COMPARATIVE STUDY ON THE PREVALENCE OF TRYPANOSOMES AMONG CATTLE IN SELECTED ABATTOIRS AND CONFINED FEEDLOTS (FARMS) IN PARTS OF KADUNA STATE, NIGERIA

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By

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JULY, 2016.

DECLARATION

I declare that the work in this dissertation titled "COMPARATIVE STUDY ON THE PREVALENCE OF TRYPANOSOMES AMONG CATTLE IN SELECTED ABATTOIRS AND CONFINED FEEDLOTS (FARMS) IN PARTS OF KADUNA STATE, NIGERIA'' was performed by me in the Department of Microbiology, under the supervision of Professor S.E. Yakubu and Professor S.A. Ado. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

Fatima SAMBO _____ _____

Signature Date

CERTIFICATION

This dissertation titled COMPARATIVE STUDY ON THE PREVALENCE OF TRYPANOSOMES AMONG CATTLE IN SELECTED ABATTOIRS AND CONFINED FEEDLOTS (FARMS) IN PARTS OF KADUNA STATE, NIGERIA meets the regulations governing the award of the degree of MSc. Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the Almighty Allah, whose kind Permits and Blessings made this an easy accomplishment for me.

I also dedicate this thesis to my Parents Malam Sani Sambo and Hajiya Binta Sani Sambo whose unending support and encouragement knows no bound. Thank You.

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ABSTRACT

The prevalence of trypanosome infection among cattle in selected abattoirs and confined feedlots (farms) in parts of Kaduna State, Nigeria was investigated. Wet mount, thin films, thick films, animal inoculation, Haematocrit Centrifugation Technique and Buffy Coat Methods were used to detect trypanosomes from the jugular blood of the animals. The Packed Cell Volume (PCV) was also determined. A total of 450 cattle were examined from the selected abattoirs. The prevalence in cattle among the selected Abattoirs studied showed that *Trypanosoma brucei, T. congolense*, and *T. vivax* had a prevalence of 33.87%, 30.65%, and 35.48% respectively, and the prevalence in all the selected farms studied showed that *T. brucei, T. congolense,* and *T. vivax* has a prevalence rate of 33.33% each. Mean PCV of all the cattle in the selected abattoirs is 38.44%, while the mean PCV of all the cattle in the selected farms is 40.63%. Sex did not significantly ($p>0.05$) affect infection rates. PCR using specific primers for *T. brucei, T. congolense* and *T. vivax* showed that 11.5% of the 26 tested animal blood were infected by at least one of these *Trypanosoma* species studied except *T. vivax*.

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CHAPTER ONE

1.0 INTRODUCTION

Animal Trypanosomiasis is a disease complex caused by several species of protozoan parasites of the genus *Trypanosoma*. The parasite is transmitted to mammalian hosts through the bite of an infected tsetse fly which causes African sleeping sickness, a fatal disease in humans, and "nagana" in cattle. Trypanosomes are unicellular protozoan parasites that belong to the genus *Trypanosoma*. These include many species which causes several diseases in humans and livestock, species of major threat to cattle, sheep and goats include *Trypanosoma vivax* (*T. vivax*), *Trypanosoma congolense* (*T. congolense*), *and Trypanosoma brucei* (*T. brucei*) (Luckins and Mehlitz, 1978; Joshua and Ige, 1982). Although, small ruminants may not often show clinical signs of the disease, it is assumed that the ruminants are rarely affected under natural conditions and that trypanosomosis of sheep and goats is not a serious problem (Kalejaiye *et al.,* 1995). Several experimental studies have shown that small ruminants are fully susceptible to infection with pathogenic trypanosomes (MC Guire *et al.,* 1985; Whitelaw *et al.,* 1985). In addition, infection in sheep and goats is frequently reported from field surveys (Kanyari *et al.,* 1986; Mawuena, 1986; Mewuena, 1988) and the economic impact of trypanosomiasis on small ruminants is substantial (Kanyari *et al.,* 1986).

In an attempt to facilitate treatment and/or control of trypanosomosis, it is imperative that early diagnosis be made to ascertain the prevalence of the disease. This can only be possible through the use of reliable and sensitive diagnostic procedures. The

epizootiology of the disease in Nigeria (Kalu and Agu, 1984; Jordan, 1986) and other parts of Africa (Griffin and Allonby, 1979) indicate increases in infection rates and losses resulting from naturally acquired infections despite decades of attempted control. The clinical manifestation of trypanosomosis in animals is influenced by the host as well as the trypanosome species and "strain". In general, the disease is characterized by severe anaemia, weight loss, reduced productivity, infertility and abortion, with death occurring in some animals during the acute phase of the disease. Animals which survive often remain infected for several months or years, exhibiting a low level of fluctuating parasitaemia which serves as a reservoir for the disease. However, the infected animals may undergo spontaneous recovery (Nantulya *et al.,* 1984, 1986). Owing to these varied clinical manifestations, diagnosis of trypanosomosis cannot be based on clinical signs alone. Laboratory confirmation of the diagnosis is an absolute necessity.

Introduction of DNA based methods such as species specific DNA probes and group/species specific polymerase chain reaction (PCR) into diagnostic tests for the detection of infection with African trypanosomes in humans, animals, as well as in tsetse fly has improved the specificity and sensitivity of parasite detection and identification (Kukla *et al*., 1987; Gibson *et al*., 1988; Moser *et al*., 1989; Masiga *et al*., 1992; Katakura *et al*., 1997; Malele *et al*., 2003; Adams *et al*., 2008; Konnai *et al*., 2008). The use of PCR for detecting trypanosome DNA is a reliable and accurate technique available for the specific identification of natural animal infections for most trypanosome species and sub-species (Welburn *et al.,* 2001; Njiokou *et al.,* 2004). Species-specific DNA targets have been identified for the most important pathogenic *Trypanosoma* species that occur in cattle

(*Trypanosoma brucei , Trypanosoma congolense and Trypanosoma vivax*) and PCR based methods for their amplification developed.

1.1 Statement of Research Problem

The current threat of African animal trypanosomosis (AAT) is ranked among the top 10 Neglected Tropical Diseases. The disease affects sustainable livestock production and mixed farming, from an economic point of view, tsetsefly-transmitted trypanosomosis is particularly important in cattle where it is mainly caused by *Trypanosoma congolense*, *T. vivax* and to a lesser extent, *T. brucei brucei* (Morlais *et al*., 1998). It is estimated that not less than 46 million cattle are at risk of becoming infected by tsetsefly-transmitted trypanosomiasis (Swallow, 2000).

Animal trypanosomosis has been known to cause not less than 3 million livestock deaths each year, 20% less in calving, 25% reduction in milk yields, 50% reduction in livestock numbers (PAAT, 2000) and reduces work efficiency of animals thus hindering crop production (Swallow, 2000). Due to the absence of surveillance, the exact prevalence situation of the disease in many part of Africa is not well known. This has led to a break down in the control strategy which has contributed to the current upsurge in both human and animal trypanosomosis in several parts of Africa (Bauer *et al*., 1999; Hendrickex *et al.*, 2000).

The failure of vector control as well as chemotherapy/chemoprophylaxis to control the present resurgence of the disease, presents a major constraint in the development of the African continent (Abenga *et al.*, 2002) and also a threat to attaining food security in several parts of subsaharan Africa and Nigeria (Samdi *et al*., 2010b). In Nigeria, the

disease currently ravages several parts of agro ecological zones of the country (Onyiah, 1997; Ogunbanwo, 1998).

1.2 Justification of the Study

It is estimated that about 48 million cattle (Kristjanson *et al*., 1999; Samdi *et al*., 2010a) are at risk of contracting African trypanosomosis from the 23 species and 33 subspecies of tsetse flies infesting 10 million km^2 of Africa stretching across 40 countries. The *Trypanosoma* species of economic importance in cattle are *T. congolense, T. vivax* and *T. brucei*. Tsetsefly transmitting the disease is responsible for 55,000 human 3 million livestock deaths annually (Abenga *et al*., 2002; Samdi *et al*., 2010a) and hinders mixed farming through reduced work efficiency of draft animals.

The loss in livestock production and mixed agriculture alone is valued at 5 billion US dollars yearly in Africa however effective and sustainable control measures can result in up to 3 fold increase in the current estimated livestock population in Nigeria (Onyiah, 1997). The decrease in national and international funding for research and surveillance of trypanosomosis has resulted in insufficient information on the current status of the disease (Maikaje *et al*., 2009).

1.3 Aim:

To determine the prevalence of trypanosomes among cattle within selected abattoirs and confined feedlots in parts of Kaduna State, Nigeria.

1.4 Objectives

The specific objectives are to:

1. determine the prevalence of the infection among cattle in both the selected abattoirs and confined feedlots (Farms) by parasitological analysis.
2. determine the PCV levels among cattle in the study areas
3. determine the risk factors associated with trypanosome infection in the study areas using a structured Questionnaire
4. confirm the rate of the infection among cattle in both the selected abattoirs and confined feedlots (Farms) by Polymerase Chain Reaction (PCR).

CHAPTER TWO

2.0 LITERATURE REVIEW

African Animal trypanosomosis (AAT) is an infectious disease of animals of similar aetiology and epidemiology. The causative agents of the disease are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies (*Glossina* sp). The distribution of trypanosomosis in Africa corresponds to the range of tsetse flies and comprises currently an area of 8 million km^2 between 14 degrees North and 20 degrees South latitude (Molyneux *et al.*, 1996). Throughout history, African trypanosomosis has severely repressed the economic and cultural development of Central Africa. African animal trypanosomosis or “nagana” disease is caused by *T. congolense*, *T. vivax* and *T. brucei*. In wild animals, these parasites cause relatively mild infections while in domestic animals they cause a severe, often fatal disease. All domestic animals can be affected by the disease and the symptoms are fever, listlessness, emaciation, hair loss, discharge from the eyes, oedema, anaemia, and paralysis. As the illness progresses the animals become weak and eventually become unfit for work, hence the name of the disease "Nagana" which is a Zulu word meaning "powerless/useless" (Winkle, 2005) and causing animal farming to be very hard to manage within the tsetsefly belt.

Human African Trypanosomosis (HAT) on the other hand or sleeping sickness is caused by two subspecies of *T. brucei*, *T. brucei gambiense* and *T. brucei rhodesiense*, while the third subspecies, *T. brucei brucei*, is only infectious to animals. *T. brucei gambiense* is responsible for the chronic form of sleeping sickness in West and Central

Africa, whereas *T. brucei rhodesiense* gives rise to the acute form of the disease in East and Southern Africa. There are two distinct stages during the course of sleeping sickness. The first or early stage of the disease, also known as the haemolymphatic phase, is defined by the restriction of the trypanosomes to the blood and lymph system (WHO, 2006). The symptoms of this stage are fever, headaches, joint pains and itching. The second or late stage of the disease, also known as the neurological phase, is characterized by the presence of the parasites in the cerebrospinal fluid (WHO, 2006). In general, this is when the typical signs of the disease occur: confusion, disturbed sleep pattern, sensory disturbances, extreme lethargy, poor condition and coma. If left untreated, sleeping sickness patients die within months when infected with *T. brucei rhodesiense* or within years when infected with *T. brucei gambiense*. Wild and domestic animals may play a major role as parasite reservoirs for human infections with trypanosomes (Njiokou *et al.,* 2006; Simo *et al.,* 2006; WHO, 2006).

Trypanosomosis in dogs otherwise known as canine trypanosomiasis comes in two main forms: American trypanosomiasis also known as Chagas disease is caused by *Trypanosoma cruzi* (*T. cruzi*), and African trypanosomiasis (surra or sleeping sickness) caused by *Trypanosoma evansi* (*T. evansi*) (Jones *et al.,* 2000). Trypanosomosis is originally an enzootic disease circulating in wild animals such as mammals and birds, which served as reservoirs. The disease became a zoonosis due to contact between rural inhabitants and natural foci, resulting from ecological imbalance (Gomes *et al.,* 2007; Rosypal *et al.,* 2007).

