogens.

Positive bulls should be culled and cows exposed to either pathogen isolated for 3 oestrus cycles to control positive herd. However, due to the production system of farming in communal and semi-commercial area this might be difficult to implement and hence vaccination of the herd to improve immunity can be instituted. Double dose of vaccination with 4 weeks interval reduce the risk of infection at herd level for *C. fetus* but not for *T. foetus.*

REFERENCES

Abril, C., Vilei, E. M., Brodard, I., Burnens, A., Frey, J., & Miserez, R. (2007). Discovery of insertion element ISCfe1: a new tool for Campylobacter fetus subspecies differentiation. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases*, *13*(10), 993–1000. https://doi.org/10.1111/j.1469-0691.2007.01787.x

Adeyeye AA, Ate I U, Bale J O and Lawal A I. (2012). Survey for Bovine Trichomoniasis in cattle at slaughter in the Sokoto metropolitan abattoir Sokoto State, Nigeria. Sokoto Journal of Veterinary Science, 8(1&2): 18-21

Alavi-Shoushtari SM, Abedizadeh R, Khaki A, Mokarizadeh A, Dorostkar K. (2014). A study on the effects of the estrous cycle on uterine fluid and blood serum immunoglobulin G (IgG) content in the cow. Vet Res Forum. Spring;5(2):115-9. PMID: 25568704; PMCID: PMC4279631.

Balzan C, Ziech R.E, Gressler L.T, Vargas A.P (2020). Bovine genital campylobacteriosis: main features and perspective for diagnosis and control. Ciencia Rural. 50(3):e20190272. Doi:10.1590/0103-8478cr20190272. Access on 15/09/2022

Bielanski, A., Ghazi, D. F., & Phipps-Toodd, B. (2004). Observations on the fertilization and development of preimplantation bovine embryos in vitro in the presence of Tritrichomonas foetus. Theriogenology, 61(5), 821–829. https://doi.org/10.1016/s0093-691x(03)00229-2

BonDurant, R. H. (1997). Pathogenesis, diagnosis and management of trichomoniasis in cattle. Veterinary Clinics of North America: Food Animal Practice, 13(2), 345 – 361.

Buller N. & Corney B (2013). Bovine Trichomonosis. Australian New Zealand Standard Diagnostic Procedure. http://www.agriculture.gov.au/SiteCollectionDocuments/animal/ahl/ANZSDP-Bovine-trichomoniasis.pdf

Cagnoli, C. I., Chiapparrone, M. L., Cacciato, C. S., Rodríguez, M. G., Aller, J. F., & Catena, M. D. C. (2020). Effects of Campylobacter fetus on bull sperm quality. Microbial pathogenesis, 149, 104486. https://doi.org/10.1016/j.micpath.2020.104486

Calvani NED, Slapeta J, Onizawa E, Eamens K, Jenkins C, & Westman ME (2021). Not gone nut forgotten: Tritrichomonas foetus in extensively managed bulls from Australia's Northern Territory. Current Research in Parasitology & Vector – borne Diseases, 1,100012.

Casteriano, A., Molini, U., Kandjumbwa, K., Khaiseb, S., Frey, C. F., & Šlapeta, J. (2016). Novel genotype of Tritrichomonas foetus from cattle in Southern Africa. Parasitology, 143(14), 1954–1959. https://doi.org/10.1017/S003118201600158X

Chaban B, Chu S, Hendrick S et al (2012). Evaluation of a *Cambylobacter fetus subspecies venerealis* real-time quantitative polymerase chain reaction for direct analysis of bovine preputial samples. Can J Vet Res; 76: 166 – 173.

Chi- square statistical calculator**.** https://www.socscistatistics.com/ retrieved on 07/09/2022

Circular No. 6 of 2017 of the Directorate of Veterinary Services, Ministry of Agriculture, Water and Land Reform, Namibia. Access through Ministry intranet.

Coetzee, J.A., Thomson G.R, Tustin R.C, (2004), Infectious Diseases of Livestock With Special References to South Africa. Vol. 1, Oxford University Press. Cape Town.

Cobo E.R, Corbeil L.B & BonDurant R.H, (2011). Immunity to infection in the lower tract of bulls. Journal of Reproductive Immunity. Volume 89, pg 55-61. https://doi.org/10.1016/j.jri.2011.02.002

Cobo, E.R, Favetto. V.M.L, Friend. VanHooster. K, Mitchel. J, et al…(2007). Sensitivity and specificity of culture and PCR of smegma samples of bulls experimentally infected with Tritrichomonas foetus. Theriogenology, 68, pp 853 – 860

Cobo ER, Morsellac C, Canoc D, Cipollac A, Campero CM. (2003). Immunization in heifers with dual vaccines conatining Tritrichomonas foetus and Campylobacter fetus antigens using systemic and mucosal routes. Theriogenology 62(2004) 1367-1382. DOI:10.1016/j.theriogenology.2003.12.034.

