STUDIES ON THE EPIDEMIOLOGY OF *TRYPANOSOMA EVANSI* INFECTION IN CAMELS IN SELECTED AREAS OF KENYA

by

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D E C L A R A T I O N

This Thesis is my original work and has not been presented

for a degree in any other university

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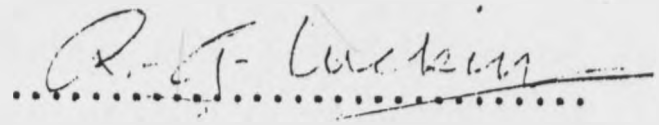

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and

MY SISTER, ESTHA L. OJYAMBO

with

GREAT LOVE

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ABSTRACT

Studies on the Epidemiology of Trypanosoma evansi infection in Selected areas of Kenya:

The study covered four camel herds namely: KULAL, NGURUNIT, OLMAISOR and GALANA. All these herds, except GALANA, are north of the Equator. The Indirect haemaggulutination test (IHA) was developed and used as the main serological test. In addition, the mercuric chloride (MC) and mouse inoculation (MI) were used. In the IHA Test, Trypanosoma evansi protein extract was used as the antigen. Sheep red blood cells were used in the IHA Test. The cells were fixed with 1% buffered glutaraldehyde solution. The antigen (T. evansi) was coupled to such cells after tanning them with 1:20,000 tannic acid solution.

Altogether, 2,100 camel serum samples were tested using the two serological tests and blood from the same number of camels inoculated into mice (in the MI test). Camel bleeds were done at monthly intervals or longer intervals.

The IHA detected the largest number of positives. 47.9% of all the cases were positive by the IHA test, 31.8% by the MC test and 6.3% by the MI test. Of the MI positive cases, the IHA detected 94.7% and MC 66.4%.

Therefore, the IHA test was found to be more sensitive in detecting patent cases.

Point prevalence rates (PPR) and Incidence rates (IR) are given. High PPR and IR rates were observed in all herds. Therefore, the disease is endemic in the camels of Kenya. Isoenzyme characterization revealed that 95.2% of the isolates were T. evansi and 4.8% T. congolense. It was possible to characterize the disease into 4 types namely: Type 1 - acute trypanosomiasis, Type 2 - chronic trypanosomiasis (showing some clinical signs), Type 3 - chronic trypanosomiasis (showing no clinical signs and characterized by low patency rate) and Type 4 - chronic trypanosomiasis (showing no clinical signs and characterized by a nil patency rate on MI and a normal PCV and WBC count values).

From the PPR, IR results and the variance of PCV and total WBC counts between the 4 herds over the whole study period, disease stability occurs in NGURUNIT and GALANA herds. It was hard to explain why the disease should be stable in some herds and unstable in others. However, management differences, fly incidence, trypanosome species and strain differences may be some of the reasons.

LIST OF ABBREVIATIONS

1.	E.S.G.	:	EDTA Saline Glucose
2.	g	:	relative centrifugal force ($g = 118 \times 10^{-7} \times r \times n^2$) (n = revolutions per minute) (r = rotating radius)
3.	r.p.m.	:	Revolutions per minute
4.	EDTA	:	Ethylene Diamine Tetraacetic Acid (disodium salt)
5.	$^{W}/_{V}$	:	Weight per Volume

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1:1 INTRODUCTION

1:1:1 THE CAMELS OF KENYA

Most of Northern Kenya is arid. Conditions here do not favour arable farming. The camel (Camelus dromedarius) is essential for the livelihood of the nomadic pastoralists of this area. Goats and sheep are also kept in a similar biomass to camels. The population of camels in Kenya has been determined by several workers. Morgan and Shaffer (1966) estimated the population of camels in Kenya to be 1.26 million, for the year 1964. The FAO report (1976) estimated the population to be at 335,000 camels. However, the most reliable figure is about 600,000 camels (Stelfox et al, 1979). Despite the fact that Gatt Rutter (1967) claimed that the camel in Africa does not thrive south of the line drawn from the Senegal River through Northern Nigeria, Lake Chad, Bahrel Ghazal and Northern Kenya, camels are kept as further south as Rumuruti and Galana Ranch, much nearer to Equator than he thought. The Government of Kenya has embarked on intensive Arid Land Development Programme. There are indications that the camel will be given a high degree of attention.

The camel can utilise flora which is otherwise less appealing to other herbivores. This means that this animal can do exceptionally well in the marginal

lands of Kenya. The productivity of the camel is very promising. It is estimated that an average lactating camel can produce about 2,000 litres of milk per lactation. Camel milk is rich in protein, fat and vitamins especially vitamin C. Camel meat is tender and is very much liked by the tribes of Northern Kenya, Somalia, Ethiopia and Arab lands. Camel hair, wool and hides have a steady market both locally and at international level. It is therefore expected that the camel can contribute substantially to the solution of food problems in arid and semi-arid regions of Kenya, especially Northern Kenya where starvation and malnutrition are most prevalent. Furthermore, camel products could be harnessed for the export market, especially in the Middle East.