Maywald *et al.* (1996) diagnosed a dog as positive for *T. cruzi* with acute natural infection in the rural zone of a municipal area in Uberlândia, Brazil. Recently, trypanosomosis has become urbanized due to increased migration into cities and increases in the number of blood transfusions (Rosypal *et al.*, 2007).

2.1 Aetiology of Trypanosomes:

The morphology of African trypanosome has been described in details (Leeflang, 1975; Ugochukwu, 1983). Trypanosomes are unicellular, microscopic and elongated protozoa that move by the help of a single flagellum at the base of which is found a characteristic structure known as kinetoplast. They are obligatory parasites usually having two hosts, they multiply in the body fluids especially blood vertebrate host (Table 2.1) and live in the digestive tract of invertebrate host which is generally a biting insect (Houre, 1976; ILRAD, 1989). The pathogenic trypanosomes have been classified either as Stercoraria (posterior station trypanosomes) *Typanosoma theleria* which is mildly pathogenic to domestic and 20 wild ruminants (Table 2.1) and Salivaria (anterior station trypanosomes which is pathogenic to both domestic and wild animals as shown in Table 2.1.

Species of the genus, *Trypanosoma* are found in a wide variety of vertebrates. The majorities are not pathogenic but some species are of considerable economic importance causing disease in man and animals. These trypanosomes mainly belong to three subgenera *Trypanozoon*, *Dutonella*, and *Nanomas* (Epstein, 1975; Clarkson, 1976; Obidike *et al*., 2005).

Table 2.1: Distribution of Trypanosoma Species among Vertebrate Hosts

Species	Host	Area	Reference
T. congolense	Goats	Nigeria	Ugochukwu (1983)
T. vivax	Cattle, Sheep, Goats, Horses	Nigeria East Africa	Roderick *et al*. (2004)
T. simiae	Pigs, Monkeys	Nigeria	Killick-Kendrick and Geoffery (1963)
T. gambiense	Man	West Africa	ILRAD (1990)
T. rhodesiense	Man	East Africa	ILRAD (1990)
T. brucei	Dogs,Cats, Ruminants, Monkeys	West Africa East Africa	Mulligan and Potts (1969) Nantulya (1990)
T. suis	Pigs	Nigeria	Killick-Kendrick and Geoffery (1963)
T. evansi	Camels, Horse	West Africa	Mulligan and Potts (1969)
T. equiperdium	Horse	West Africa East Africa	Mulligan and Potts (1969); Nantulya (1990)

(Houre, 1976; ILRAD, 1989).

2.2 Transmission of Trypanosomes:

Animal trpanosomosis has a sylvastic transmission cycle. The disease is maintained in ecological system which includes tsetse flies, woody vegetation and game or wild life. It is only when livestock is introduced into this system that tsetse fly will use the livestock as their food source and infect them with trypanosome. Trypanosomes except *T. equiperdium*, and *T. evansi* are transmitted cyclically by tsetse flies. Additionally, it is believed that biting flies including Tabanidae and Stomoxys also transmit the parasite mechanically. This activity is responsible for the persistence of *T. vivax* in areas of Africa free from tsetse flies as well as in several South American countries like Brazil, Colombia, and Guyana. For instance, two fatal outbreaks of bovine trypanosomaisis due to *T. vivax* were described in Maiduguri and Wadara both in Nigeria in closed herds maintained in the tsetse free Sahelian region (Maxie *et al.*, 1979), also in Tanzania, pseudo-lumpy skin disease and acute *T. vivax* infections occurred simultaneously in a dairy herd which had no previous history of trypanosomosis. Whereas tsetse fly might have introduced *T. vivax*, the failure to trap tsetseflies and the presence of large number of biting flies *(Stomoxys* and *Tabanus taenicola*) strongly suggested that in the outbreak both aetiologial agents were transmitted mechanically. The trypanosomes that cause disease in livestock and humans also infect some wild species which serve as a reservoir of infection that may in turn infect domestic animals and people. (Connor and Mukangi, 1986).

Many wild animals carry trypanosomes with no apparent ill-effect as in humans and most domestic livestock. However where such a harmless relationship with trypanosome, and their vector has not evolved, the pathogenic effects of infection are

severe (Desowitz, 1960; Conner and Mulkangi, 1986). Wild life plays an important role as natural reservoir of trypanosome infection for domestic animals and man. It is generally believed that game animals can harbour trypanosomes and hardly suffer from the disease and the mechanism of this is unknown, although differences in the electrophoretic pattern of serum protein have been described (Desowitz, 1960). A similar tolerance is possessed by certain breeds of cattle, notably Ndama and Muturu which can be maintained in endemic areas where it is impossible to keep zebu cattle (ILRAD, 1989). Blood meal analysis of tsetse flies caught in the wild shows that they feed on a wide variety of wild life host including primates (baboons, monkeys), suids, warthog, bush pig, red river hog, giant bush pig and various bovidae especially antelopes. Other studies have shown that pathogenic trypanosomes including. *T. vivax, T. congolense* and *T. brucei* have been isolated from wild life in East Africa (Ashcroft, 1959; Anosa and Isoun, 1983).

However, various game animals such as red buck, giraffe, bush pigs, kudo and bush buck are known reservoir hosts of pathogenic Africa trypanosome and transmission is by bite of flies in the wild life. The trypanosome undergoes cyclical development in flies lasting 12 -35 days before they become infective. Though all species of tsetse fly are capable of cyclically transmitting trypanosomes, their importance as vectors depends on feeding habit, relative infectivity and distribution in relation to domestic stock. Additionally, it is believed that biting flies including tabanids and stomoxys also transmit the parasite mechanically. This is by direct transmission of the infection by blood contaminating the mouth part of biting flies which are distributed during feeding. The life cycle of the single-celled trypanosome is complex in both the tsetse fly vector and

mammalian host, trypanosomes undergo a series of transformations into different forms. As flies feed on animals infected with the parasite, they take up blood containing trypanosomes which then completes the life cycle (Houre,1976; ILRAD, 1990).

2.3 Prevalence and Epidemiology of Trypanosomes:

Large areas of Africa, approximately 4 million km^2 have been rendered unsuitable for livestock production by trypanosomes. Climate and vegetation play a major role in the distribution of Tsetse fly and other factors include presence of wild-life for food, types of soil for breeding, presence of predators and diversity of human population(ILRAD, 1990). The behaviour of tsetse flies varies from species to species in the same vegetation zone. The knowledge of tsetse flies ecology is important in the control measures most adopted in a particular zone or locality. Some workers have shown that as one moves from the north to the south, the feeding pattern of *Glossina. tachinoides* changed from preference for man to one for domestic and wild animals.

The genus, *Glossina* can be divided according to their habitat into (a) the forest species (*Fusca* group) comprising, of 12 species, (b) The riverine species (*Palpalis* group) consisting of 5 species and (c) the savanna (the *Mortisan* group) consisting of 5 species. The group name already give an indication of their distribution, the forest species are found in 500 km^2 wide coastal belt of tropical rainforest which stretches from Guinea Bissau to Nigeria in West Africa and further to Cameroon, the central African Republic, large regions of Zaire and Angola as well as Congo, Gabon and Equatorial Guinea (ILRAD, 1990).

The distribution of riverine species on one hand coincides with that of forest flies, but on the other hand it clearly goes beyond the North and South along rivers on gallery forest, and even beyond the distribution of savanna species. Savanna species appear in the savanna belt which links up with the forest species region to the North and East especially, but primarily in the South-East. The four most important species of *Glossina* in Nigeria are *G. palpalis, G. tachinoides, G. mortisans* a submortisans and *G. longipalpis*. Surveys have shown that there is a wide geoecological distribution of animal trypanosomiasis in Nigeria stretching from the mangrove forest to the Sudan savanna, owing to the presence of tsetse flies in these areas. Only about 1/5 of the northern Sahel savanna and the plateau of Mambilla, Jos and Obudu are free from testes fly (ILRAD, 1990). However, cases of Trypanosomiasis have been reported on the Jos plateau (Anene *et al.,* 1991) and in the Sahel around Maiduguri (Maxie *et al*., 1979). These unusual occurrences of animal trypanosomiasis have been attributed to movement of cattle from tsetse fly infested to tsetse fly free areas (Anene *et al*., 1999).

2.4 Parasitological Diagnosis

The easiest technique for detection of trypanosomes in peripheral blood is by direct microscopic examination of blood, either by the wet film method to detect motile trypanosomes or, as stained thick and thin smears, when parasites are identified on the basis of their morphology by light microscopy. Examination of wet blood films is quick and the method is suitable for screening large numbers of animals which is insensitive as half of the infected animals may be missed (Barnett, 1947). The sensitivity of direct microscopic examination can be improved through concentration of the parasites by centrifugation. When unclotted blood is spun in a haematocrit centrifuge, the trypanosomes

are concentrated at the buffy coat. Examination of the buffy coat is thus more sensitive than examination of blood films. Initially it is used for the detection of avian trypanosomes (Bennett, 1962), this technique has gained wide application through various modifications (Woo, 1969;Murray *et al.*, 1977; Kelly and Schillinger, 1983).

The microhaematocrit centrifugation technique is particularly useful in the condition of anaemia to be evaluated in animals, however, it requires the use of electricity which limits its application in the field. There are prospects for circumventing this shortcoming through the use of the battery-operated minicentrifuge described by Kelly and Schillinger (Kelly and Schillinger, 1983), but this has not yet received widespread appraisal. Improvement in the sensitivity of the concentration methods can be achieved if red blood cells are removed from the blood sample prior to centrifugation,the cells are removed by lysis either by hypotonic shock or through the use of a detergent (N. van Meirvenne, personal communication). Erythrocytes can also be removed from the test blood sample by diethyl amino-ethyl (DEAE) anion exchange chromatography (Lumsden *et al.*, 1979).

The miniature anion-exchange chromatography technique (MAECT) as it is known (Lumsden *et al.*, 1979) is widely used to diagnose *T. gambiense* infections in man but is yet to be established as a routine test for diagnosis of animal trypanosomosis due to its more cumbersome procedure. The technique likely to gain wider application due to its simplicity is that employing sodium dodecyl sulphate for lysis of the red cells prior to centrifugation (N. van Meirvenne, personal communication). Blood from infected animals can also be

inoculated into susceptible laboratory animals, usually mice or rats. This technique is more sensitive than direct microscopic examination of the blood sample (Paris *et al.*, 1982).

Animal inoculation has the added advantage that trypanosome isolates can be collected for other studies in the laboratory. This, however, is not a practical technique because diagnosis is not immediate. In addition, the cost of maintaining the animals makes the method prohibitively expensive for routine diagnosis, especially in the field. Furthermore, some trypanosome isolates, notably East African *T. vivax, T. simiae* and, to a lesser extent, *T. congolense,* do not infect laboratory rodents. It can thus be seen that despite several improvements in the techniques for trypanosome detection, a high proportion of infections still go undetected as the majority of infections are chronic and often aparasitaemic. Also, the intermittent parasitaemia arising from the phenomenon of antigenic variation (Doyle, 1977) may preclude detection of the parasites even in acute infections (Murray *et al.,* 1977). Moreover, some of the techniques are not practical enough to be applied in the field. These drawbacks have necessitated the development of alternative methods of diagnosis.