Collantes-Fernández, E., Moreno-Gonzalo, J., Sánchez-Sánchez, R., García-Bocanegra, I., Horcajo, P., & Ortega-Mora, L. M. (2019). Prevalence of bovine trichomonosis and associated risk factors in bulls from Spanish beef herds. Theriogenology, 128, 116–121. https://doi.org/10.1016/j.theriogenology.2019.01.030

Collantes-Fernandez, E., Fort, M.C., Ortega-Mora, L.M and Schares, G. (2017). Trichomonas. Parasitic Protozoa of Farm Animals and Pets, 313 – 388. DOI:10.1007/978-3-319-70132-514 access on 13/09/2022

Corbeil L. B. (1994). Vaccination strategies against Tritrichomonas foetus. Parasitology today (Personal ed.), 10(3), 103–106. https://doi.org/10.1016/0169-4758(94)90009-4

Da Silva M.S.F (2021). Bovine Genital Campylobacteriosis: New insights into Molecular and Pathogenesis. Faculty of Veterinary Medicine, University of Lisbao. https://www.repository.utl.pt/bitstream/10400.5/21243/1 access on 17/09/2022

Erickson NEN, Lanigan E, Waug T, Gesy K, Waldner C. (2017). Evaluation of Long-acting oxytetracycline and a commercial monovalent vaccine for control of Campylobacter fetus subsp. venerealis infection in beef bulls. Can Vet J. 58(10):1051-1058. PMID:28966354; PMCID: PMC5603938

FAO. (2021), World Food and Agriculture – Statistical Yearbook 2021. Rome. https://doi.org/10.4060/cb477en Online access on 28/08/2022

Foscolo CB, Pellegrin AO, Leite RC, Stynen APR, and Lage AP. (2018). Vaccination of bulls against Bovine Genital Campylobacteriosis: a therapeutic approach. Animal Reproduction (AR). 2(2). pp. 122 – 127

Fuunel, BJ (2022). Bovine trichomoniasis overview and testing. In American Association of Bovine Practitioners Conference Proceedings (No. 55. pp, 22-25). https://doi.org/10.21423/aabppro20228592

Gharban, HAJ. (2023). Molecular prevalence and phylogenetic confirmation of bovine trichomoniasis in aborted cows in Iraq. Vet World. 16(3):580-597. Doi:10.14202/vetworld.2023.580-587. PMID:37041823; PMCID: PMC10082752

Gregory, M. W., Ellis, B., & Redwood, D. W. (1990). Comparison of sampling methods for the detection of Tritrichomonas foetus infection in bulls. The Veterinary record, 127(1), 16.

Government Gazette of the Republic of Namibia No 4694 (2011). Animal Health Act 1 of 2011. https://www.lac.org.na/laws/2011/4694.pdf access on 14/09/2022

Government Gazette of the Republic of Namibia No 2926 (2002). Communal Land Reform Act, 5 of 2002. https://www.lac.org.na/laws/2002/2926.pdf access on 14/09/2022

Guerra GA, Chaban B, Hill JE, Waldner CL, Hendrick SH (2014). Clinical sensitivity and specificity of real-time PCR assay for Campylobacter fetus subsp venerealis in preputial samples from bulls. Theriogenology. Volume 75. Issue 9. https://doi.org/10.2460/ajvr.75.9.851

Haas. D.J, Miranda-Guimaraes K.L, Dorneles E.M.S, and Lage. A.P (2020). Prevalence of Bovine Genital Campylobacteriosis in Beef Cattle in Brazil. EC Veterinary Science.5. pp 45-53

Habibzadeh F, Habibzadeh P, Yadollahie M, (2022). The apparent prevalence, the true prevalence. Biostatistics. Volume 32. Issue 2. http://doi.org/10.11613/BM.2022.020101

Hairgrove, Y., and Gill, R. (2009). Bovine Trichomoniasis (Trich) in Cattle – Texas A&M AgriLife Extension. Texas A&M AgriLife Extension Service. https://agrilifeextension.tamu.edu/library/ranching/bovine- trichomonas/ access on the 15/07/2022

https://www.socscistatistics.com/tests/ztest/default2.aspx retrieved on the 13/03/2023

https://en.wikivet.net/Campylobacter_species_-_Overview retrieved on the 20/08/2023

Hum.S, Hornitzk. M, Berg. T, (2009), Bovine Genital Campylobacteriosis. Australia and New Zealand Standard Diagnostic Procedures. Australia. https://www.agriculture.gov.au/sites/default/files/sitecollectiondocuments/animal/ahl/ANZSDP-Bovine-genital-campylobacteriosis.pdf access on 24/06/2022

Hum S, Quinn K, Brunner J, et al. (1994) Evaluation of a PCR assay for identification and differentiation of Campylobacter fetus subspecies. Aus Vet J. 75: 827 - 831

Irons. P.C, Schutte. A.P, van der Walt. M.L, and Bishop. G.C, (2004). Genital Campylobacteriosis in Cattle In: Coetzer. J.A.W, Tustin. R.C, (Eds). Infectious diseases of Livestock with Special Reference to Southern Africa, Oxford University Press. Kitching JP (1999).

