DIAGNOSTIC ACCURACCY OF *EHRLICHIA RUMINANTIUM* TEST FOR HEARTWATER SCREENING IN MASVINGO DISTRICT

BY

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Abstract

Background

We evaluated the diagnostic accuracy of the *Ehrlichia Ruminantium test* (Post Mortem vs Direct Brain Smear) to screen for Heartwater in Masvingo District.

Methods

Between July 2016 and July 2017, cattle and goat carcasses were screened for heartwater (tick abundance, characteristic post mortem lesions and direct brain smear for microscopy). Sensitivity and specificity and predictive values of E Ruminantium were calculated using microscopy results as our gold standard. Epi info software version 7 was used.

Findings

145 animal carcasses were selected, 101 were positive by post mortem examinations and 44 were negative by post mortem examinations. Samples had to underdo tentative diagnosis by direct brain smear in the lab. Among all the participants, sensitivity was found to be 88% and 82% respectively. Sensitivity and specificity was calculated seperately for cattle and goats, for cattle sensitivity and specificity was 91% and 70.3% respectively for goats sensitivity and specificity was 82% and 72.2% respectively.

Conclusion

The diagnostic tests used have adequate sensitivity for heartwater screening in this population.

Keywords: *Diagnostic accuracy, sensitivity, specificity, negative predicitive value, positive predictive value, Ehrlichia ruminantium, Masvingo district.*

Acknowledgement

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Last but not least all the gratitude I give it to God from whom I draw my inspiration.

Declaration of Thesis

I hereby declare that this thesis is composed of work carried out by myself unless otherwise acknowledged and that this thesis is of my own composition. The research was carried out during the period of July 2016 to July 2017. This thesis has not in whole or in part been previously submitted for any other degree or professional qualification.

Witness Zhou

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DATE

14/ 05 / 2018

Certification of Thesis

I the undersigned, certify that Witness Zhou, a candidate for the degree of Bachelor of Science (Honours) Animal and Wildlife Sciences has presented this thesis with title:

Diagnostic accuracy of Ehrlichia ruminantium test for heartwater screening in Masvingo District.

That this thesis is accepted in form and content and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on the 27th of May 2018.

Major Supervisor

Mr. J.V. Muzvondiwa

Signature……

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CHAPTER 1

1.0 INTRODUCTION

Correct diagnosis is an important and necessary part of disease control in both humans and animals .(Aggarwal, 2003). Assessment of validity and reliability of a diagnostic and screening test is essential to provide appropriate and effective health care for animals and for research. Diagnostic testing and screening have been the centre of discussion by various authorities globally. In most cases it is about assessing whether the test implemented can accurately distinguish between individuals who do and who do not have the specific disease, and also whether a valid, accurate diagnostic test can be applied to a specific individual. The quality of a given test comes into play in order to make reasonable decision regarding its utilization and interpretation. In this research the diagnostic accuracy of post mortem examinations vs direct brain smear as a reference standard was assessed by calculating sensitivity (Sn) and specificity (Sp). Data was collected at two stages of diagnosis which are necropsy or post mortem examinations where characteristic lesions were used to aid in confirming the presence or absence of *E ruminantium*, and also during direct brain smear and microscopic observation of samples.

An understanding of heartwater is essential because signs are not always pathognomic. Heartwater is an infectious disease caused by rickettsial agent *E ruminantium. Ehrlichia ruminantium* is transmitted to domestic and wild ruminants by *Amblyoma hebraeum* ticks (Mahan, Simbi and Burridge, 2004). Epidemiology of the disease has also been reported to be affected by the migration patterns of cattle egrets which feed on *A hebraeum* ticks. Heartwater is known to be endemic in the southern lowveld region of Zimbabwe and is characterised by large abundance of *A hebraeum* ticks species (Peter et al, 1999). Prevalence of *A hebraeum* in the southern low veld of Zimbabwe has been found to be very high with ticks being found in almost every diptank (Sungirai et al, 2016). The disease is also endemic in sub Saharan part of Africa were it is a main obstacle to livestock development (Mboloi et al, 1999). Heartwater is identified by the World Organisation for Animal Health as a notifiable disease of ruminant animals (OIE, 2014).According to (Carmus et al 1996) the disease is responsible for 90% of mortalities and heavy economic loses in domestic animals. The causative agent *Ehrlichia ruminatium* has got a number of characteristics, it is an intracellular gram negative bacterium in the order ricketsialles and family Anaplasmataceae. The distribution of the causative agent coincides with that of its vector species, (Walker and

Olwage 1987), when stained with Giemsa the parasite stains purplish blue (Cowdry, 1825). *A Variegatum* another widespread vector of heartwater was introduced onto some Caribbean island during the 18th or 19th centuries (Maillard and Maillard 1998), Heartwater was recognised to be a tickborne infection in 1900 (Lounsbury,1900), in 1925 the causative agent was identified as a rickettsia, originally named rickettsia ruminantium (Cowdry ,1925) and subsequently renamed *Cowdria ruminantium*, (Mashkovski, 1947), Heartwater is endemic in the lowveld and prevalence is influence by seasonal variations. Geographical co-ordinates of Masvingo are latitude 20°03′49″S, longitude 30°49′39″E, Elevation above sea level is 1091m.