2.4.1 Antibody detection tests

Several techniques have been developed for immunodiagnosis of trypanosomiasis. Most of these are based on the detection of immune responses of the animal to the infection. As early as 1899, it was shown that sera from trypanosome-infected animals caused lysis of trypanosomes (Wenyon, 1926). Trypanosome lysis was later shown to be both complement- and antibody-dependent. This was the basis for the development of the complement fixation (CF) test for the diagnosis of trypanosomiasis. The CF test was

successfully applied to the diagnosis of *T. evansi* infections in several domestic animal species (Schoening, 1924) with the aim of guarding against introduction of trypanosome-infected animals into the United States. The same test was central to the design of a successful eradication campaign against *T. equiperdum* infections in horses in Canada and South Africa. The main diasdvantage of CF test is the difficulty encountered in preparation and standardization of the antigen for use in the test (Gill, 1970), furthermore, the test itself is difficult to perform; it is cumbersome and requires supplies of sheep red blood cells, complement a centrifuge and a refrigerator. Thus, it is frequently unsuitable as a routine diagnostic tool. The next generation of tools for immunodiagnosis of trypanosomiasis emanated from the observation that sera from trypanosome-infected animals were gelatinized on treatment with various chemicals, such as formalin and mercuric chloride (Hornby, 1923). This observation, first recorded with sera from syphilitic patients, was the basis for the development of the formol gel test and the mercuric chloride test for the diagnosis of animal trypanosomosis. Both tests were later adopted as a routine diagnostic tool for camel trypanosomosis (Knowles, 1924; Bennet, 1929). In experimental camel infections, Leach (1961) observed that the mercuric chloride test became positive ten to fifteen days postinfection and negative reactions occurred two to three months following treatment with a trypanocide. The basis for the reactions observed when formalin or mercuric chloride were added to serum was that these chemicals precipitate immunoglobulins (Horgan and Bennett, 1929), irrespective of the antigens responsible for the production of the immunoglobulins. Hence, the tests were not specific for any one disease (Bennet, 1929), later investigators failed to reproduce, let

alone correlate, the results of these tests with patent parasitaemia in subsequent studies (Killick-Kendrick, 1968; Luckins *et al.,* 1979).

Major improvements in the specificity and sensitivity of immunodiagnostic techniques were recorded through the introduction of the indirect haemagglutination test (Gill, 1964; Gill, 1966). The test was applied to the diagnosis of trypanosomosis in camels (Jaktar and Singh, 1971; Olaho and Wilson, 1981) and other ruminants (Schoening, 1924). The basic problems with this technique were that the antigens used were not well-defined and as a result, the test was difficult to standardize with regard to sensitivity and specificity (Clarkson *et al.,* 1971). Coupling of the antigen onto the red blood cells can be irregular; the cells may be unstable on storage and nonspecific agglutination can be encountered due to heterophile antibodies. One of the most significant improvements in trypanosomiasis serodiagnosis was the introduction of the indirect immunofluorescent antibody test (IFAT) (Williams *et al.,* 1963).

The IFAT is one of the most commonly applied serodiagnostic tests for trypanosomiasis (Wilson, 1969; Wery *et al.,* 1970). The antigens used are usually prepared by fixing smears of parasitized blood using a variety of fixatives (Zwart *et al.,* 1973), but there are two major problems associated with this method of antigen preparation. First, preparation of large numbers of blood smears to provide a standardized antigen preparation is cumbersome and the slides require ultra-low temperatures for storage and transportation. Second, the antigens so prepared provide substantial non-specific reactions. The technique for antigen preparation has recently been greatly improved (Katende *et al.,* 1987) so that the test can give results which are specific enough to differentiate, to a limited extent,

between infections with different trypanosome species in ruminants. The antigens are fixed in a mixture of acetone and formaldehyde in suspension (Katende *et al.*, 1987) and can be stored and transported with greater ease. A major disadvantage of IFAT is its requirement for sophisticated equipment and cannot be performed in the field.

The introduction of enzyme immunoassays was a major breakthrough in the field of immunodiagnosis. Enzyme immunoassays require simple equipment, the technique is straightforward and sensitive, and can be used for large-scale screening of samples. In trypanosomiasis, the test more commonly known as ELISA (enzyme-linked immunosorbent assay) was first used for diagnosis of *T. rhodesiense* infections in man (Voller *et al.*, 1975).

Luckins *et al.* (1978; 1979) later used it for diagnosis of *T. evansi* infections and obtained results comparable to those of the immunofluorescent technique with regard to sensitivity and specificity. A major limitation of ELISA in its application as a routine diagnostic test is the nature of the antigens used in the assay. The antigen is usually a crude trypanosome lysate, the quality of which is ill-defined. This makes the test difficult to standardize with regard to specificity and sensitivity. A recent report, however, has demonstrated a much improved test which under laboratory conditions has been reported to be specific enough to distinguish between infections with different trypanosome species through the use of purified antigens (Ijagbone *et al.*, 1989). With selection and purification of suitable antigens for use in the assay, ELISA is, therefore, likely to become the antibody-detection test of choice for trypanosomiasis diagnosis.

The persistent disadvantage of trypanosomiasis serodiagnosis has always been the lack of well-defined, standardized antigens. This problem, however, was solved for *T. b. gambiense* to some extent, by the introduction of the card agglutination test for trypanosomiasis (CATT) (Magus *et al.*, 1978). This test predominantly detects antibodies to the surface coat antigens of a commonly occurring trypanosome antigenic variant (VAT). The antigens are fixed and stabilized on the parasite using formaldehyde (Nantulya and Doyle, 1977) so that whole trypanosomes can be used in a direct agglutination test. The *T. b. gambiense* test has been applied to the diagnosis of camel trypanosomosis with some success (Zweygarth *et al.*, 1984), though not in the field. This is because the VAT used in the test is found in the repertoire of VAT's expressed by *T. b. brucei, T. b. gambiense* and *T. evansi* (Van Meirvenne *et al.*, 1977). Applied to the diagnosis of *T. evansi* in water buffaloes, however, the test was found to give good results only after the inclusion of a specific anti-buffalo immunoglobulin (Bajyana-Songa *et al*., 1987). Further improvement in the sensitivity of the test with regard to the diagnosis of *T. evansi* infections has recently been introduced by Bajyana-Songa and Hamers (1988)) through substitution of the VAT used in the present test with a predominant VAT of *T. evansi.* The test is at present the simplest serological test for diagnosis of *T. evansi* infections. Its application to the diagnosis of the other species of pathogenic trypanosomes in livestock, however, is unlikely to succeed due to difficulties in stabilizing their surface antigens by fixation. All the serological tests described above measure host antibody responses to antigens of the infecting trypanosomes, specifically (i.e. using trypanosome antigens) or non-specifically (i.e. measurement of immunoglobulin rise). Antibody detection systems can provide only a presumptive diagnosis as they do not differentiate between current and

past infections. Therefore, a positive antibody detection test does not necessarily form a good basis for the decision to treat the animal. This is because anti-trypanosomal antibody levels may persist for several months following successful trypanocidal therapy (Luckins *et al.*, 1978, 1979) or spontaneous self-cure (Nantulya *et al.*, 1984, 1986). They are thus more useful as epidemiological tools rather than as diagnostic procedures. For this reason, attention has continued to be focused on the development of tests that can differentiate between exposure and a current infection.

2.4.2 Antigen detection tests

An alternative approach to antibody detection would involve the use of assays to detect trypanosome-specific antigen in the blood of infected animals as a means of diagnosis. Demonstration of trypanosomal antigens in the blood of the infected animal would be synonymous with parasitological diagnosis (Voller and De Savigny, 1981) and hence an indicator of current infection. The first attempt to detect circulating trypanosome antigens was made in Chagas' disease (Araujo, 1982) but the sensitivity obtained was low. Later, Rae and Luckins (1984) developed a *T. evansi* antigen detection system, using polyclonal anti-T. *evansi* antibodies. This polyclonal antibody system has, however, been found to have low specificity because cross-reactions occur with non-targeted trypanosome species and possibly with other parasitic diseases. In this regard, the development of trypanosome species-specific monoclonal antibodies first reported by Nantulya and co-workers (Nantulya, 1981; Nantulya *et al.*, 1984, 1986, 1987), and later, by Richardson *et al.* (1986), has been a major breakthrough in the diagnosis of African trypanosomosis. The antibodies derived by Nantulya and colleagues were extensively cross-tested against

several common haemoparasites of animals (Nantulya *et al.,* 1987, 1989) or man, and cross-reactivity was not observed. Using the *T. brucei* group-specific monoclonal antibody, a sandwich ELISA was developed for diagnosis of *T. brucei* infections in cattle and *T. rhodesiense* and *T. gambiense* infections in man (Nantulya *et al.,* 1985, 1989). Applying the same sandwich ELISA to the diagnosis of *T. evansi* infections in various animal species, circulating antigens were detected as early as six days following experimental infection and the "antigenaemia" persisted as long as the animals remained infected (Nantulya *et al.,* 1989). The antigenaemia correlated well with parasitological findings and it was applied to the diagnosis of *T. evansi* infections in camels from an endemic area (Nantulya *et al.,* 1989), the test was able to detect circulating antigen in 92% of the animals with parasitologically confirmed diagnosis. Perhaps more importantly, the test also detected antigens in 55% of the animals in the same herd which had been missed by parasite detection techniques. Several camels from a trypanosomosis-free area were all negative for antigens, indicating the high specificity of the assay. A tube-ELISA, as opposed to a microtitre plate-ELISA, has since been developed. This test gave similar results (Nantulya *et al.,* 1989), thus providing, for the first time, a potentially suitable test for immunodiagnosis of individual animals in the field. The principle of the assay is that the monoclonal antibody is used to coat a micro-ELISA plate or polystyrene tubes and excess coating antibody is discarded, test serum is then added to the coated plates and the antigen in serum is captured by the coating antibody. Since the antigen trapped has several combining sites, a second antibody which is enzyme-labelled is introduced. The labelled antibody will bind to the free combining sites on the captured antigen. Any excess free labelled antibody is washed off and the reaction is then revealed by the addition of

substrate and chromogen. From the protocol it can be seen that the same reagents can be used for diagnosis of the disease in several animal host species, since host species-specific antiimmunoglobulin reagents are not required. The species-specific monoclonal antibodies against *T. congolense* and *T. vivax* have also been used to develop antigen-capture sandwich ELISA for the diagnosis of these infections in cattle (Nantulya *et al*., 1986; Nantulya and Lindqvist, 1989). It was applied to the diagnosis of trypanosomosis in cattle in an endemic area, the antigen-trapping ELISA gave positive results in 96% of the animals with parasitologically confirmed diagnosis (Nantulya *et al.,* 1989). Sera from control cattle from a trypanosomosis-free area were all negative for antigen. Interestingly, a high proportion of animals had mixed infections and a significant number (52.6%) of the animals from the same herd which had been missed by parasite detection techniques, were found to be infected as shown by the tests for antigen (Nantulya *et al.,* 1989). Thus, these tests may miss a small fraction of the early infections as it is compensated for by its ability to detect those cases which would be missed by parasitological diagnosis (Barnett, 1947). These antigen-trapping assays are easy to perform; the results can be read visually; large numbers of serum specimens can be analyzed at a time; they can be applied to the diagnosis of individual animals; and, they are more sensitive than the techniques for parasitological diagnosis. Thus, these assays seem to provide an answer to some of the important questions regarding diagnosis of animal trypanosomosis. The assays for diagnosis of bovine and camel trypanosomosis are currently under field evaluation in several African countries, as part of a collaborative project involving the International Atomic Energy Agency of the Food and Agriculture Organization of the United Nations

and ILRAD, funded by the Government of the Netherlands. Although these tests are still under evaluation, certain conclusions can be drawn at this stage.

First, there are a small proportion of animals in which antigens cannot be detected despite the patent parasitaemia. These results have also been observed under experimental conditions where very early in infections occur (Nantulya and Lindqvist, 1989; Nantulya *et al*., 1986); high parasitaemia may not be associated with the presence of antigen in serum. Since the basis for this test is that trypanosomes have to be destroyed to release the antigens in circulation, it is conceivable that the test will not be positive until after a critical number of trypanosomes have been destroyed by the immune response of the host. Second, there are a high proportion of animals without parasitaemia which test positive for antigens. Considering that the control animals from trypanosomosis-free areas do not test positive for antigen, the cases detected by this test may represent true infections that cannot be diagnosed by parasitological techniques. The most effective diagnostic strategy, therefore, will be to combine antigen-trapping ELISA with one of the more sensitive standard trypanosome detection methods.

2.5 Molecular Technique (Polymerase Chain Reaction).

Routine diagnosis of trypanosomiasis using classical parasitological approaches shows poor sensitivity under field conditions (Wastling and Welburn, 2011). This is in part, due to the normally very low peripheral parasitemia of naturally infected animals (Masake *et al.,* 2002; Picozzi *et al.,* 2002). In recent years, PCR has been widely applied for the detection of trypanosomes and has been shown to be highly sensitive and specific in the laboratory (Gibson, 2009). Species-specific primers show good sensitivity and

specificity against their target species. However, to screen large numbers of samples such as are derived from naturally infected hosts to determine prevalence data, multiple PCRs need to be undertaken for each sample which is both time consuming and expensive. It would be desirable to be able to apply a single PCR reaction that could simultaneously differentiate all of the trypanosome species present within a sample, making screening of a large number of samples viable in terms of time and cost.