Janzen, E. (2020). Trichomoniasis in Cattle – Reproductive System. Merck Veterinary Manual. https://www.merckvetmanual.com/reproductivesystem/trichomoniasis/trichomoniasis-in-cattle access on the 11/08/2022

Jine Y, Schumaker B, Logan J & Yao C (2014). Risk factors associated with bovine trichomoniasis in beef cattle identified by a questionnaire. Journal of medical microbiology, 63(6), 896-902

Karli, G. Polava, R. Varada, K. (2020). Comparative Omics Based Approach to Identify Putative Immunogenic Proteins of Trichomonas Foetus. In: Jyothi, S., Mamatha D, Satapathy Raju, K., Favorskava, M (eds). Advances in Computational and Bio-Engineering. CBE 2019. Learning and Analytics in Intelligent Systems, vol 15. Springer, Cham. https://doi.org/10.1007/978-3-030-46939-9 51

Kimsey. P.B, Darien. B.J, Kenderick. J.W, & Franti. C.E (1980). Bovine trichomoniasis: diagnosis and treatment. J Am Vet Med Assoc 177, 616 - 619

Love, D., Fajt, V. R., Hairgrove, T., Jones, M., & Thompson, J. A. (2017). Metronidazole for the treatment of Tritrichomonas foetus in bulls. BMC veterinary research, 13(1), 107. https://doi.org/10.1186/s12917-017-0999-2

Madoroba, E. Gelaw, A. Hlokwe, T and Mnisi, M (2011). Prevalence of Campylobacter foetus and Trichomonas foetus among cattle from Southern Africa. African Journal of Biotechnology vol. 10 (50) pp. 10311-10314. DOI: 105897/AJB11.1259. ISSN 1684-5315 @2011 Academic Journal. Online http://www.academicjournals.org/AJB access on the 20/08/2022

Mai, H. M., Irons, P. C., Kabir, J., & Thompson, P. N. (2013). Prevalence of bovine genital campylobacteriosis and trichomonosis of bulls in northern Nigeria. Acta veterinaria Scandinavica, 55(1), 56. https://doi.org/10.1186/1751-0147-55-56

Mardones, F. O., Perez, A. M., Martínez, A., & Carpenter, T. E. (2008). Risk factors associated with Tritrichomonas foetus infection in beef herds in the Province of Buenos Aires, Argentina. Veterinary parasitology, 153(3-4), 231–237. https://doi.org/10.1016/j.vetpar.2008.01.038

Martin KA, Jesudos Chelladurai JR, Bader C, Carreiro E, Long K, Thompson K, Brewer MT (2020). Repurposing the open access malaria box reveals compounds with activity against Trichomonas foetus trphozoites. Int- Journal for Para: Drugs and Drug Resistance, Vol 13. 89 – 93. ISSN 221-3207. https://doi.org/10/1016/j.ijpddr.202.06.003

Martin, K. A., Henderson, J., & Brewer, M. T. (2021). Bovine Trichomonosis Cases in the United States 2015-2019. Frontiers in veterinary science, 8, 692199. https://doi.org/10.3389/fvets.2021.692199

MAWF. (2016). Ministry of Agriculture, Water and Forestry Annual Report 2015/2016. Windhoek, Namibia: Ministry of Agriculture, Water and Forestry.

McGoldrick A, Chanter J, Gale S, Parr J, Toszeghy M, Line K. (2013). Real Time PCR to detect and differentiate Campylobacter fetus subspecies fetus and Campylobacter fetus subspecies venerealis. J Microbiol Methods. 94(3): 199 – 204. Doi:10,1016/j.mimet.2013.06.014

Meat Board of Namibia. (2022). Annual Report 2020-2021. www.nammic.com.na Online Access on 24/08/2022

Mendelson, J. M. (2008). Customary and legislative aspects of land registration and management on communal land in Namibia. Windhoek, Namibia: Research & Information Services of Namibia (RAISON).