Like any domestic animal, the camel is a victim of a number of diseases of which little is known. The camel has been neglected in livestock development (Gatt Rutter, 1967 and Fazil, 1977). Camel pox and camel trypanosomiasis have been cited as outstanding diseases of camels (Gatt Rutter, 1967). In Kenya, there have been cursory reports of camel surra (Trypanosoma evansi infection) (Gatt Rutter, 1967 and Fazil 1977). However, the extent to which this disease affects Kenyan camels is not known. In neighbouring Ethiopia and Sudan, camel surra is endemic (Sudan Ministry of

Animal Resources, 1960; Pegram and Scott, 1976). It is therefore possible that camel surra in Kenya may be more grave than it was thought.

1:1:2 AIMS OF THE STUDY

(1) To develop the indirect haemagglutination (IHA) test and use it for serodiagnosis of *T. evansi* infection in camels in selected areas of Kenya. In addition, to compare the results of IHA with those obtained by mouse inoculation and the mercuric chloride (MC) test.

(ii) To use the results of the above serological tests to assess the epidemiological situation of *T. evansi* infection in camels in Kenya.

(iii) To make recommendations for the strategy of control of *T. evansi* infection in Kenyan camels.

1:2 LITERATURE REVIEW

Despite all other attempts that have been made to improve camel production, little has been achieved as far as disease control aspect is concerned. This calls for a closer attention. Since knowledge of camel diseases is essential in all efforts to advance camel husbandry. In recent years, a number of workers have made attempted to study the disease problem of camels.

Gatt Rutter and Mack (1963) and Gatt Rutter (1967) reviewed the diseases affecting camels, Of the flagellate protozoa, trypanosomes are the most important pathogenic parasites affecting camels (Gatt Rutter, 1967). Trypanosoma evansi is the most important trypanosome affecting camels (Gatt Rutter, 1967). This flagellate was first described by Evans (1880, 1881-1882). Other trypanosomes also affect camels. These are T. simiae (Pellegrini, 1948), T.vivax (pricolo and Ferraro, 1920; Didomizio, 1918), T. congolense and T. brucei (Bennett, 1929). In Kenya, Fazil (1977) reported the occurrence of T. evansi in camels. Earlier. Gatt Rutter (1967) and Pellegrini 1948) reported the occurrence in Kenya of T. brucei and T. simiae, in camels respectively. However, due to improper diagnostic and identification procedures, it is possible that some of the T. evansi infections could have been mistaken for T.brucei. Tsetse-transmitted trypanosomes play a less important role in the pathogenesis of camel trypanosomiasis because camels are not normally kept in tsetse fly areas.

1:2:1 TRYPANOSOMIASIS DUE TO TRYPANOSOMA EVANSI (STEEL, 1885, BALBIAN, 1888)

Trypanosoma evansi was the first pathogenic trypanosome to be described and identified as the causative agent of mammalian trypanosomiasis. Evans (1880, 1881-82) associated the trypanosome with an endemic disease in equines and camels known as "Surra" in the Dera Ismail Khan, in the Punjab in India. McFadyean (1894) reported that John Henry Steel made similar observations during an outbreak of a disease in transport mules in Burma in 1885. Since then, a number of workers have reported T. evansi as being the chief causative agent of camel trypanosomiasis (Pease, 1906; Theiler, 1906; Yakimoff, 1921, 1923; Hoare, 1972; Mahmoud and Osman, 1979).

About thirty-three different names are ascribed to the trypanosomes causing "Surra". However, Hoare (1972) was of the opinion that these "Surra"-causing trypanosomes could all be classified under Trypanosoma evansi. If Hoare's classification is accepted, then the trypanosomes causing the following diseases in camels are under the name T. evansi:-
"Murrina" (T. hippicum), "Mal de Caderas" (T. equinum), "Derrengadera" (T. venezuelensis) in Central America and "Su-auru" (T. ninaekohlyakimovi) in Russia.

1:2:2: THE PARASITE (Trypanosoma evansi)

The classification of Trypanosoma evansi is given below (See Fig. 1).