2.6 Treatment of Trypanosomosis

The satisfactory treatment of trypanosomosis requires more than a correctly administered trypanocidal injection, and the rate of an animal's recovery is largely determined by the plane of nutrition and amount of exercise during convalescence. Well-rested and well-fed animals recover more rapidly after trypanocidal therapy than undernourished animals which have to trek long distances to pasture and water. However, chronic trypanosomosis often fails to respond to therapy; the ferrokinetic disturbances and accompanying dyshaemopoeisis appear to be irreversible, and affected animals may remain thin and anaemic despite trypanocidal treatment.

It is common practice for many owners to treat their animals with trypanocides on a presumptive diagnosis of trypanosomosis based solely on recognition of clinical signs. In such cases sub-curative treatment is often given, the effect of the trypanocide being merely sufficient to restore the host-parasite equilibrium and effective pre-immunity. Clinical recovery often follows such treatment but the exposure of trypanosomes to low concentrations of trypanocide increases the risk of drug resistance developing. Nevertheless, this enables livestock to be maintained in trypanosomosis-enzootic areas at

minimal cost, although it is not easy to defend the practice scientifically. The therapeutic approach to the control of animal trypanosomosis is mainly practised when the incidence of infection is low. Diagnosis may be confirmed before treatment in some cases although it is more usual to take specimens at the time of treatment for subsequent examination. Diaminazene and homidium are the commonly used drugs. An accurate estimation of live weight is important for curative treatment, however, the pack sizes in which these compounds are presented can encourage under-dosing. There is a temptation not to use a second sachet or tablet to treat an animal requiring only slightly more than the dose contained in one sachet or tablet. The treatment of individual animals to control trypanosomosis can be very successful but requires a high level of surveillance for which diagnostic equipment and materials are needed. Under low tsetsefly challenge, the cost of diagnosis and treatment may be considerably less than prophylactic treatment of the whole herd (Wissocq *et al.,* 1983; Dowler *et al.,* 1989). A significant advantage of diagnosis and treatment is that it promotes drug-assisted acquired immunity, which is manifested as the reduced frequency of infection in older cattle (Boyt *et al.,* 1963; Wilson *et al.,* 1976; Logan *et al.,* 1984; Trail *et al.,* 1985), while the acquisition of immunity facilitates maintenance of livestock in tsetse-infested areas, it can interfere with surveillance of trypanosome transmission. Challenge would be underestimated since infections would not become established in animals resistant to certain serodemes. To overcome this problem sentinel animals have to be periodically replaced with trypanosome-naive stock. There is considerable variation between individual animals in their susceptibility to trypanosomal infection and some individuals are parasitaemic more frequently than others. Since an animal's ability to control parasitaemia and anaemia has a major effect on weight gain,

monitoring infection rates and haematocrit of individuals in a herd is recognized as a useful tool for the selection of trypanotolerant breeding stock (Trail *et al.,* in press). Judicious use of trypanocides is indicated in these circumstances.

2.7 Prophylaxis

The diagnosis and treatment of trypanosomosis is not a practical way to control the disease when the challenge is high. The incidence of infection under these conditions necessitates such frequent treatment of so many animals that a prophylactic approach to control is needed and all animals at risk should be treated to control trypanosome transmission in an area. To maintain protection repeated treatments are needed the interval between which is mainly determined by the dosage rate used and the level of challenge. Of the trypanocides now available isometamidium is the only true prophylactic. There are many reports confirming the efficacy of isometamidium in maintaining livestock (mainly cattle) in tsetse-infested areas, and the advantages of herd protection with isometamidium over a therapeutic approach have been well demonstrated (Wilson *et al.,* 1975; Blaser *et al.,* 1979; Logan *et al.,* 1984; Takken *et al.,* 1988). Direct comparisons between trials cannot be made: not only did experimental designs differ but different breeds of animals were challenged by different species of tsetseflies in different parts of Africa. Irrespective of these differences the common decision had to be made as to when to retreat animals to provide continued protection.

2.7.1 Duration of prophylaxis

This is a vexing issue, to which there is no easy answer. The duration of protection afforded by isometamidium has been shown experimentally to be as long as five months (Whitelaw *et al.,* 1986). However, numerous factors modify the duration of protection (Ogunyemi and Ilemobade, 1989). Experimentally, the dose of isometamidium given has been shown to have a significant effect; higher dosage rates confer longer protection. To obtain prolonged protection the dosage rate of isometamidium cannot be increased greatly because of its toxic effects. Normally this drug is used at between 0.5mgkg^{-1} and 1.0 mgkg^{-1}; while a dosage rate of 2.0 mgkg^{-1} is not tolerated by all cattle. Numerous reports from the field indicate that the period of protection is reduced by high tsetsefly challenge. Experimental evidence does not confirm this common observation, but it has been shown that the sensitivity of the parasites to isometamidium affects prophylaxis. The manner in which high trypanosomal challenge reduces prophylaxis is obscure, but may be related to the presence of a large number of different serodemes with a range of sensitivities to the trypanocide. The physiological state of animals also influences the duration of prophylaxis. Animals stressed by poor nutrition, intercurrent disease, lactation or physical exertion receive shorter protection than non-stressed animals. This occurrence may reflect altered pharmacokinetics in physiologically disturbed animals, a condition not found in the animals used for the experiments of Whitelaw *et al.* (1986), or those of Peregrine *et al.* (1988). Under experimental conditions it has been shown that cattle protected by isometamidium do not develop anti-trypanosomal antibodies (Whitelaw *et al.,* 1986; Peregrine *et al.,* 1988) unless under heavy metacyclic challenge. In the field, the host's

immune response may well contribute to apparent prophylaxis, especially if infection becomes established when residual levels of isometamidium confer partial protection.

2.7.2 Interval between prophylactic treatments

This aspect of prophylaxis is obviously directly related to the duration of prophylaxis, in practice it is not easy to determine the precise timing of repeated herd treatments. The need to maintain protective levels of isometamidium at all times to prevent the establishment of trypanosomal infections, has to be balanced against both the practicality and economics of doing so. Each field situation may require a different approach, and in the several studies of maintaining animals by chemoprophylaxis, the criteria determining retreatment varied. One of the most unequivocal demonstrations of the maintenance of cattle by chemoprophylaxis under tsetsefly-challenge was provided by the analysis of the data collected from Mkwaja ranch in Tanzania (Trail *et al.,* 1985). Cattle received an average of 4.4 treatments with isometamidium and 0.6 treatments with diminazene per year, and were impressively productive. The decision on when to repeat treatment was based, not only on parasitological parameters, but incorporated an element of clinical judgment. The criteria for deciding when to repeat isometamidium treatments have to be simplified for most situations. Repeat group treatments may be given when the first animal in a group of 20 regularly monitored cattle becomes parasitaemic. This criterion was used by Wilson *et al.* (1975). However, they concluded that it would have been more economical to delay retreatment until three of the 20 animals were found positive. In other cases repeat treatments were made at arbitrary three monthly intervals. In the absence of regular parasitological or entomological monitoring to determine the

probable risk or challenge animals face in tsetsefly-infested areas, the arbitrary inter-treatment interval has been widely used. There is a need to relate prophylaxis more closely to challenge in order to achieve high drug levels at a time when challenge is high.

2.7.3 Strategic chemoprophylaxis

Local epidemiology determines the design of strategic chemoprophylaxis. For example, cattle entering a tsetsefly-infested dry season grazing reserve would benefit from prophylactic treatment before exposure to challenge. Similarly, animals in sedentary herds should receive protection immediately before the anticipated onset of high risk. Attempts have been made to calculate challenge in terms of a Berenil index (Rogers, 1985). For practical purposes it is important to know when to expect an increase in risk so that preventive measures such as chemoprophylaxis can be implemented. The incidence of infection often increases soon after the onset of the rainy season in areas with unimodal rainfall and the efficacy of strategic prophylaxis before the onset of high risk has been demonstrated (Njogu *et al.,* 1985; Connor *et al.,* 1989). A similar approach in which prophylaxis was provided to village cattle over the high risk period, as three treatments with isometamidium at intervals of three months, produced good control of trypanosomiasis and led to improved productivity (Maloo *et al.,* 1988). A sanative diminazene treatment was included in this regime during the period of lowest trypanosomiasis risk.

2.7.4 Tactical chemoprophylaxis

Chemoprophylactic treatment of a whole herd has been found to enhance all production parameters. However, on a cost recovery basis, few owners will be prepared to

adopt this approach to the control of trypanosomiasis, even strategically. There is a need to evaluate the efficacy and acceptability of a tactical approach to the use of trypanocides in selected and economically important categories of animals. The overall objective should be to achieve, on a limited basis, maximum benefit from restricted trypanocide usage. An obvious category for such an approach is draught animals. Under conditions of light to medium challenge, the protection of all work oxen in the area, just before the onset of the ploughing season, could be expected to achieve a better output from the "work force". Oxen represent a considerable investment for all farmers, and the threefold improvement in crop yields as a result of using oxen sharpens the farmer's perception of their importance to his household's economy. A sadative diminazene treatment throughout the area would be a wise, if not mandatory, requirement to guard against the rapid emergence of resistant trypanosomes. Another important category is the breeding cow. Improved calving rates may be achieved by prophylaxis before the breeding season. A study at OGAPROV, Gabon has shown that trypanosomiasis impaired the fertility of lactation-stressed N'Dama cows, particularly those failing to maintain control of anaemia (Rowlands *et al.*, 1990). Trypanocidal treatment with diminazene, followed two weeks later by isometamidium, prior to the breeding season, improved the average calving rate of cows lactating at the time of breeding. It must be noted that this improvement, confined to lactating cows, may well have been associated with the level of stress to which they were exposed in the particular environment of the station at the time.

2.8 Failure of Trypanocides

The use of trypanocides does not always produce the expected cure or protection and there is then a tendency to assume that drug resistance has arisen. Whilst this may be true, there are many other reasons which contribute to their failure (Table 2.2). Only after carefully investigating the practical points of drug administration and eliminating them as causes of failure, is it valid to investigate the likelihood of there being true drug resistance. Before trypanocides can be said to have failed, there must be parasitological evidence that trypanocidal treatment either has not removed an established infection or has not provided protection against infection or reinfection. Thus, parasitological monitoring is a prerequisite to drug usage. From an analysis of properly kept records of routine monitoring it is possible to determine whether administration of a therapeutic dose of a trypanocide cures an infected animal. There may be complete cure, temporary aparasitaemia followed by a relapse of parasitaemia or no abatement in parasitaemia. A report from Ethiopia (Rowlands *et al.*, 1990) has demonstrated the value of a field appraisal to determine the efficacy of trypanocidal drugs in an area where trypanocide failure occurred.

2.9 True Drug Resistance

Resistance to trypanocides is a well known phenomenon (Williamson, 1979; Leach and Roberts, 1981). The introduction of every new trypanocide has been followed by the emergence of resistance to it, and in the cases of dimidium, quinapyramine and prothidium, has led to their withdrawal from the market. Resistance seems to develop in a stepwise manner; trypanosomes resistant to a low dose of a trypanocide can be removed by a higher dose of the same compound. The problem is that, because of the narrow therapeutic indices

of the trypanocides, there is only limited scope to overcome resistance by increasing the dosage. An effective trypanocidal dose may also kill the animal. The mechanisms of resistance are unclear and probably involve gene amplification and spontaneous shifts in sensitivity. Resistance may appear in an area but may also spontaneously disappear (Whiteside, 1960), which may be linked to unstable genetic components or poor tsetse transmissibility of some resistant strains. On the other hand, transmission of drug resistant strains of trypanosomes in sylvatic cycles is known to occur. Reports of resistance to the four remaining trypanocides are increasingly common, although few reports have been substantiated. The difficulty in confirming resistance is a result of the numerous factors which may lead to the failure of trypanocides (Table 2.2), and of the difficulty in obtaining reliable field data. Sometimes the intervals between repeat treatments can be so short that signs of trypanocide overdosage occur before an assessment of drug resistance is made.