Mendozo-Ibarra, J.A, Pedraza-De\\iaz. S, Garc\\ia-Pena. F.J, Rojo-Mentojo S, Ruiz Santa-Quitera J.A, San Miguel-Ibanez E,…….Collantes Fernandez E (2012). High prevalence of Trichomonas foetus infection in Asturiana de la Montana beef cattle kept in extensive condition in Northern Spain. The Veterinary Journal, 193(1), 146-151

Michi, A. N., Favetto, P. H., Kastelic, J., & Cobo, E. R. (2016). A review of sexually transmitted bovine trichomoniasis and campylobacteriosis affecting cattle reproductive health. Theriogenology, 85(5), 781–791. https://doi.org/10.1016/j.theriogenology.2015.10.037

Molina, L. L., Angón, E., García, A., Caballero-Villalobos, J., Giorgis, A. O., Moralejo, R. H., & Perea, J. (2020). Response to letter of comments: Comments on a retrospective epidemiological analysis of shared risk factors for bovine trichomoniasis and bovine genital campylobacteriosis in La Pampa province (Argentina). Highlighting a neglected agent: Leptospira sp. Preventive veterinary medicine, 178, 104754. https://doi.org/10.1016/j.prevetmed.2019.104754

Mshelia, G. D., Amin, J. D., Egwu, G. O., Yavari, C. A., Murray, R. D., & Woldehiwet, Z. (2010). Detection of antibodies specific to Campylobacter fetus subsp. venerealis in the vaginal mucus of Nigerian breeding cows. Veterinaria italiana, 46(3), 337–344.

Mukhufhi. N, Irons. P.C, Michel. A & Peta. F, (2003). Evaluation of a PCR test for the diagnosis of Tritrichomonas foetus infection in bulls: effects of sample collection method, storage and transport medium on the test. Theriogenology 60, 1269 – 1278.

Najera J. (2022). Investigating the Immune Mechanism in the Death of Tritrichomonas foetus. Thesis Dissertation. California State Polytechnic University, Pomona. https://scholarworks.calstate.edu/concern/theses/028712706

Noakes D.K, Parkinson T.J, England G.C, 2001. Specific Infectious Diseases Causing Infertility in Cattle. 8th edition. Arthur's Veterinary Reproduction and Obstetrics. p. 473 – 509

Ondrak. J.D, (2016). Tritrichomonas foetus prevention and control in cattle. Vet Clin North Am Food Anim Pract. 32:411-423. Doi: 10.1016/j.cvfa.2016.01.010

Ortega-Mora, L.M. Sanchez-Sanchez. R, Rojo-Montejo. S, Roman-Trufero. A, Montenegro-Gregorio. D, Puentes-Colorado. E……& Collantes-Fernandez. E (2022). A new inactivated Tritrichomonas foetus vaccine that improves genital clearance of the infection and calving intervals in cattle. Frontiers in Veterinary Science. 9.1005556

Oyhenart, J. (2024). Positive cases of bovine genital campylobacteriosis and bovine trichomonosis in the eradication program of La Pampa, Argentina: 2008 – 2021. Tropical Animal Health and Production, 56 (2). 96

Parsonson, I. M., Clark, B. L., & Dufty, J. H. (1976). Early pathogenesis and pathology of Tritrichomonas foetus infection in virgin heifers. Journal of comparative pathology, 86(1), 59–66. https://doi.org/10.1016/0021-9975(76)90028-1

Pena-Fernández, N., Cano-Terriza, D., García-Bocanegra, I., Horcajo, P., Vázquez-Arbaizar, P., Cleofé-Resta, D., Pérez-Arroyo, B., Ortega-Mora, L. M., & Collantes-Fernández, E. (2021). Prevalence of Bovine Genital Campylobacteriosis, Associated Risk Factors and Spatial Distribution in Spanish Beef Cattle Based on Veterinary Laboratory Database

Records. Frontiers in veterinary science, 8, 750183. https://doi.org/10.3389/fvets.2021.750183

Perez. A, Cobo. E, Martinez. A, Campero. C & Spath. E (2006). Bayesian estimation Tritrichomonas foetus diagnostic test sensitivity and specificity in range beef bulls. Vet Parasitol. 142, 159 162

Pica-Ciamarra. U, Baker. D, Morgan. N, Zezza. A, Azzarri. C, Ly. C, P, Sserugga. J. (2014), Investing in the Livestock Sector: Why Good Numbers Matter, World Bank Report Number 85732-GLB. FAO

Polo C, Garcia-Seco T, Fernandez V, Hernandez M, Briones V, Diez-Guerrier A & Perez-Sancho M, (2022). Molecular detection of Tritrichomonas foetus in bovine samples: a novel real-time polymerase chain reaction (PCR) assay targeting EF1-alpha-Tf1 and a comparative study of published PCR techniques. Parasitology Research, 121(6), 1725-1733.