1.1 Problem statement

The pathognomic nature of heartwater calls for improved diagnostic accuracy. The use of antibiotics by livestock farmers compromises diagnostic test results resulting in false negative results, only a few colonies can be found in per acute cases. Reporting on the checklists available for the assessment and critical appraisal of diagnostic test studies is scarce in the animal husbandry field (Bossuyt *et al*).

1.2 Justification of the study

The study has been carried out to create awareness about the validity and the reliability of the brain smear diagnostic test and its related basic concepts (Aggarwal, 2003). For the test to be considered valid and reliable it has to be of higher sensitivity than specificity. This is so because we need to identify the true cause of mortality. Diagnostic tests produce results which aid in making a diagnosis, we need a test that will give a correct diagnosis (Altman, 2007).

1.3 General objective

To evaluate the diagnostic accuracy of the direct brain smear test for diagnosis of heartwater.

1.4 Specific objectives

- To test the sensitivity and specificity as well as PPVs and NPVs of direct smear examinations versus post mortem examinations.
- To compare the validity and reliability of this test in cattle and goat.
- Calculate and evaluate risk of bias and associated factors that contribute to bias.

1.5 Hypothesis

H^1 - There is no difference between post mortem diagnostic results and laboratory results.

CHAPTER 2

2.0 LITERATURE REVIEW

The diagnosis of heartwater is difficult because its clinical signs are pathognomic, meaning it is quiet easy to confuse with other diseases (Songer et al 2005). The use of antibiotics also supresses the parasite such that it might not be visualised under the microscope (OIE, 2014). Correct diagnosis is sometimes based on a case history, in most cases veterinarians gather information such as tick type and abundance on the carcase, the geographical location of the area from which the case was recorded (is heartwater endemic?), the vaccination and tick control method implemented. The species, breed, age and origin of the animal play a major role in confirming the presence of a disease (Van de Pypekamp and Prozesky, 1987).

However these alone are not reliable sources of information to guarantee us success in diagnosing the disease. There is need for valid and reliable diagnostic tests with higher accuracy. Various technologies such as the PCR and antibody immune fluorescence assay have been developed to diagnose and identify the presence of *E ruminantium* in live animals. The validity of a test is determined but factors such as cost of implementation, level of accuracy, and timeliness (Streiner, 2003). There are four possible results from a diagnostic test and these are, animals which actually have the condition and were correctly detected by the test (TP, true positives) or missed by it (FN, false negatives), whereas animals that do not have the condition and were erroneously labelled as having it (FP, false positives) or correctly labelled as not having it (TN, true negatives) (Schmidt and Factor, no date). False positive and false negative diagnoses are rarely equally important, thus missing a life threatening disease will be regarded as much more important than a false positive diagnosis in a healthy patient. A number of attributes of the test, collectively known as diagnostic efficiency statistics, can be derived from these four possible results. We have, sensitivity, specificity, and the likelihood ratio which are conditional on the population (Akobeng, 2007). Explained below are fundamental attributes which help in validating a diagnostic tests.

2.1 Diagnostic Accuracy

This is the ability of a diagnostic tests to identify an individual with disease or rule out an individual without disease, it is a simplification of sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV). Mathematically diagnostic accuracy is the sum of those correctly identified as ill and well divided by all those tested (Petersburg and Teitelbaum, 2012).

$$\text{Diagnostic} = \frac{TP+FN}{TP+FP+TN+FN}$$

Sensitivity of a test is defined as the proportion of individuals with disease who will have a positive result,

$$\text{Sensitivity} = \frac{TP}{TP+FN}$$

Whereas specificity is the proportion of individuals without the disease who will have a negative result.

$$\text{Specificity} = \frac{TN}{TN+FP}$$

Positive and Negative predictive values describe a patient's probability of having disease once the results of his or her tests are known (Akobeng, 2007).

$$\text{PPV} = \frac{TP}{TP+FP}, \qquad \text{NPV} = \frac{TN}{TN+FN}$$

The whole point of a diagnostic test is to use it to make a diagnosis, hence the need to know the probability that the test will give the correct diagnosis. Sensitivity and specificity do not give this information, instead there is need to approach the data from the direction of the test result using predictive values. However both PPV and NPV vary according to the prevalence of a disease in a given situation. The diagnostic accuracy of a test can also be evaluated using summary Receiver Operator Characteristic (ROC) curve/plots (Cross et al., 2015). ROC AUC is the area under the ROC curve of sensitivity versus 1- specificity. The ROC AUC for each test corresponds with the probability that of two randomly selected samples or patients, one with and the other without disease, the diagnostic test will rank the person with disease wi8th a higher suspicion of disease than the one without disease. For example a ROC AUC of 0.8 means that of two randomly selected samples, there is an 80% chance that the sample with disease will be ranked with higher suspicion than the sample without disease. An alternative interpretation of the ROC AUC is the average sensitivity given that all values of specificity are equally likely. ROC are also used within studies to compare different tests, to compare different groups of patients and to investigate variability between different test observers. (Zhu, Zheng and Wang, 2010) also stipulate that ROC curves help determine the threshold for a given diagnostic test. The Receiver Operator Characteristic curve presents the relationship between sensitivity and specificity by plotting sensitivity on the Y axis and 1-specificity on the X axis. However the ROC AUC usually averages all possible thresholds but

not all thresholds are clinically relevant, also threshold offering sensitivity greater than 80% are not clinically useful because specificity will be too low .