Table 2.2: Causes of apparent drug resistance in trypanosomes: failure of trypanocides

1. Underdosage	underestimated bodyweight
	overdiluted solution of trypanocide
	Incorrectly calculated dose volume
	deliberate underdosage to enable treatment of more animals
	incorrect injection technique
	- short needles
	- non-sterility/abscessation
	- early withdrawal of needle
	- elastic recoll of tissue produces excessive "leak-back"
2. Incorrect strategy	irregular treatment
	prolonged intervals between treatments (challenge when drug levels have waned)
	lack of knowledge of seasonality
3.Stress-induced metabolic changes	Malnutrition
	Lactation
	intercurrent disease
	breed-associated stress
	work and trekking
4. Increase in challenge	Seasonal
	changed grazing area
	absolute increase in tsetsefly numbers
5. Relapsing infections	
6. True drug resistance	

(Whiteside, 1960).

2.10 Assessment of Resistance to Trypanocides

An ideal method to assess drug resistance should be accurate, should give reproducible results, and should be simple, rapid and inexpensive. None of the available methods meets these criteria. All methods require the isolation of suspect trypanosomes and subsequent testing. The curative effect of a trypanocide is exerted on bloodstream trypanosomes in the presence of the host's immune apparatus. Prophylaxis is achieved by the action of a trypanocide against metacyclic trypanosomes at the level of the skin (Peregrine *et al.*, 1988). These conditions can only be reproduced *in vivo,* and for the determination of prophylaxis animals should be challenged by the bite of infective tsetseflies. Precise simulation of field conditions is never possible. A single animal may be infected with more than one trypanosome species or serodeme, and animals frequently have some acquired immunity.

2.10.1 *In vitro* assay of resistance

The potential advantages of *in vitro* techniques to assess drug resistance of trypanosomes relate to savings in time and money compared with *in vivo* tests. There are, however, significant obstacles still to be overcome before *in vitro* assays can replace *in vivo* tests (Kaminsky, 1990). The first difficulty is to isolate trypanosomes representative of the population of parasites in infected animals. Mixed infections commonly occur and parasitaemias are often low. The sub-inoculation of other livestock or laboratory animals with blood from an infected animal does not always result in the establishment of an infection. Similarly, it is not always possible to culture trypanosomes from blood. There is therefore inevitably selection for those trypanosomes which adapt more readily to the

culture system, with the faster growing trypanosomes being more abundant. No universally standardized culture system is in use and some systems utilize a feeder layer of, for example, bovine aorta endothelial cells, others have no feeder layer but incorporate serum or serum components. The variation between batches of sera can considerably affect the growth of trypanosomes in otherwise identical culture conditions. Furthermore, trypanosomes may be cultured as epimastigotes or bloodstream trypanosomes, depending on culture conditions; promastigotes and metacyclics may also be cultured and by varying the conditions, transformation through all stages of the trypanosome life cycle can be induced (Brun and Jenni, 1987; Gray *et al.,* 1987). There are indications that the expression of resistance by trypanosomes is not an entirely stable characteristic (Peregrine *et al.,* 1988). It is possible that prolonged culture may be associated with alterations in the level of sensitivity expressed to a particular trypanocide.

2.10.2 *In vitro* techniques

The medium requires changing at regular intervals of approximately 48 hours. The exposure of trypanosomes to the test compound should be for a time short enough to produce a measurable effect, yet not long enough to select for the more resistant organisms. Solutions of trypanocides are added to culture medium to give final concentrations in the range of 0.01 $ngml^{-1}$ -100 $ngml^{-1}$. Exposure should be in the absence of a feeder layer, these cells may take up the trypanocide. Additionally, an effect of the trypanocide on the cells of the feeder layer may indirectly affect the trypanosomes. After trypanosomes have been incubated with the trypanocide, the assay is completed by measurement of an end-point, of which several have been used. The results of these tests

will indicate that, compared with laboratory reference stocks, the test trypanosomes are more, or less, sensitive to a particular concentration of a trypanocide. To interpret these results in the light of clinical findings is not straightforward. It is important to be able to state that a trypanocidal effect exerted *in vitro* at a concentration of, say 1.5 $ngml^{-1}$ is equivalent to a curative dose of, say, 3.5 $mgkg^{-1}$ bodyweight. Too few data are currently available for this to be done. The *in vitro* technique will be much improved when bloodstream trypanosomes can be adapted rapidly to culture, and exposed to trypanocides (and possibly their metabolites) for a minimum time to give an easily measurable end-point. The result obtained may then be correlated with results of curative treatment. In the case of prophylaxis, metacyclics should be used for the *in vitro* assay and it is unlikely that a single assay system will be suitable for all stages of all species of trypanosomes. Studies are therefore needed to compare results from the different systems. The development of *in vitro* assays to determine drug resistance of trypanosomes is still at an early stage. In view of the potential of *in vitro* techniques for screening for new trypanocidal compounds, marked improvements in culturing trypanosomes are likely in the near future.

2.11 Assessment of Drug Resistance in the Field

Initially, the sensitivity of trypanosomes to a drug is appraised clinically: the response of trypanosomal infections to treatment, or the prevention of infection is determined. The time taken for infection to relapse, or the duration of protection, is taken as broad indicators of the degree of resistance. Without placing animals in fly-proof accommodation there is the risk of continued challenge, which raises the problem of distinguishing relapse from re-infection. Isolation of trypanosomes for further testing

inevitably requires some degree of selection, and the results obtained have then to be related to the field situation. The assessment of drug resistance seeks to determine the level of trypanocide lethal to trypanosomes, which should be related to a plasma concentration in a treated animal. The closest approximation to these conditions might be achieved by simultaneously demonstrating trypanosomes in an animal and measuring the concentration of the trypanocide, or its metabolites, in the plasma of that animal. A rapid result would then be obtained at low cost which would be easily interpreted, once normal ranges were established. Measurement of trypanocides is not easy because they are active at extremely low levels. High performance liquid chromatography (HPLC) detects isometamidium at levels of 2 ng per gram of tissue (Kratzer, Turkson, Karanja and Ondiek, in press) whilst a competition micro-ELISA to detect plasma levels of isometamidium has a limit of detection of 10 $pgml^{-1}$ (Whitelaw *et al.*, 1989). Refinements to sample preparation and pre-analysis extraction improve the level of detection of this ELISA to the femtogram ($10^{-15}g$) range. The further development of drug detection ELISA's could offer a simple tool for direct assays of drug resistance. Improvements to the *in vitro* techniques would not render them as suitable for field use, but would enable baseline data to be collected on the ranges of sensitivity of characterized trypanosomes to drugs. In assessing drug resistance there are strong arguments for working at field level.

2.12 Epidemiology of Resistance to Trypanocides

Little is known of the prevalence and incidence of acquired resistance to trypanocides. The appearance of resistance to all trypanocides has been documented, and in view of their severely restricted range, a better understanding of the epidemiology of

resistance is needed. The natural resistance of *T.simiae* to most trypanocides presents special problems, but it is acquired resistance to trypanocides by other species of trypanosomes which has received greatest attention. The expression of resistance is not entirely stable but the underlying mechanisms are not yet understood. Genetic recombination in the tsetsefly may contribute to the emergence of resistance, although the dilution of resistant trypanosomes by other populations of trypanosomes in transmission cycles may influence the extent of the problem. Methods to detect and quantify resistance quickly and economically are needed to enable measures to be taken to control the growing menace.

2.13 Control of Drug Resistance

At present, diminazene and isometamidium are used widely as a sanative pair to slow down the development of drug resistance. Nevertheless, there are reports of confirmed multiple resistance in *T. congolense* and *T. vivax* to both of these compounds. The efficacy of the sanative pair is also reduced once the resistant trypanosomes enter the tsetsefly population and become cyclically transmitted. Treatment of, say, an isometamidium-resistant infection with diminazene would soon be followed by repeated challenge with resistant trypanosomes. The removal of livestock from an area in which resistance is a clinical problem has been used to reduce transmission whilst the resistant trypanosomes die out. However, this does not always happen, and even after two years, the reintroduction of livestock may be followed by the re-emergence of resistance (Gray and Roberts, 1971). Destocking policies are only likely to be used today as a desperate last resort, and other methods are needed. It is most important to reduce transmission of

resistant trypanosomes and, therefore, in the event of resistance emerging as an area problem, vector control is essential. The recent failure of isometamidium on Mkwaja ranch in Tanzania - evidenced by decreasing prophylaxis has been overcome, as a clinical problem, by vector control. Trypanocide use on the ranch has decreased and productivity has improved following a reduction in tsetsefly challenge. Tsetsefly control has been achieved by deltamethrin treatment of cattle and limited deployment of odour-baited, insecticide-treated targets, but there is constant reinvasion pressure. Consequently, the sustainability of vector control in this situation is in doubt. In other circumstances, a rapid reduction in tsetse numbers may be sufficient to reduce the problem of resistance to manageable levels. An important measure to limit the spread of resistant trypanosomes is to restrict livestock movement. The arrival of an animal infected with resistant trypanosomes in a tsetse-infested area may represent a serious risk to other livestock already there and is potentially an expensive problem for livestock owners. Selection of trypanosomes is also inevitable. In a mixed infection one species or serodeme may become more readily established in the new host, especially if a different host species is used; for example, *T.vivax* and *T.simiae* are not infective to mice. Furthermore, not all serodemes are transmitted with the same ease by tsetsefly. The disadvantage of using cattle to assess the sensitivity of trypanosomes to trypanocides is largely related to cost. The purchase and maintenance of a large number of cattle for observation periods of 100 days or longer in fly-proof housing is beyond the resources of most laboratories. Consequently, other laboratory animals are used, and assessment of drug resistance of trypanosomes in mice is widely used. However, the results of tests with drugs administered by the intraperitoneal route to cure syringe-passaged trypanosomal infections in mice, cannot be extrapolated

directly to the situation in cattle. Another major difference is that of pharmacokinetics. A dosage rate of 0.5mgkg^{-1} of isometamidium in a bovine is not the same as 0.5 mgkg^{-1} in a mouse, which has a much higher metabolic rate. At best, the results obtained from tests in mice can only broadly indicate the sensitivity of a strain or stock of trypanosomes to a drug. Despite the advantage of the lower cost of assessing sensitivity to trypanocides in mice, an observation period of between 30 and 60 days is needed. As a result of these factors, more suitable methods are needed to assess drug resistance and are being sought. The *in vitro* assay has obvious potential in this respect.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Kaduna state is located at the center of northern Nigeria with the coordinates 10°31′N 7°26′E 10.517°N 7.433°E. The state shares boundaries with Niger state to the west, Zamfara, Katsina and Kano state to the North, Bauchi and Plateau state to the east and FCT Abuja and Nassarawa states to the south (Kaduna state, 2010). The study was conducted within three selected Abattoirs and three confined Feedlots in parts of Kaduna State, Nigeria.

3.2 Study Population

A total of 450 cattle; 300 from the Abattoirs and 150 from the Feedlots were sampled and examined. Five mililitres (5mls) of blood was collected at the sites from the cattle into Ethylene tetra-acetic acid (EDTA) bottle. Each sample was immediately transported to the laboratory for analysis.

3.3 Study Design

This study was cross-sectional. It was aimed to provide data on the entire cattle population under study and make comparison at a single point in time. The study was used to describe the relative risks from the prevalence and also to compare the different variables at the same time.