Rae, D. O., & Crews, J. E. (2006). Tritrichomonas foetus. The Veterinary clinics of North America. Food animal practice, 22(3), 595–611. https://doi.org/10.1016/j.cvfa.2006.07.001

Sahin, O., Yaeger, M., Wu, Z., & Zhang, Q. (2017). Campylobacter-Associated Diseases in Animals. Annual review of animal biosciences, 5, 21–42. https://doi.org/10.1146/annurev-animal-022516-022826

Samkange, A., van der Westhuizen, J., Voigts, A. S., Chitate, F., Kaatura, I., Khaiseb, S., Hikufe, E. H., Kabajani, J., Bishi, A. S., Mbiri, P., Hawanga, N. N., & Mushonga, B. (2022). Investigation of the outbreaks of abortions and orchitis in livestock in Namibia during 2016-2018. Tropical animal health and production, 54(6), 346. https://doi.org/10.1007/s11250-022-03342-0

Schmidt. T, (2008), Detection of Campylobacter fetus in Bovine Preputial Scrapings Using PCR and Culture Assays. University of Pretoria. South Africa

Schmidt, T., Venter, E. H., & Picard, J. A. (2010). Evaluation of PCR assays for the detection of Campylobacter fetus in bovine preputial scrapings and the identification of subspecies in South African field isolates. Journal of the South African Veterinary Association, 81(2), 87–92. https://doi.org/10.4102/jsava.v81i2.111

Schneider. H, (1994), Animal Health and Veterinary Medicine in Namibia, Journal of the South African Veterinary Association 83(1): E1-E9. Doi:104102/jsava.v83i1.4 access on the 17/06/2022

Seid A (2019). Bovine Campylobacteriosis and its Economic Importance: A Review. Journal of Dairy and Veterinary Sciences. https://doi.org,10.19080/JDVS.2019.11555807 access on 15/09/2022

Skirrow, S. Z., & Bondurant, R. H. (1988). Treatment of bovine trichomoniasis with ipronidazole. Australian veterinary journal, 65(5), 156. https://doi.org/10.1111/j.1751-0813.1988.tb14446.x

Szonvi. B, Srinath. I, Schwartz. A, Clavijo A, Ivanek R. (2012). Spatio-temporal epidemiology of Tritrichomonas foetus infection in Texas bulls based on state-wide diagnostics laboratory data. Vet Parasitol. 186, pp. 450 - 455

Taylor, M. A., Marshall, R. N., & Stack, M. (1994). Morphological differentiation of Tritrichomonas foetus from other protozoa of the bovine reproductive tract. The British veterinary journal, 150(1), 73–80. https://doi.org/10.1016/S0007-1935(05)80098-3

Thompson SA, Blaser MJ, (2000). In: Nacham I, Blaser MJ, eds. Campylobacter. 2nd ed. Washington DC: American Society for Microbiology; pp.321 - 347

Thrusfield. M, (2008), Veterinary Epidemiology, 3rd Edition. Blackwell Publishing. London, ISBN 97-1-405-15627-1

Tolbert K, (2021). Trichomonosis: Greene's Infectious Diseases of the Dog and Cat. 5th Edition. W.B Saunder. pp. 1278 – 1284. ISBN 9780323509343. https://doi.org/10.1016/B978-0-323-40934-3.00102-6

Truyers I, Luke T, Wilson D, Sargison N. (2014). Diagnosis and management of venereal campylobacteriosis in beef cattle. BMC Vet Res, 10(1): 1-7. Doi:101186/s12917-014-0280-x

Yao C. (2013). Diagnosis of Tritrichomonas foetus-infected bulls, an ultimate approach to eradicate bovine trichomoniasis in US cattle?. Journal of medical microbiology, 62(Pt 1), 1–9. https://doi.org/10.1099/jmm.0.047365-0

Yao. C, and Koster. L.S (2015). Tritrichomonas foetus infection, a case of chronic diarrhea in domestic cat. Veterinary Research. 46:35. https://doi.org/10.1186/s13567-015-0169-0

Youngquist, R., and Threlfael, W. (1997). Bovine Venereal Diseases. In Large Animal Theriogenology. 2nd edition. pp. 355 – 363.

Van Bergen M.A, Linnane S, van Putten JP, Wagenaar JA (2005). Global detection and identification of Campylobacter fetus subsp. venerealis. Rev Sci Tech. 24(3): 1017-26. PMID: 16642772

van der Graaf-van Bloois, L., van Bergen, M. A., van der Wal, F. J., de Boer, A. G., Duim, B., Schmidt, T., & Wagenaar, J. A. (2013). Evaluation of molecular assays for identification Campylobacter fetus species and subspecies and development of a C. fetus specific real-time PCR assay. Journal of microbiological methods, 95(1), 93–97. https://doi.org/10.1016/j.mimet.2013.06.005