2.2 Likelihood Ratios (LR)

LR = sensitivity/ (1-specificity)

Likelihood ratios tell what the odds are that a positive test result has come from an individual who has the attribute and the other way round in the case of a negative test. Sensitivity and specificity can be combined in an equation to calculate the likelihood ratio, likelihood ratios can be negative or positive. The LR is also an index of the accuracy of a test, meaning that it is one of the most important parameters measured when a test is being validated (Sackett et al 1991, Streiner and Norman, 1996). LRs just like sensitivity and specificity are fixed properties of a test, meaning, these attributes remain the same if the test is used with similar groups of people or animals, but in a case when the individuals being tested have a different amount of the attribute, sensitivity and specificity will have to be re calculated. An example is the validation of a test on patients with severe depression, the test is more likely to have a different LRs compared to when used with outpatients with dysthymia (Meyer, 2002). When likelihood ratios are used with scales of continuous outcomes like in our case, they can be calculated from different cut points. Thus the higher the score, the more likely it is to have come from an individual with the disease. When likelihood ratio is 1, the test is useless,

2.3 Bias

There has been increasing awareness of deficiencies in study design and reporting of diagnostic test accuracy studies and it is now recognized that diagnostic accuracy studies are subject to unique sources of bias (Schmidt and Factor, no date). For any diagnostic review to be standard it has to include an assessment of the risk of bias. There are a number of sources of bias that affect diagnostic accuracy. According to (Whiting 2011), the Quality Assessment for Diagnostic Accuracy Studies (QUADAS) consists of four domains which can be assessed for risk of bias and these are, patient selection criteria, index testing, reference standard and flow and timing.

Difference in patient populations can affect the accuracy of a test and also the comparison of studies conducted in different populations raises questions of applicability. The extent to which the results of the study apply to the study target population depends on how well the

actual study participants match the target population defined in the study question (Schmidt and Factor, 2010).

It is recognised however that the sensitivity of a test can be considerably reduced by poor sampling techniques. Diagnostic tests are often complex processes involving many steps that can be performed in many different ways. There are a number of bias factors that affect the final result of a test, these are **sample acquisition** which depend largely on personnel experience and also use of rapid on-site evaluation, **sample processing** (type of stain and use of ancillary techniques) and **interpretation** (number of lab technicians who interpret the results, their level of experience and availability of clinical information) Each of these factors has the potential to affect test accuracy, and each variation can be thought to be a different test with different performance characteristic

Bias can be a result of incorrect measurement, when measurements are unreliable, they may fail to represent the true value of the disease being measured. A study is said to lack internal validity when it fails to measure the true value of the phenomenon being measured (Schmidt and Factor, 2010).

Bias can be a result of the reference standard used in a test, (Kohi et al., 2013) argues that sensitivity and specificity are determined in research studies that use a reference standard to confirm the true disease the reference standard can therefore be imperfect or it could be a clinical determination that partly relies on the diagnostic test in question. This gives the possibility that the reference standard is not entirely the reference but could be a potential source of bias.

To be useful, a study must address a clinical question. To formulate a question we use the PICO format. For a diagnostic study, the PICO parameters are **population, index test (the test under examination), comparator or reference test (the gold standard), and outcomes**. In this study our population comprises of cattle and goats suspected to have died of heartwater, the index test is post mortem examination, the reference test is direct smear and microscopy procedure and lastly the outcome is the measure of the diagnostic accuracy of the test. The value of a study is a function of its capacity to resolve a clinical question. A clinical question can arise (like in our case) in the context of clinical work (Can this study help me to diagnose this patient's condition?), or in meta-analysis (Can this study help to resolve the question of the meta-analytic study?). To answer a clinical question correctly, a

study must provide information that is both reliable (internal validity) and applicable (external validity). Internal validity is a function of bias and precision.

2.4 Diagnostic tests for heartwater

The most common and probably oldest method to diagnose heartwater post mortem is observing *E ruminantium* colonies in the brain. The diagnosis process involves a post mortem examination, where classical signs are hydro pericardium, hydrothorax and also oedema of the lungs and brain (Allsopp, 2015). However in some cases post mortem might fail to diagnose heartwater due to nature of the disease (acute, per acute or sub-acute), only a few colonies may be found in animals with per acute disease. Some of the gross lesion depend on species type for example straw coloured to reddish pericardial fluid which is more consistently found in sheep and goats than in cattle. In both cases veterinarians depend on a tentative diagnosis that is determined by observation of *E. ruminantium* colonies in the cytoplasm of brain endothelial cells. The detection of *E ruminantium* in capillary endothelial cell membrane smears was a major breakthrough in the diagnosis of heartwater (Purchase, 1945). Until then, the demonstration of *E. ruminantium* in smears from the intima of large blood vessels and in tissue sections were used to make a diagnosis (Jackson, 1931). *E.ruminantium* occurs as clumps of reddish-purple to blue, coccoid to pleomorphic organisms in the cytoplasm of capillary endothelial cells (Allsopp, 2015). These organisms are often found close to the nucleus, and may be in a ring or horseshoe. The number of colonies in brains smears may also vary between strains with very rare colonies even with some very virulent strains. Colonies are still visible 2 days after death in a brain that has been stored at room temperature (20–25°C) and up to 34 days in a brain that has been stored in a refrigerator at 4°C. *E. ruminantium* can also be detected in formalin-fixed brain sections using immunoperoxidase techniques. Preparation of brain smears has been described by various researchers such as (Matos 2008, Purchase 1945, Ulienburg 1987) Detection of *bacterium* can be done using culture preparation which act as standard gold for diagnosing heartwater (Dhingra et al., 2003).