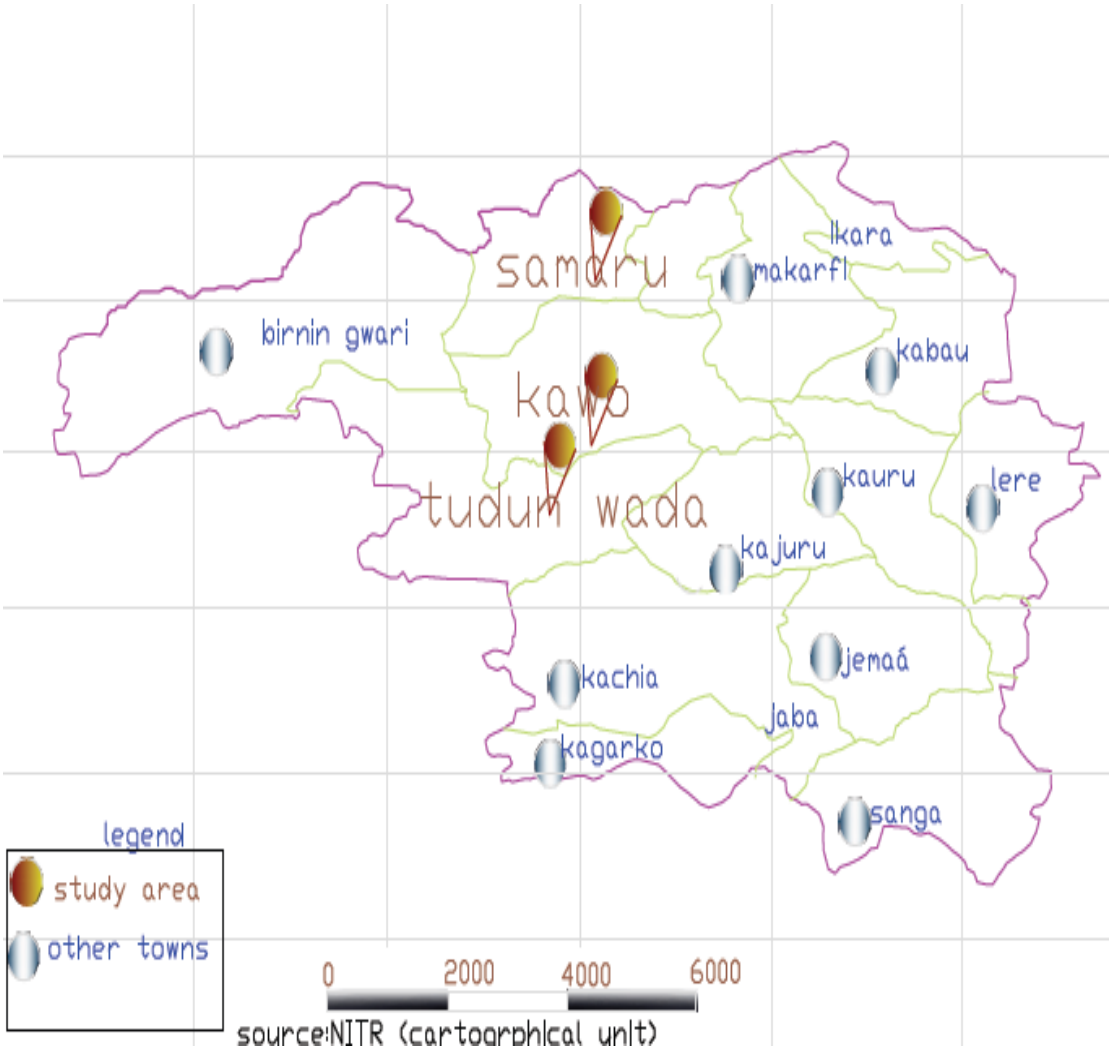


Figure 3.1: Map of Kaduna State showing study areas and other satelite towns

3.4 Sample Methodology

3.4.1 Sample Size

The sample size was determined using the formula described by Naing *et al.* (2006)

$$n = \frac{Z^2 x (p) x (1-p)}{d^2}$$

n = Sample size

Z = Standard normal distribution (1.96 for 95% confidence level)

p = Percentage expressed as decimal.

d= Precision, confidence interval expressed as decimal.

$$n = \frac{(1.96)^2 * 0.5 * (1- 0.5)}{0.05* 0.05}$$

n = 384

For the purpose of this study, a total of 450 samples were collected. One hundred (100) samples were collected from each of the three (3) selected abattoirs, while fifty (50) samples were collected from each of the three (3) selected confined feedlots (farms), making the total of four hundred and fifty samples collected using non-probability sampling procedure (convenience).

3.5 Collection of Samples

3.5.1 Blood samples**:**

A total of 450 blood samples were collected from cattle; Three hundred (300) from the three abattoirs (100 from each) and 150 from the confined feedlots (50 from each) were collected and examined. Five milliliters (5ml) of blood was collected into Ethylene tetra-

acetic acid (EDTA) bottle. Each sample was kept cool by placing in a box containing ice pack and transported to the laboratory for examinations.

3.6 Parasitological Diagnosis

The 5ml of the sample collected was subjected to diagnostic techniques of the Standard Trypanosome detection methods (STDM) (Wilson, 1969). These were Wet Film (WF), Thin Film (TF), Thick Film (THF), Animal inoculation (AI) and Concentration Techniques (Swallow, 2000) then, Haematocrit Centrifugation Techniques (HCT) (Woo, 1971) and Buffy Coat Method (BCM) (Kalu *et al.*, 1991) were employed

3.6.1 Wet mount technique

Two (2) microlitre of blood was dropped on a clean microscope slide and covered with a coverslip (22 x 22 mm). Blood samples were examined using the x 40 magnification with condenser aperture. Parasites were seen directly between blood cells as they cause blood films to move.

3.6.2 Thin film

Smears of the blood samples were made by placing a drop of blood from a microhaematocrit capillary tube on a clean microscope slide, using the edge of another clean slide at 20milimeters from one end the smear was spread at 30 degrees to the first slide and drawn back to make contact with the blood droplet. The blood was allowed to run along the edge of the spreader and pushed to the other end of the slide in a fairly rapid but smooth motion. The slide was air dried, fixed with methanol for 2min and stained with Giemsa reagent and viewed using the x100 oil immersion objective lens.

3.6.3 Thick film

The blood sample was placed on a clean grease free microscopic slide and spread over an area of 2 cm in diameter using a spreader. The film was air dried and dehaemoglobinized by immersing in distilled water for two seconds and dried before staining. The film was stained for 30 mins with 4% diluted Giemsa stain in phosphate buffered saline and examined using the x 100 magnification for the presence of trypanosomes.

3.6.4 Haematocrit centrifugation technique (HCT)

The microheamatocrit capillary tube was filled to ¾ mark with uncoagulated blood and sealed using plasticine and placed in a centrifuge with the sealed ends pointing outward. The tubes were loaded symmetrically to ensure balance. The rotary cover was screwed and the centrifuge lid was closed. The capillary tubes were centrifuged for 5minutes at 12000 rpm to obtain a buffy coat. The plasma / white blood cell (Buffy Coat) interface was examined by slowly rotating the capillary tube and placed on a tube carrier. Trypanosomes movement was detected using the x40 objective lens. This method was used to determine parasitaemia.

3.6.5 Buffy coat

The fresh blood was collected into a heparinized capillary tube and centrifuged at 12000rpm for five minutes. The capillary tube was cut around 1mm below the buffy coat to include the top layer of the Red Blood Cells. The Buffy coat and the top layer of the RBC were extruded unto a clean microscopy slide and covered with a slip. The slide was examined using a phase contrast microscope (Dark ground) using X40 magnification for

the presence of motile trypanosomes. Trypanosomes were identified based on their morphology and mobility under the microscope field.

3.7 Differential Morphology

Any organism with a free flagellum, very well developed undulating membrane and a small sub-terminal kinetoplast was classified as *T. brucei*, while an organism with absence of free flagellum, inconspicuous undulating membrane, and kinetoplast marginal and subterminal was classified as *T. congolense* Also for *T. vivax* are those with a medium sized marginal kinetoplast but without a free flagellum and inconspicuous undulating membrane (Onyiah, 1997).

3.7.1 Packed cell volume (PCV)

The Pack Cell Volume was determined for all blood samples as a haematological index. This was carried out using the Haematocrit Centrifugation Technique and after spinning for 5 minutes, the length of the columns of the fluid (Plasma) plus red blood cells, was taken as direct measurements of the relative amount of the solid and fluid portions of the sample since the diameter of the bore is constant. The column of packed red cells is stated as a percentage of the whole and it expresses the proportion of red cells.(Cheesbrough, 2004). It is used as a quantitative expression of anaemia, a most useful index in assessing the progress of trypanosomosis.

3.8 Detection of the Trypanosome Infection among Cattle in both the Selected Abattoirs and Confined Feedlots (Farms) by Polymerase Chain Reaction (PCR)

3.8.1 DNA extraction

Two hundred and fifty microlitres (250µl) of blood was put into 1.5µl of effendorff tubes, 5µl of lysis buffer was added and mixed thoroughly by votexing. The sample was then incubated at room temperature for 30mins, and then mixed by invasion and put into spin column and centrifuged at 12,000g for 5min. The flow through was discarded and 500µl of wash buffer I was added and centrifuged at 12, 000g for 2min, and the flow through was discarded. 500µl of wash buffer II was added and centrifuged at 12,000g for 2min, and the flow through was discarded. The spin column was centrifuged empty at 12,000g for 2min. The column was then placed in microcentrifuge tube and 80µl of elusion buffer was added, and was incubated for 5mins at room temperature, then centrifuged at 12,000g for 2min. Seventy microlitres (70µl) more of the elusion was added and centrifuged at 12,000g for 2min. The extracted DNA was then stored in the freezer for downstream application.

3.8.2 Polymerase chain reaction for the isolates

3.8.2.1 Cocktail preparation per reaction

The cocktail was prepared using 12.5µl of Master mix. This was mixed with 1µl of Reverse Primer, 1µl of Forward Primer, 3.5µl of Nuclease-free water, and 7µl of DNA Template. The Tubes were incubated at 94°C for 5min in an initial denaturation step,

followed by 30 cycles of 30sec at 94°C, followed by annealing step at 56°C for 30sec, 72°C for 30sec for initial extension step, and final extension was processed at 72°C for 7min. The PCR reaction was started by adding the *Taq* polymerase before the first annealing step.

3.8.3 Detection of PCR products on agarose gel

A mixture of 9µl PCR products and 1µl of loading buffer, was applied onto 1.5% agarose gel stained with ethidium bromide. Electrophoresis was run at 120V for 1hr in an electrophoresis set containing 1X Tris Borate EDTA and photographed under UV illumination. DNA ladder was used to determine the size of the PCR products as shown in Table 3.1.

Table 3.1 List of primer and primer sequences for PCR amplification of trypanosome DNA

Primer	Primer sequence (5´-3´)	Size	Reference
TC1	5'-CGA GAA CGG CAC TTT GCG A-3'	**316 bp**	Masiga *et al.* (1992)
TC2	5'-GGA CAA ACA AAT CCC GCA CA-3'	**316 bp**	Masiga *et al.* (1992)
TV1	5'-GTG CTC CAT GTG CCA CGT TG-3'	**210 bp**	Masiga *et al.* (1994)
TV2	5'-CAT ATG GTC TGG GAG CGG GT-3'	**210 bp**	Masiga *et al.* (1994)
TB1	5'-GAA TAT TAA ACA ATG CGC AG-3'	**230 bp**	Masiga *et al.* (1992)
TB2	5'-CCA TTT ATT AGC TTT GTT GC-3'	**230 bp**	Masiga *et al.* (1992)

TCS - *Trypanosoma congolense*

TV - *Trypanosoma vivax*

TB – *Trypanosoma brucei*

CHAPTER FOUR

4.0 RESULTS

The Comparative analysis on the prevalence of trypanosomes infection among cattle in selected abattoirs and confined feedlots (Farms) in parts of Kaduna state are shown in the tables below.

Table 4.1 shows the Trypanosome infection rates and the mean packed cell volume (PCV) among cattle in the selected abattoirs and confined feedlots (Farms). Out of 450 samples collected, a total of 33(7.33%) of Bulls (Male cattle) and 32(7.11%) of Cows (female cattle) were infected. There were 12(2.67%) of Bulls infected with *T. brucei*, and 10 (2.22%) Cows 10(2.22%) Bulls were infected with *T. congolense*, similarly, 10(2.22%) of Cows were also infected with *T. congolense*. For *T.* vivax, 11(2.44%) of Bulls were infected, and 12 (2.67%) of Cows were infected. A mixed infection was observed in 3(0.67%) of Bulls and 8(1.78%) of Cows. The total infection rate of the cattle in all the abattoirs studied was 62(13.78%), while that of all the Farms was 3(0.67%).