WOAH, 2021. Bovine Genital Campylobacteriosis. Chapter 3.4.4. Terrestrial Manual

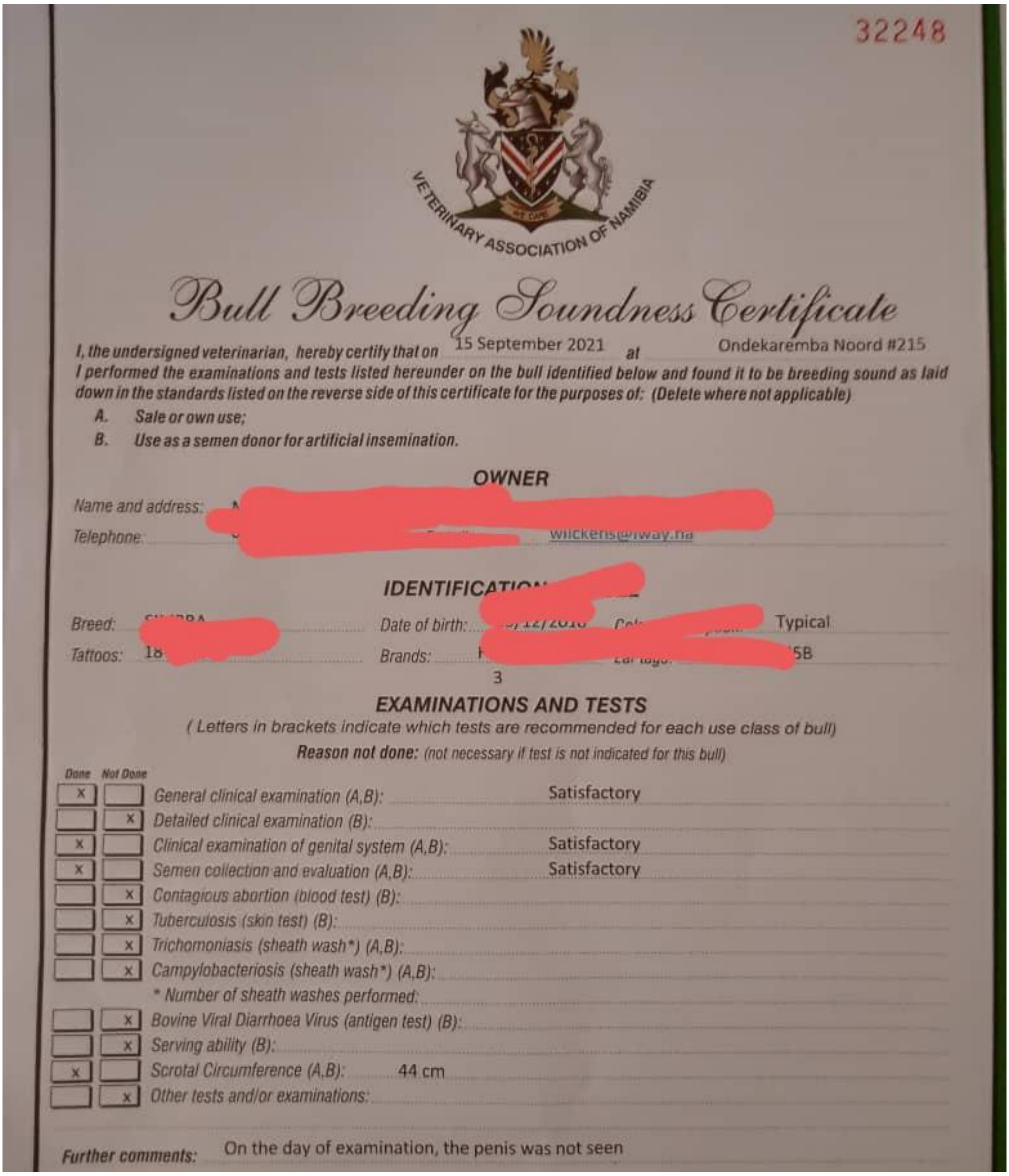
WOAH, 2018. Trichomonosis. Chapter 3.4.15. Terrestrial Manual

Woldehiwet. Z, Odiawo. G.O and Pawadiwa. A (1989). Isolation of Campylobacter fetus subsp. venerealis from Bulls in a Beef Herd in Harare, Zimbabwe Vet J. pp. 37-40

Zangure. T.A (2019). A voluntary trichomonosis inter-laboratory comparison study in South Africa. University of Pretoria. South Africa. URI:http:///hdl.handle.net/2263/76456

ANNEXURES

Annexure A: Breeding Soundness Certificate



32248

VETERINARY ASSOCIATION OF NAMIBIA

Bull Breeding Soundness Certificate

I, the undersigned veterinarian, hereby certify that on 15 September 2021 *at* Ondekaremba Noord #215
I performed the examinations and tests listed hereunder on the bull identified below and found it to be breeding sound as laid down in the standards listed on the reverse side of this certificate for the purposes of: (Delete where not applicable)

A. Sale or own use;
B. Use as a semen donor for artificial insemination.

OWNER

Name and address: [redacted]
Telephone: [redacted] wilckens@iway.na

IDENTIFICATION

Breed: [redacted] Date of birth: [redacted] Typical
Tattoos: 18[redacted] Brands: [redacted] 5B
3