2.5 Gold standard for detection of *Ehrlichia ruminantium*

Results obtained from a diagnostic accuracy study are expressed by comparison with a reference standard of the true disease status for each individual sample/patient. A gold standard is a best test available at the moment, gold standard test is usually diagnostic test or benchmark that is the best available under reasonable conditions (Versi, 1992). A

hypothetical ideal "gold standard" test has a sensitivity of 100% with respect to the presence of the disease, it identifies all individuals with a well-defined disease process, it does not have any false-negative results and a specificity of 100% it does not falsely identify someone with a condition that does not have the condition, it also does not have any false-positive results. In practice, there are sometimes no true gold standard tests. As new diagnostic methods become available, the gold standard test may change over time. In this study the gold standard for the diagnosis of Heartwater disease is a brain biopsy. However, because this can be done only after the patient has died, there is need for considerable efforts to develop tests that can be used to identify *E ruminantium* colonies in the brain while the animal is still alive, (Chen et al., 2000) reported the use of brain imaging and psychological test batteries to detect Alzheimer's disease in humans and there is also a possibility of applying such procedures in animals.

2.6 Serological diagnosis of *E. ruminantium*

In Zimbabwe disease control is hampered by absence of a specific, sensitive diagnostic assay which can quickly differentiate between those animals recovering from heartwater and those requiring protection. The first serological test for heartwater used peritoneal macrophages from *E ruminantium* infected mice to detect antibodies in sera from infected animals (Allsopp, 2015), however false positive feedbacks were common. The IFA and enzyme linked immunosorbent assay (ELISA) test, used on serum from live animals, have a low specificity for E ruminantium organisms as cross reactions with antibodies against Anaplasma and other Ehrlichia species can occur, resulting in false positives. Current serological tests are based on the detection of antibodies to the immunodominant fragment of MAP1 (MAP1B) in an indirect enzyme linked immunosorbent assay (ELISA) format (Van vleit et al, 1995). It must be emphasised, however that all serological tests for *E ruminantium* may exhibit false positive reactions owing to the presence of closely related homologs of MAP1 in other Anaplasmataceae species (Van Heerden, 2004: Zhang et al 2008 and Noh SM, 2008). Serological tests for heartwater also exhibit false negative results, mostly in cattle as antibody levels are often too low to be detected, even in animals that are under continuous natural challenge by infected ticks (De Waal, 2000 and Kelly et al 2011). It has been confirmed that results from MAP1B ELISA do not correlate well with those from a nested Pcs20 polymerase chain reaction test. Assays are available, including indirect fluorescent antibody, polyclonal competitive ELISA, major antigenic protein (MAP-1) ELISA, and

Western blot, but often lack specificity because of cross-reactivity with closely related ehrlichiae.

2.7 PCR diagnosis of *E. ruminantium* parasite

The first *E. ruminantium* specific genetic target used for a diagnostic test was in the form of a plasmid clone, selected pCS20, from a genomic library of the virulent Crystal Springs isolate from Zimbabwe (Waghela et al, 1991). The target region consists of two overlapping genes (Van heerde et al, 2004). The tests that have been developed to detect this region all use a variation of PCR amplification, whether directly (Van heerde, 2004), in a nested format (Martinez D, 2004), in a quantitative real-time format (Steyn et al, 2008), or in a loop-mediated isothermal amplification (LAMP) format (Nakao et al, 2010). There are sequence polymorphisms, mostly single nucleotide polymorphisms, among the pCS20 regions of different E. ruminantium isolates, but there are also more divergent homologs in all known *Ehrlichia* spp. The pCS20 test can therefore give positive signals with DNA from organisms other than E. ruminantium, most notably with E. chaffeensis and E. canis; nevertheless, when the test is properly calibrated, these signals are an order of magnitude lower than those given by an equivalent concentration of E. ruminantium DNA. Extensive use of the pCS20 test over more than 20 years has shown that it can specifically identify E. ruminantium in domestic animals, wild game and ticks. The quantitative real-time format for this test appears to be the most sensitive for examining field samples, while the LAMP format may be more convenient for less well- equipped laboratories.

A new quantitative real time polymerase chain reaction (qPCR) test has been developed for rapid and accurate diagnosis of heartwater in the blood of live animal as well as in ticks. (Steyn, 2010), coupled with the in vitro culture of new genotypes (qPCR) has been useful in facilitating molecular epidemiological studies which aim to contribute to better control of heartwater and the development of a more effective vaccine.

The diagnosis of heartwater depends largely on the demonstration of organisms in endothelial cells in brain either in smears or section; brain biopsy can be used to establish the diagnosis in the living animal. The pCS20 PCR assay is the most reliable and specific assay for the detection of E. ruminantium in ruminants and *Amblyomma* ticks. Various antibody detection.