Table 4.2 shows the mean Packed Cell Volume (PCV) rate for all the Abattoirs and farms studied. The abattoir had 38.44% while the farm had 40.63%. The low PCV rate observed for the abattoirs was statistically significant.

Table 4.3 shows the prevalence of trypanosomes among cattle between selected abattoirs and the confined feedlots (farms). Tudun-Wada abattoir had the highest number of infected cattle with 23 cases, while Zango abattoir in Zaria had the lowest number of infected cattle with 19 cases. All the confined Feedlots (Farms) studied had the same number of infected cattle with 1 case each.

Table 4.1: The trypanosome infection rates among cattle in three selected abattoirs and three confined feedlots.

Sample size = 450

Study area	Total number of Samples	Sex	Mean PCV(%)	*T. brucei*		*T. congolense*		*T. vivax*		Mixed Infection		Exact Sig.
				Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	
Tudun-Wada	100	Bull (72)	39	3	69	5	67	4	68	1	71	0 .756
Abattoir		Cow (28)	38	4	24	3	25	4	24	3	25	
Kawo	100	Bull (78)	39	5	73	4	74	4	74	2	76	0.456
Abattoir		Cow (22)	37	2	20	2	20	3	19	2	20	
Zango	100	Bull (76)	39	4	76	1	79	3	77	0	80	0.221
Abattoir		Cow (24)	34	3	17	4	16	4	16	3	17	
Simbafid	50	Bull (12)	41	0	12	0	12	0	12	0	12	1.001
Farm		Cow (38)	40	0	38	1	37	0	38	0	38	
Hairi	50	Bull (9)	42	0	9	0	9	0	9	0	9	1.000
Farm		Cow (41)	40	0	41	0	41	1	40	0	41	
Malonagge	50	Bull (12)	41	0	12	0	12	0	12	0	12	1.002
Farm		Cow (38)	40	1	37	0	38	0	38	0	38	
Total	**450**			**22**		**20**		**23**		**11**		

Pos=Positive, Neg=Negative. P≤0.05 (Fisher's Exact Test)

Table 4.2: The comparative analysis using mean Packed Cell Volume (PCV) (%) of trypanosome infection rates among cattle in the selected abattoirs and confined feedlots.

Sample size = 450

Location	Total	Mean PCV (%)	Sig. (2-tailed)
Abattoirs	300	38.44	0.000
Feedlots (Farms)	150	40.63	

$P \leq 0.05$ (Paired Samples T-Test)

Table 4.3: The percentage prevalence of *Trypanosoma* species between each Study Area

Study area	*Trypanosoma brucei* (%)	*Trypanosoma congolense* (%)	*Trypanosoma vivax* (%)	Number examined	Number Positive	P-value
Tudun-Wada Abattoir	7 (30.43)	8 (34.78)	8 (34.78)	100	23	0.320
Kawo Abattoir	7 (35.00)	6 (30.00)	7 (35.00)	100	20	
Zango Abattoir	7 (36.84)	5 (26.32)	7 (36.84)	100	19	
Simbafid Farm	0 (0.00)	1 (100.00)	0 (0.00)	50	1	0.412
Hairi Farm	0 (0.00)	0 (0.00)	1 (100.00)	50	1	
Malonagge Farm	1 (100.00)	0 (0.00)	0 (0.00)	50	1	

$P \leq 0.05$ (Paired Samples T-Test)

Table 4.4 shows the percentage prevalence of *Trypanosoma* species within the selected Abattoirs and confined feedlots. *T. brucei*, *T. congolense*, and *T. vivax* had a prevalence rate of 21(33.87%), 19(30.65%), and 22(35.48%) within the abattoir respectively, while in all the farms *T. brucei*, *T. congolense*, and *T. vivax* had prevalence of 1(33.33%) each.

Table 4.5 shows the risk factors associated with infection among cattle from selected abattoirs and confined feedlots indicating body condition, age, sex, and anaemia. Among the risk factors assessed body condition was statistically significant. However, there was no statistical significant difference between the infection with sex and age. The association between the infection and anaemia was statistically significant.

Plate I shows the extracted Genomic DNA of the species of Trypanosomes: *T. brucei, T. congolense and T. vivax.*

Plate II shows the agarose gel electrophoresis obtained after amplification of *T. congolense* specific sequences with 316 bp. 20 blood samples were positive using Microscopy from the 450 samples collected. Nine blood samples were tested with three positive sample (lane 1,2 and 3) and two negative samples (lane 4 and 5) from abattoirs, one positive sample (lane 6) and three negative samples (lane 7, 8 and 9) from farms detected from parasitological analyses, only lane 4 was confirmed positive for *T. congolense* on PCR.

Plate III shows the agarose gel electrophoresis obtained for the amplification of *T. brucei* specific sequences with 230 bp. Out of 450 samples collected, 22 were positive for *T. brucei* using Microscopy. Nine (9) blood samples were selected and subjected to PCR.

Three of the nine samples were positive (lane 1, 2 and 3) and two negative samples (lane 4 and 5) from abattoirs and one positive sample (lane 6) and three negative samples (lane 7, 8 and 9) from Farms detected from parasitological analyses, only lane 2 and 7 were confirmed positive for *T. brucei* on PCR.

Plate IV shows the agarose gel electrophoresis obtained after amplification of *T. vivax* specific sequences with 210 bp. Of the 23 samples tested positive for microscopy, eleven blood samples ; four positive samples (lane 1, 2,3 and 4) and two negative samples (lane 5 and 6) from abattoirs and three positive samples (lane 7,8 and 9) and two negative samples (lane 10 and 11) from Farms detected on parasitological analyses, Lanes 3,8 and 10 were confirmed positive for *T. vivax* on PCR.

Table 4.4: The percentage prevalence of *Trypanosoma species* within abattoirs and confined feedlots

Location	*Trypanosoma brucei*	*Trypanosoma congolense*	*Trypanosoma vivax*	Number examined	Number of Positive	P-value
Abattoirs	21 (33.87)	19 (30.65)	22 (35.48)	300	62	0.340
Feedlots (Farms)	1 (33.33)	1 (33.33)	1 (33.33)	150	3	0.764

P≤0.05 (Paired Samples T-Test)

Table 4.5: Risk Factors associociated with the infection among cattle from selected abattoirs and confined feedlots.

Risk Factor			Prevalence (%)	X^2 value	p-value
Body condition	Normal	409	90.89	42.265	0.000
	Emaciated	41	9.11		
Age	Adult	375	83.33	1.323	0.250
	Young	75	16.67		
Sex	Bull	263	58.44	0.232	0.603
	Cow	187	41.56		
Anaemia	≥39	411	91.33	207.088	0.000
	≤38	39	8.67		

x^2 = chi square , P≤ 0.05

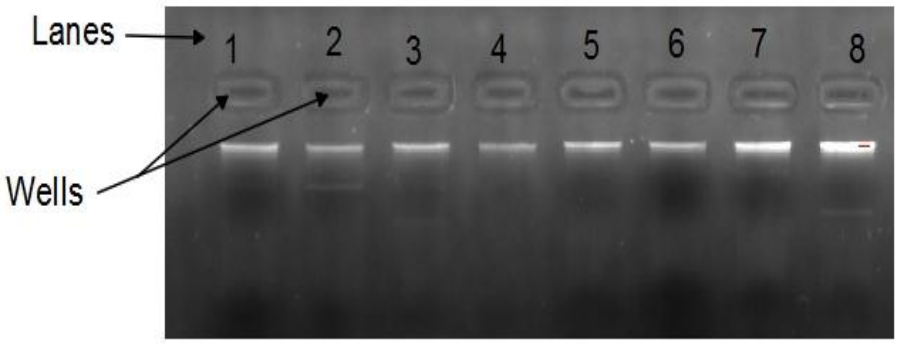


Plate I :Genomic DNA

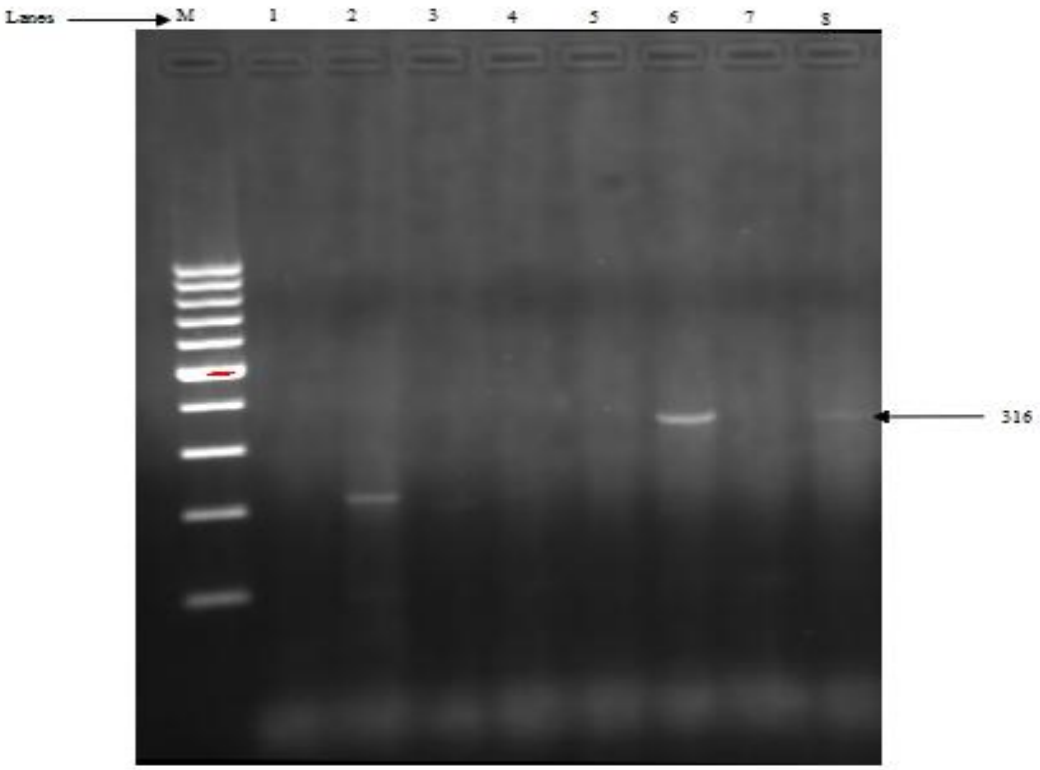


Plate II: Lane M 100bp ladder, lanes 1-8 PCR amplicons of *Trypanosoma congolense*