EXAMINATIONS AND TESTS

(Letters in brackets indicate which tests are recommended for each use class of bull)

Reason not done: *(not necessary if test is not indicated for this bull)*

Done	Not Done	Examination	Result
x		General clinical examination (A,B):	Satisfactory
	x	Detailed clinical examination (B):	
x		Clinical examination of genital system (A,B):	Satisfactory
x		Semen collection and evaluation (A,B):	Satisfactory
	x	Contagious abortion (blood test) (B):	
	x	Tuberculosis (skin test) (B):	
	x	Trichomoniasis (sheath wash*) (A,B):	
	x	Campylobacteriosis (sheath wash*) (A,B):	
		* Number of sheath washes performed:	
	x	Bovine Viral Diarrhoea Virus (antigen test) (B):	
	x	Serving ability (B):	
x		Scrotal Circumference (A,B):	44 cm
	x	Other tests and/or examinations:	

Further comments: On the day of examination, the penis was not seen

Annexure B: Questionnaire

QUESTIONNAIRE GUIDED INTERVIEW

My name is Israel Kaatura, a student at the University of Namibia, pursuing a Masters Degree in Veterinary Medicine. I am currently conducting a research on the “PREVALENCE AND RISK FACTORS ASSOCIATED WITH BOVINE CAMPYLOBACTERIOSIS AND TRICHOMONOSIS IN DORDABIS AND OVITOTO AREAS, NAMIBIA”

This research is purely for academic and responses will be treated confidential and hence individual anonymity will be safeguarded.

SECTION A

PERSONAL INFORMATION

1. Name of the owner: …………………………………………………………………..
2. Name of the farm/village ………………………………………………………..……
3. Duration of farming on this farm / village ………………………………………………
4. District: ………………………………………………………………………………
5. Stock brand number (s): ………………………………………………………………..
6. Contact number: ……………………………………………………………………….
7. Postal address: ………………………………………………………………………

SECTION B

BULL INFORMATION

Ear tag number	**Age (years)**	**Breed**	**Duration of being with bull**	**Bought or bred at farm**	**Breeding Soundness previously done**	**If bought from which region and district**

8. Have someone borrowed or used your bull (s) in the last two years………………

9. Did you borrow or used other farmer's bulls (s) in the last two years: …………..

10. Have you removed a bull (s) out of your herd due to infertility problems…………….

11. If yes to question 10, what infertility problem was diagnosed on the bull (s)

………………………………………………………………………………………………
………………………………………………………………………………………………

12. Are the bulls kept separately in a camp or are just grazing together with the cows:

……………………………………………………………………………………………………..

13. What type of feed is given to your bull(s) per year ………………………………………………………………………………………………… ………………………………………………………………………………………………… ………………………………………………………………………………………………… ……………....

SECTION C

HERD INFORMATION

14. Total number of cows: …………………………………………
15. Total number of bulls…………………………………………..
16. Total number of heifers………………………………………..
17. Total number of oxen ……………………………………
18. Total number of calves ……………………………………….

Year	Estimate number of cows pregnant	Abortion	Retained Placenta	Vaginal discharge	Repeat breeders	Calving rate
2020						
2021						

Annexure C: Names of research areas were sample was collected

Ovitoto communal villages

No.	Name of Village
1	Bulskop
2	Okamboro
3	Ombupaouri
4	Okatjosorui
5	Oruua
6	Otjivaijona
7	Ouhave
8	Ovanduwongwe
9	Ombungururu
10	Okaokokunja
11	Okouwe
12	Otjiserandu
13	Ombeto
14	Okomakwara
15	Otjitarazu
16	Okandjira
17	Otjongombe
18	Ondamekondo
19	Okandjira
20	Okamuina
21	Ozoserekaze
22	Okatjongoro
23	Otjiuapeke

Okakarara communal villages

No.	Name of Village
1	Okatemba Otjitanda
2	Oruirupe

3	Outemba uaTjikango
4	Outemba uaMbijomenje
5	Outemba uaMatupa
6	Outemba Uatuvingo
7	Otuvingo
8	Okahua
9	Otjase
10	Otjorusuo
11	Otjounjingandu
12	Okaepe
13	Okahua

List of Dordabis/ Nina Farms

No.	Name of Farm	Areas
1	Heimat	Dordabis
2	Onverwarcht	Dordabis
3	Autabib	Dordabis
4	Josefine	Nina
5	Marie	Nina
6	Alice	Nina
7	Margaruite	Nina

Annexure D: Ethical Clearance

UNAM

UNIVERSITY OF NAMIBIA

ETHICAL CLEARANCE CERTIFICATE

Ethical Clearance Reference Number: NEC0002 **Date:** 28/03/2022

This Ethical Clearance Certificate is issued by the University of Namibia Ethics Committee (REC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the ethics committee.