The epidemiology of heartwater disease is not fully understood, but the agent is known to be transmitted by ticks of the genus Amblyomma (Songer et al 2005). These three host ticks have a variety of hosts, ranging from birds to tortoises to mammals, Amblyomma hebrium

(the bont tick) and *Amblyomma variegatum* are the major vectors in Africa. Transstadial transmission also occurs in ticks but transovarian transmission is infrequent.

Heartwater has an average incubation period in susceptible animals of less than 2 weeks (Van de Pypekamp and Prozesky, 1987), adult cattle have a subsequent mortality of up to 82% (Du Plessis and Malan, 1987b) and Merino sheep of up to 95% (Neitz, 1964). The disease is a major obstacle to the introduction of high-producing animals into sub-Saharan Africa to upgrade local stock, and is of particular importance when susceptible animals are moved from heartwater-free to heartwater-infected areas (Simpson et al., 1987). The occurrence of heartwater is frequently taken for granted in the endemic areas of Africa, and definitive diagnoses are not often performed. The economic impact of the disease is therefore difficult to quantify, although estimates which have been made indicate that the losses can be enormous (Mukhebi et al., 1999). *Ehrlichia ruminantium*, the causative agent of heartwater, is transmitted by *Amblyomma* spp. ticks. *Amblyomma variegatum* ticks, which are found in the Caribbean and sub-Saharan Africa, except in certain areas of southern Africa, are major vectors of *E. ruminantium*.

CHAPTER 3

3.0 RESEARCH METHODS

A total of 145 brain samples were collected from cattle and goats during the July 2016-july 2017 period, from the total 81 were cattle and 64 were goats. Samples were examined by post mortem and direct brain smear in the laboratory. The method used in this research is similar to that of Dhingra et al (2003) except that the researcher was assessing the validity and reliability of sputum smear examination as diagnostic and screening test for tuberculosis in humans.

Diagnosis started at post mortem examination where carcasses examination was supervised by the District veterinary and lab specialists. Characteristic post mortem lesions included a straw coloured to reddish fluid in the heart sac (abdominal) fluid accumulation, fluid in the chest cavity, and fluid filled lungs. Animals also had enlarged spleens, damaged heart muscles, enlarged liver and kidneys, swelling and bleeding in the stomach and intestines. Characteristic post mortem lesion of heartwater were more consistently found in sheep and goats than in cattle. Presence of *Amblyoma Hebraeum* ticks also helped in diagnosis of the disease. Ticks were collected for identification at the Central Veterinary Lab in Harare. For laboratory observation brain tissue collection was done by cutting the upper part of the skull thus exposing the brain and collecting tissue through the foramen magnum with a curette. (Infectieuse and Infecciosa, 2015) also outline that best samples to collect from the brain are well-vascularized portions such as the cerebrum, cerebellum or hippocampus.

3.1 Laboratory diagnosis

Identification of *E ruminantium* was carried out after collection of brain smears. The direct smear of brain samples was done using Diff quick a commercial Giemsa type stain which is a cost effective tool for diagnosis of rickettsia organisms. However this method had the possibility of producing false negative results because veterinarians faced with sick animals which may have heartwater normally treat them with tetracycline, which makes colonies of the organism more difficult to detect at any subsequent post-mortem (Allsopp, 2015). Brain samples collected from suspected carcases were squashed and smeared on microscopic slides. A special technique was used to spread the sample along the slide. A required amount of

sample was placed on a microscopic slide then another slide placed on top of the slide at 45°, smearing was then done by slowly pushing the sample backwards and the push forward quickly. Staining technique included fixing the slides in methanol for 5 minutes and allowed to air dry. Slides were soaked in dilute Giemsa for 20 minutes after which they were rinsed in distilled water, air dried and observed under the light microscope at x10 resolution. Reddish-purple to blue colonies were observed, most of the colonies were close to the nucleus of the infected endothelial cell.

3.2 Determination of test results

Each sample had to go through both tests that is post mortem examination and direct brain smear, direct brain smear test acted as a reference standard for validating the results of post mortem examination. Four outcomes were expected from the test. Samples which produced positives results for *E ruminantium* by both post-mortem and direct brain smear were recorded as True Positives (TP), those that produced positive results at post mortem and negative results by direct brain smear were recorded as False positives (FP), Negative results by post mortem followed by positive direct brain smear results during microscopy produced false negative results (FN) and lastly True negative (TN) results were a result of the absence of *E ruminantium* in both cases of diagnosis.

Table 1: A 2x2 table showing the relationship between post mortem and direct brain smear for diagnosis of heartwater in cattle and goats.

	Direct smear +	**Direct smear -**	**Total**
Post mortem +	TP	FP	TP+FP
Post mortem -	FN	TN	TN+FN
Total	TP+FN	FP+TN	TP+FP+FN+TN

Out of 88 samples collected from cattle carcasses for heartwater screening 56TPs, 19TNs, 8FPs and 5FNs were recorded. Out of the 64 samples collected from goats 32TPs, 13TNs, 5FPs and 7FNs were recorded.