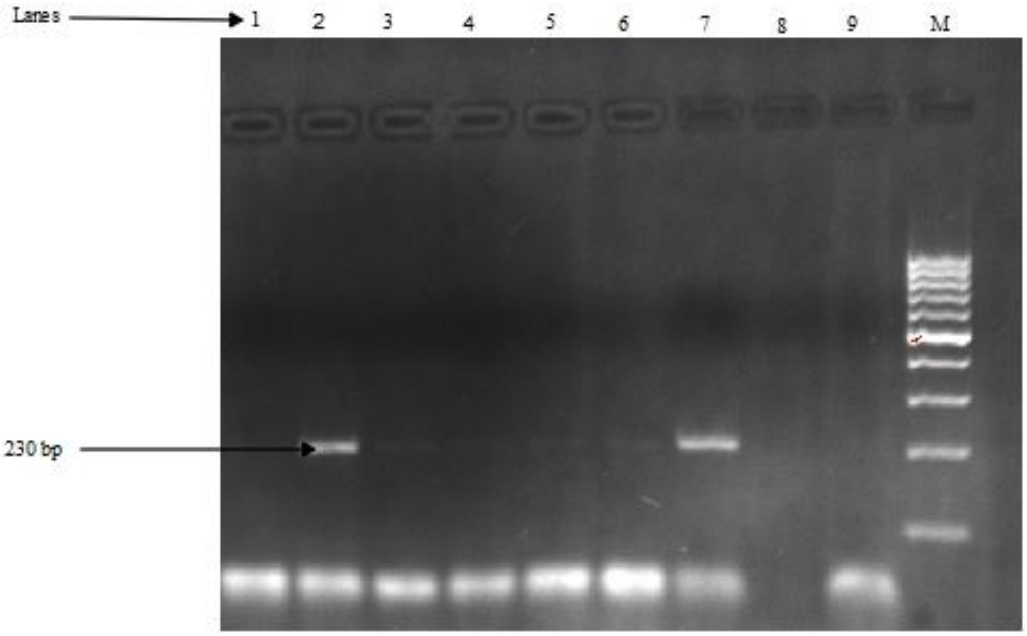


Plate III: Lane M 100bp ladder, lanes 1-9 PCR amplicons of *Trypanosoma brucei*

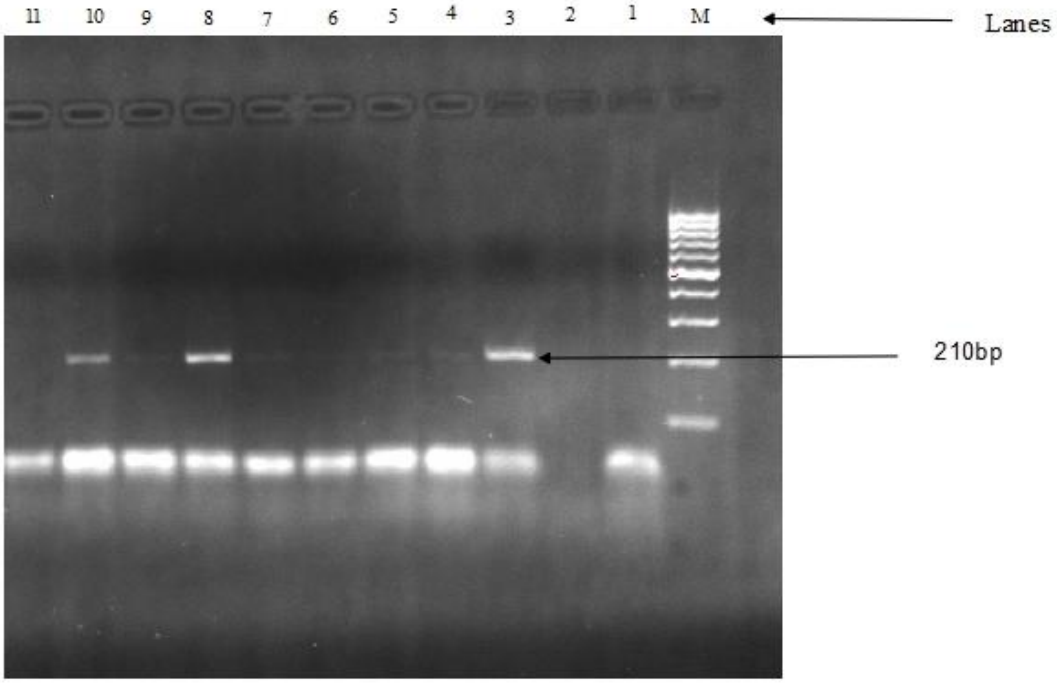


Plate IV: Lane M: 100bp ladder, lanes 1-11, PCR amplicons of *Trypanosoma vivax*

CHAPTER FIVE

5.0 DISCUSSION

A comparative study on the prevalence of trypanosomes among cattle within selected abattoirs and confined feedlots in parts of Kaduna State Nigeria was carried out. A prevalence of 62 (20.67%) for abattoir and 3 (2.00%) for Confined feedlots was observed. The prevalence of 20.67% observed in this study is similar to the report of Qadeer *et al*., (2009) who reported a prevalence of 17.8% to 50%. This could be due to improper management of the herds and the presence of large number of infective vectors in the area where they were sourced for. The prevalence of 2.00% observed for confined feedlots could be as a result of reduced animal and tsetsefly contact, abundance of pasture and water, coupled with reduced stress factors (*S*amdi *et al*., 2011a). More cases of single infection was recorded from the abattoirs than mixed infection indicating a higher prevalence of trypanosomal infection from the selected abattoirs. This is in accordance with the findings of Samdi *et al*. (2010a) who reported that the association of both parasite is scarce.

There was a significant difference between the mean Packed Cell Volume (PCV) of the infected cattle in the abattoirs and the farms. A Low mean PCV of 38.44% was recorded in the abattoir as compared to 40.63% recorded for Confined Feedlots which is statistically significant, this indicates that cattle from the three selected abattoirs were more infected which could due to different sources or treatment. This is in agreement with previous findings of Losos and Ikeda, (1972); Anosa and Obi(1980) who reported that trypanosomosis caused depressed PCV levels in infected animals.

The Body condition observed in this study was shown to be statistically significant in emaciated animals from the abattoir in relation to risk factor, as emaciation is a physical indication of trypanosomosis in animals. This is in agreement with the work of Abenga *et al.,* (2002), which showed that age of the animal was not statistically significant in the study. The prevalence of trypanosomosis was relatively higher in male cattle (Bull) than the female cattle (Cow), this was in consonant with a report of Fasanmi *et al. (*2014) who stated that gender should not play a relevant role in influencing the susceptibility of the animals to infection regarding several environmental interactions. The PCV is the most reliable indicator of anaemia in trypanosomosis (Murray, 1978; Morrison *et al.*, 1981). Anaemia was found to be statistically significant in relation to risk factors as this was caused by the level of parasitaemia recorded in the study area.

The high prevalence of *T. vivax* (23) observed using microscopy as compared to *T.brucei* (22) and *T.congolense* (20) was as a result of the level of pathogenicity of the specie. This is in agreement with the report of Fasanmi *et al.,* 2014; Nimpaye *et al.,* 2012 but in contrast with the report of Authié *et al.,* 1999, who stated that *T.vivax* infection is generally low and better controlled in sheep and goats. The prevalence of *T. congolense* was observed to be low in comparison with *T. vivax* which is accompanied by serious anaemia, leading to the rapid death of animals (Bengaly *et al.,* 2002; Sidibe *et al.,* 2002).

The more traditional methods involving microscopy have prevailed in most field studies, despite the fact that it has been suspected for some time that these classical methods are missing, a large proportion of the infections present in livestock, as a result of the presence of trypanosome-positive DNA signals from cross-checked cattle deemed

aparasitaemic by these methods (Majiwa *et al.,* 1994). The principal problem with microscopy is the lack of effectiveness and sensitivity, furthermore, Mugittu *et al.* (2001) found that trypanosomes could be detected in 43% of parasitologically negative cattle using Polymerase Chain Reaction (PCR).

Genomic DNA of all the samples was showed in Plate 4.1 showing the presence of parasites. PCR was able to confirm the presence of three trypanosomal species (*T.congolense, T.vivax and T. brucei*) as shown in plates 4.2, 4.3 and 4.4. This study is in agreement with the report of Fasogbon *et al*. (1990) and Dirie *et al*. (1993) that PCR failure on some positive samples from microscopy might be due to divergence between trypanosome sequences which can exist according to geographical location, as it was observed for all the three trypanosomes by isoenzyme characterization and PCR method.

PCR positive results indicates the presence of the parasite DNA in the vertebrate hosts and not necessarily an active infection as PCR detects also transient infections. It is therefore useful to confirm new host status by the isolation of the parasites in an appropriate medium and characterisation. The use of specific primers for other trypanosome species and sub-species could enable the identification of all trypanosomes infecting animals.

The application of DNA techniques provides much improved levels of sensitivity, such as the possibility of detection of individual organisms in samples of whole blood. Microscopy remains useful particularly as it can be carried out directly at the field level and gives immediate results. The original molecular protocols were not immediately

applicable to conditions in the field, due to the amount of processing required and the time involved prior to the testing of the samples.

The findings from this study with earlier studies of Clausen *et al.* (1998) who demonstrated that the detection rate by PCR was two times higher than the detection rate with parasitological techniques and also detection of small numbers of parasites which were not seen by microscopy is possible by PCR (Masiga *et al.*, 1992). In the abattoir, the relatively high infection rate observed might be due to higher animal and tsetsefly contact resulting from the concentration of the flies in the area (MacLennan, 1983). The lower infection rate in the farms may be as a result of the reduced animal and tsetsefly contact, abundance of pasture and water, coupled with reduced stress factors. The results of this study indicated the differences in trypanosome infection rates in the different locations. The low infection rate in the farms was due to increasing proper husbandry and the presence of few numbers of infective vectors. The high trypanosome infection rate in the abattoirs could be linked to improper management of the herds and presence of larger number of infective vectors in the area. The cumulative effects of exposure to tsetsefly and new strains of trypanosomes in the abattoir probably account for the observed infection rate (MacLennan, 1983). Single infections of *T. brucei*, *T. congolense* or *T. vivax* were encountered more frequently than mixed infections.

The results of this study shows that confined feedlots (farms) management of cattle is associated with a reduced trypanosome infection rate compared to abattoir management. The herds in abattoir are continuously exposed to new strains of trypanosomes while other adverse husbandry stress factors predisposes them to increased susceptibility and high infection rate.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

A total of 450 samples were collected from abattoir and confined feedlots, 300 blood samples from 3 selected abattoirs and 150 samples from 3 selected confined feedlots were examined to determine the prevalence of trypanosomes among cattle in parts of Kaduna state Nigeria. A comparative study on the infection rate of trypanosomes was carried out on wet mount, thick blood film , thin blood film , buffy coat and PCV using microscopy which was further subjected to PCR. The results indicated that confined feedlots management of cattle is associated with a reduced trypanosome infection rate as compared to the management of cattle in abattoir. The infection rates in Abattoirs and confined feedlots were 62(20.67%) and 3(2.00%) respectively by parasitological analysis. The species of trypanosomes observed within the abattoir in this study showed that *T.vivax* had the highest prevalence of 22 (35.48%) followed by *Trypanosoma brucei* 21 (33.87%) and *Trypanosoma congolense* with a least infection of 19 (30.65%) . PCR was used to confirm the presence of trypanosomes using specific primers.

6.2 Conclusion

In conclusion, the findings from this study showed a prevalence of 62(20.67%) and 3 (2.00%) in abattoir and confined feedlots respectively. The Packed Cell Volume showed a mean percentage of 38.44% within the abattoir and 40.63% in the confined feedlots. The risk factors associated with the infection showed a prevalence of 83.33% among adult cattle and 58.44% among bull which were statistically significant. The polymerase Chain

reaction also showed the presence of the various species of trypanosomes using the band sizes and base pairs.

The high prevalence may be a serious limiting factor to livestock production in sedentary cattle herds. Therefore an intensive commercial production of cattle coupled with good management could be encouraged in abattoirs and confined feedlots in parts of Kaduna State, Nigeria, therefore, it becomes apparent that there are far more trypanosome-infected cattle in the abattoirs than in the confined feedlots.

6.3 Recommedations

It is recommended that,

1. Abattoir workers should be acquainted with proper information about the infection and the causative agent.
2. Abattoir workers should be informed on the need to get their livestock from Tsetsefly-free areas.
3. Research should be conducted using molecular-based method to assess the further characteristics of the different species of the parasite

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APPENDICES

APPENDIX I: Questionnaire

DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, AHMADU BELLO UNIVERSITY, ZARIA

Risk Factors associated with Animal (Cattle) Trypanosomiasis Questionnaire

Dear respondent,

I am a student of the above department conducting a survey on the risk factors associated with Animal Trypanosomiasis among Cattle in abattoirs and confined feedlots (Farms) in parts of Kaduna State. Any information provided is strictly for this purpose and will be treated with utmost confidentiality. Kindly fill this questionnaire appropriately and accurately.

Questionnaire

A questionnaire addressing risk factors for trypanosome infection was given to the Abattoir and farm owners. Risk factors included body condition, age and sex using a structured questionnaire was obtained.

Risk Factors associated with Animal (Cattle) Trypanosomiasis Questionnaire

Body condition	Normal ( )	Emaciated ( )
Age:	Adult ( )	Young ( )
Sex:	Bull ( )	Cow ( )

Personal comment(s) on the health status of the animal (cattle)

_____

_____

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