Title of Project: PREVALENCE AND RISK FACTORS ASSOCIATED WITH BOVINE CAMPYLOBACTERIOSIS AND TRICHOMONOSIS IN DORDABIS AND OVITOTO AREAS, NAMIBIA

Principal researchers: Dr. Israel Kaatura

Staff Number/ Student number: 200430637

Remarks: the research proposal respects all the parameters to obtain the ethical clearance

Centre for Research Services

Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the ethics committee. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the ethics committee
3. The Principal Researcher must report issues of ethical compliance to the ethics committee (through the Chairperson) at the end of the Project or as may be requested by the ethics committee
4. The ethics committee retains the right to:

i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,

ii) Request for an ethical compliance report at any point during the course of the research.

The ethics committee wishes you the best in your research.

Dr. Umberto Molini (Chairperson Neudamm Decentralized Ethics Committee)

Prof. Davis Mumbengegwi (Head, Multidisciplinary Research)

Annexure E: Research Permission Letter

CENTRE FOR RESEARCH SERVICES
Office of the Pro-Vice Chancellor: Research, Innovation & Development
University of Namibia, Private Bag 13301, Windhoek, Namibia
340 Mandume Ndemufayo Avenue, Pioneers Park, Office F223 - Fblock, Second Floor
☎ +264 61 206 4673; E-mail:kmbulu@unam.na; URL.: http//www.unam.edu.na

UNAM
UNIVERSITY OF NAMIBIA

RESEARCH PERMISSION LETTER

Date: 18/05/2022

Student Name: ISRAEL KAATURA

Student Number: 200430637

Programme: MSC IN VETERINARY MEDICINE (BY THESIS)

Approved Research Title: PREVALENCE AND RISK FACTORS ASSOCIATED WITH BOVINE CAMPYLOBACTERIOSIS AND TRICHOMONOSIS IN DORDABIS AND OVITOTO AREAS, NAMIBIA

TO WHOM IT MAY CONCERN

I hereby confirm that the above-mentioned student is registered at the University of Namibia for the programme indicated. The proposed study met all the requirements as stipulated in the University guidelines and has been approved by the relevant committees.

The proposal adheres to ethical principles as per attached Ethical Clearance Certificate. Permission is hereby granted to carry out the research as described in the approved proposal.

Best Regards

Dr. AEE Shikongo
Head: Postgraduate Support Services
Tel: +264 61 206 3129
E-mail: aeshikongo@unam.na

CENTRE FOR RESEARCH SERVICES
Office of the Director
2022 -05- 18
University of Namibia
UNAM

Annexure F: Test Result Copy from CVL

CENTRAL VETERINARY LABORATORY
Ministry of Agriculture, Water and Land Reform
24 Goethe Street, Private Bag 13187, Windhoek, Namibia
Tel: 061-237684 Fax: 061-221099
e-mail: Siegfried.Khaiseb@mawlr.gov.na
Vat Reg. No: 0131475015

Date reported: 05-07-2022

Client Ref. No: SW 098/22 IK

Samples owner telephone: -

DR.I KAATURA
Private Bag 13301 WINDHOEK
Windhoek
YEAR 2022 Submission Number 3420

FINAL TEST REPORT

This is to report the results of the samples analyzed as per Herd Health Form from 21-06-2022 to 04-07-2022

# Samples	Specimen	Species	Sampling Date	Date Received	Owner	Owner's Address
1	Sheath scraping in Steves transport medium	Bovine	20/06/2022 20/06/2022	21/06/2022	[redacted]	

Sample: Sheath scraping in Steves transport medium - Species: Bovine

Samples	Sample Id	Test	Test Result	Method
1	37550985	Campylobacter fetus spp: Identification	Positive	PCR
1	37550985	Tri-trichomonas fetus spp: Detection	TF detected.	Isolation

Sample: Trichomonas culture - Species: Bovine

Samples	Sample Id	Test	Test Result	Method
1	37550985	Tritrichomonas foetus spp. identification (Real-time)	Positive	Real-Time PCR

Test	Method	Test procedure	Testing Laboratory
Tri-trichomonas fetus spp: Detection	Isolation	CLM SOP 04	Central Veterinary Laboratory
Tritrichomonas foetus spp. identification (Real-time)	Real-Time PCR	BIO SOP 31	Central Veterinary Laboratory
Campylobacter fetus spp: Identification	PCR	BIO SOP 28	Central Veterinary Laboratory

Sub-Division	Section	Authorized Signatory
Biotechnology	Molecular Biology : Microbiology	G. Aikukutu

Submission Number: 3420

Page 1 / 3

1.CVL is not responsible for sampling therefore the results apply to the sample(s) as received. null 2.This test report shall not be partially reproduced, except in full with the written approval from Head of CVL.

Sub-Division	Section	Authorized Signatory
Diagnostic Services	Clinical Microbiology	Georgina Ngaisiue