3.3 Data analysis

Calculation of sensitivity, specificity, PPVs and NPVs was done using the statistical analysis tool Epi info 7. Thereafter means were separated using least significant differences (LSD) at

a probability level of 5%. Diagnostic odds, likelihood ratios, Cohen's kappa and bias assessment values were also obtained using Epi info 7 software. Diagnostic accuracy was first calculated seperately for cattle and goats and then later calculated overall. A ROC plot was plotted indicating the position of the test for both cattle and goats and overall.

CHAPTER 4

4.0 RESULTS

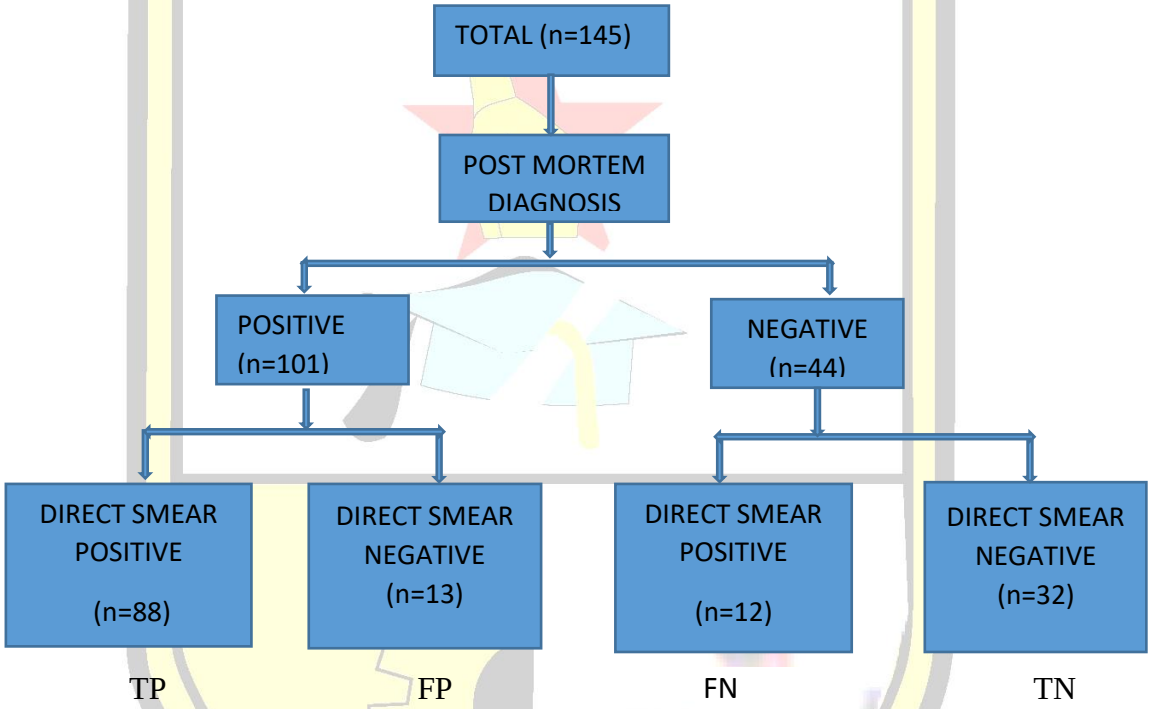


Figure 1: Participant flowchart showing the overall process implemented to determine the diagnostic accuracy of E Ruminantium test for heartwater screening in Masvingo district. Direct brain smear was used as the standard gold.

Group	Sensitivity	Specificity	PPV	NPV	DA
Overall	88	86,49	94,62	72,73	87,59
95% CI	(80,19 - 93)	(72,02 – 94,09)	(88,03 – 97,68)	(58,15 – 83,65)	(81,03 – 92,11)
Cattle	91	70,37	87,5	79,17	85,23
95% CI	(82,21- 96,45)	(51,52 - 84,15)	(77,23 - 93,53)	(59,53 - 90,76)	(76,35 - 91,16)

Goats	82	72,2	86,49	65	78,95
95% CI	(67,33 - 91,02)	(49,13 - 87,5)	(72,02 - 94,09)	(43,29 - 81,88)	(66,71- 87,53)

Table 2: Performance of direct smear test compared with post-mortem findings.

PPV= Positive Predictive Value, NPV= Negative Predictive Value, DA= Diagnostic Accuracy

Kappa is the measure of agreement between the test under validation and the reference gold. There was a substantial agreement between post mortem findings and direct brain smear for *E ruminantium* (Kappa = 0.5) for cattle and (Kappa = 0.44)] for goats.

Table 3**:** Likelihood ratios and associated Odds ratio and Kohhen's kappa.

	LR+	**LR-**	**Diagnostic odd**	**Kappa**
Cattle	3	0,285	10,59	0,527
Goats	2,528	0,39	6,5	0,4348

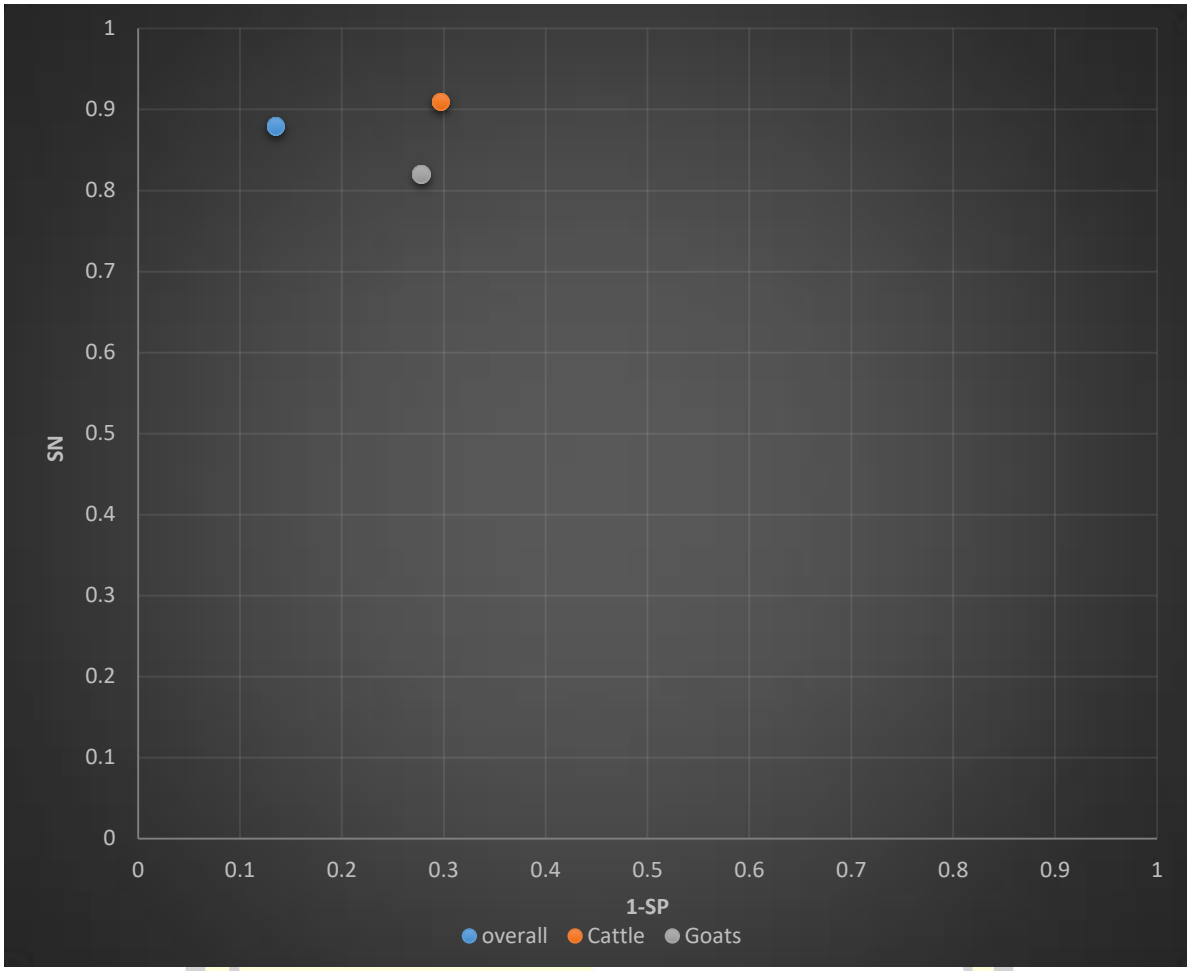


Figure 2: Receiver operator characteristic ROC analysis showing the area under the curve for cattle, goats and overall.

Receiver operating characteristics (ROC) analysis of the data showed that the two tests are approximately equivalent in sensitivity with the area under the curve (AUC) being 0.89, 0.72 and 0.8 for cattle and goats and overall respectively.

CHAPTER 5

5.0 DISCUSSION

The diagnostic calculations should have certain features like sensitivity, specificity and predictive values (NPV and PPV). The data collected at Masvingo Provincial veterinary lab has been useful in illustrating the concepts of sensitivity, specificity in relation to diagnostic methods used , which are post mortem examinations and direct brain smear examinations in the lab. The direct smear examination was taken as gold standard for evaluation of these indices.

Though less sensitive than direct brain smear, post mortem observation is a very useful and essential diagnostic technique for Heartwater. However the sensitivity is lower with specimens with higher concentrations of colonies more likely to result in positive test while a lower count of colonies is more likely to result in a false negative test. Sensitivity was found to be 91% and 82% in cattle and goats respectively. Sensitivity can however be increased if the number of samples examined for each sample is increased (Dhingra *et al.*, 2003)

The sensitivity and specificity values give a direct assessment of the usefulness of the test in practice and are not affected by the proportion of subjects with the abnormality that is the prevalence. However to know how good the test is at predicting abnormality, one has to find the probability of index test for giving correct diagnosis, whether positive or negative. These two proportions are once again expressed as the Positive Predicative Value (PPV) and the Negative Predictive Value (NPV).

Receiver operating characteristics (ROC) analysis of the data showed that the two tests are approximately equivalent in sensitivity with the area under the curve (AUC) being 0.89, 0.72 and 0.8 for cattle and goats and overall respectively. This indicates that the test would be valid and reliable for heartwater diagnosis.

Diagnostic odds for cattle were 10,6 and 6,5 for goats, odds ratios are a direct function of likelihood ratios, The likelihood ratio combines sensitivity and specificity thereby providing an estimate of how the odds of having a diseases are affected by the test results. **Table 4.3** shows the likelihood ratios and their corresponding diagnostic odds.

The likelihood ratio for a positive result (LR+) shows how much the odds of having a disease increase when a test is positive. Negative likelihood ratio (LR-) shows how much the odds of the disease decreases when a test is negative, so in this case the odds of the having disease increase by 3 and 2,528 in cattle and goats respectively and the odds decrease by 0,29 and 0,39 in cattle and goats respectively. In other words a likelihood ratio greater than 1 indicates that the test result is associated with the presence of a disease whereas a likelihood ratio less than 1 indicates that the test result is associated with the absence of disease. The further LRs are from 1 the stronger the evidence to rule in or rule out diagnoses respectively, for example LRs above 10 and below 0.1 are considered strong evidence to rule in and rule out disease. There for in our case the LRs were all fair and acceptable.

CHAPTER 6

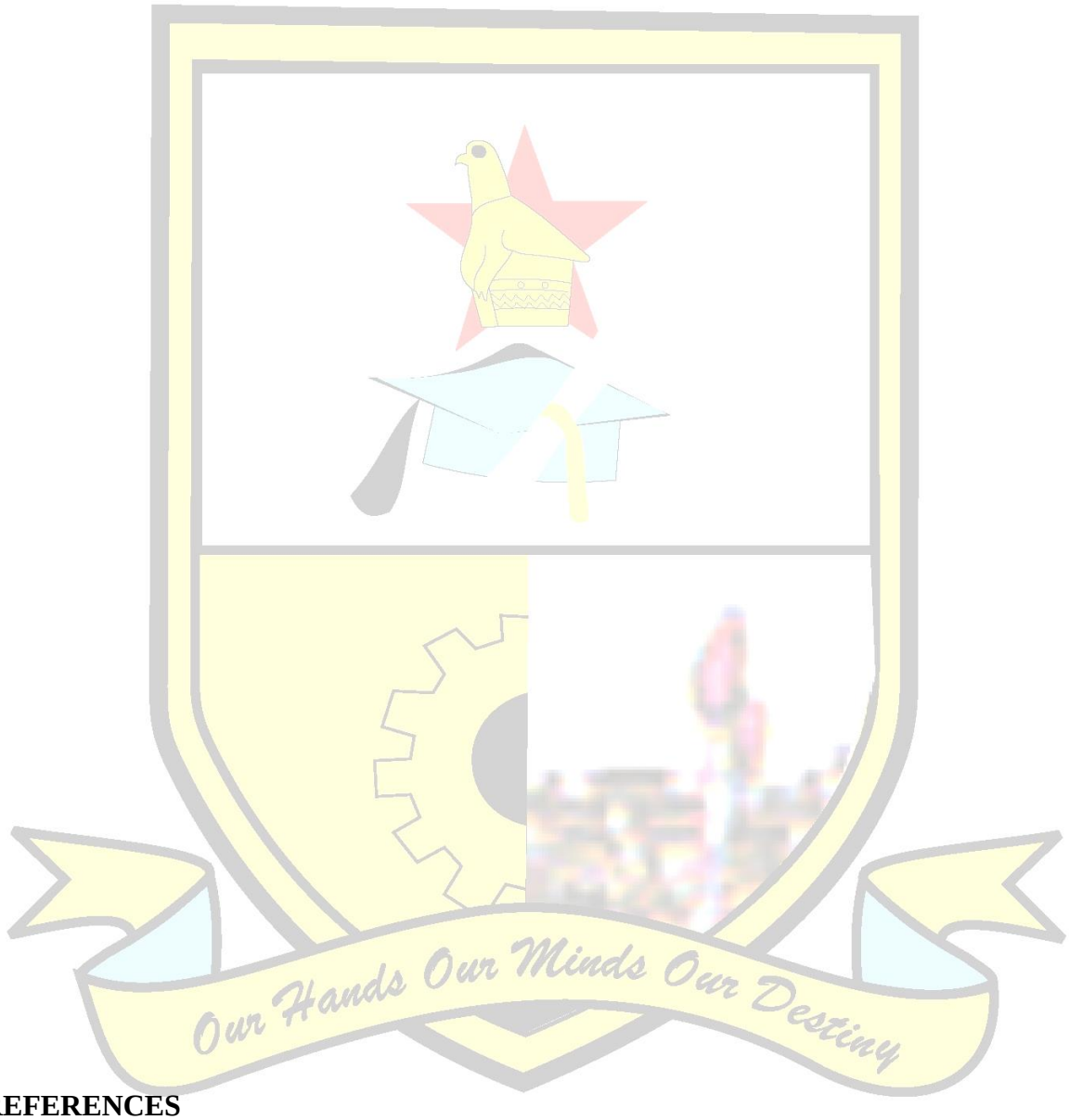
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Due to the high overall sensitivity 88% and high overall specificity 82% found in the study, I conclude that test is valid and reliable and therefore should be used for diagnosis of heartwater in cattle and goats

6.2 Recommendations

Sensitivity can however be increased if the number of samples examined for each sample is increased (Dhingra *et al.*, 2003).



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