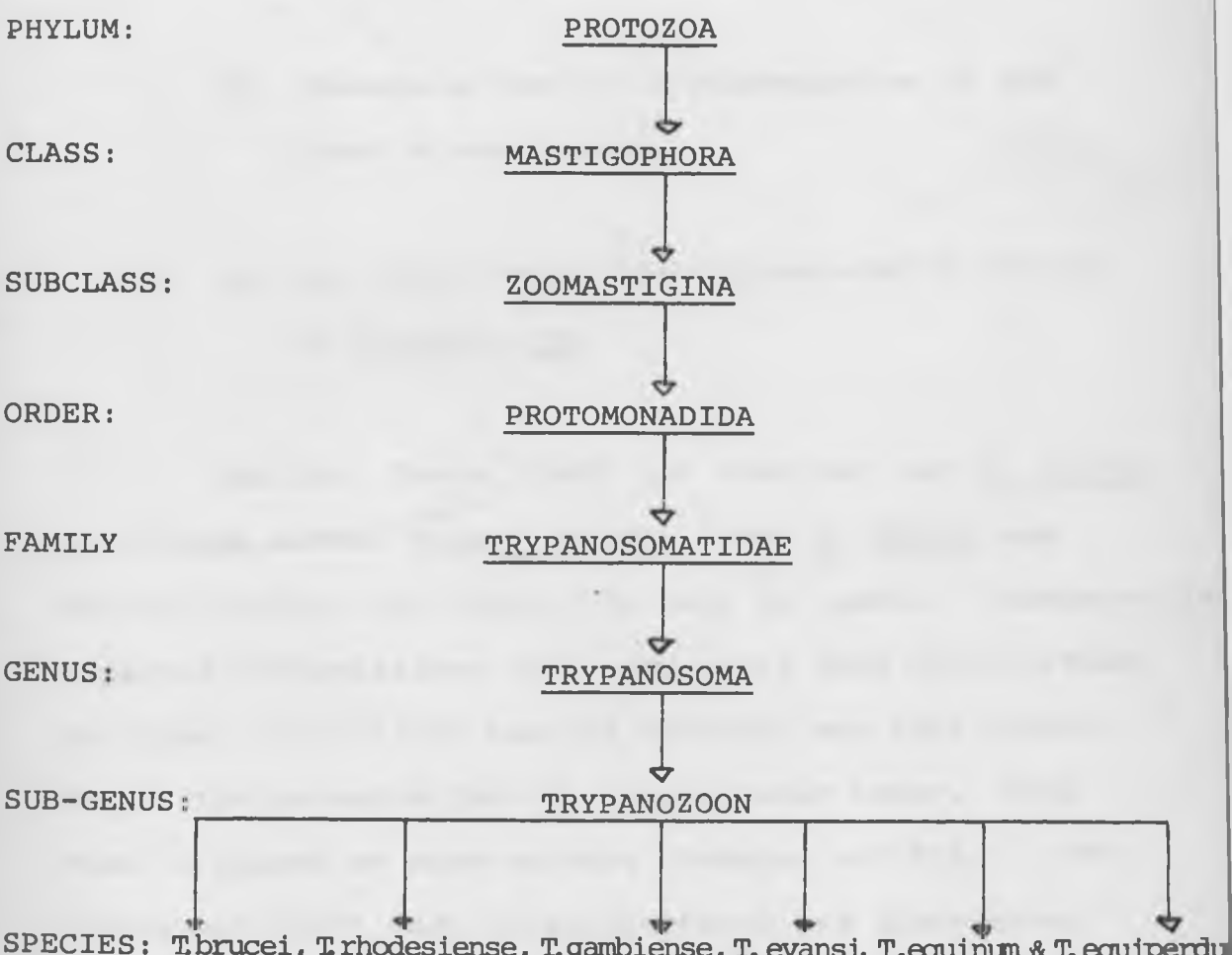

Fig 1: A simplified diagram of the classification of Trypanosoma evansi and related trypanosomes (Modified after Soulsby, 1973).

Hoare (1956) laid down certain criteria for the identification of T. evansi from other members of the subgenus of Trypanozoon to which it belongs. These criteria were:-

(a) absence of tsetse flies in the area where the trypanosome was isolated.

(b) absence or rarity of pleomorphism in the blood stream forms.

and (c) the inability of the trypanosome to develop in Glossina spp.

Earlier, Hoare (1940) had observed that T. evansi could have arisen from T. brucei, when T. brucei was carried outside the tsetse fly belt by camels. Subsequently repeated transmissions over many years from host to host by other biting flies such as tabanids may have transformed the parasite into T. evansi known today. This view is shared by many workers (Mahmoud and Gray , 1980). Vickerman (1965) made ultrastructural and biochemical studies on sleeping sickness - causing trypanosomes and T. evansi. He observed that T. evansi and old monomorphic laboratory stocks of T. brucei failed to exhibit any NAD diaphorase activity in their chondriome, while the recently isolated pleomorphic stocks of T. brucei possess such activity which is important during cultivation in vitro and for cyclical transmission.

However, Miles (1972) was able to demonstrate pleomorphism in T. evansi NS Stock. This may explain why it is possible to establish this organism in culture in the presence of Glossina spp. extract (Mahmoud and Gray, 1980). Trypanosoma evansi is susceptible to human plasma (Hawking, 1978). Hence its zoonotic potentiality is doubted.

Gibson et al. (1978) attempted to characterise T. evansi stocks with more precision using isoenzyme techniques. They were unable to detect any isoenzyme differences between the isolates. However, they noted the existence of intra-species differences in protein isoelectric points, polypeptide sizes and free amino acid content.. In spite of doubts expressed by Gibson and her colleagues regarding the usefulness of isoenzyme determinations in the characterization of T.evansi stocks. further work is required to assess this potentially valuable technique for parasite identification (Mahmoud and Gray, 1980).

Trypanosoma evansi has been adopted to represent more than thirty "species" of T. evansi-like trypanosomes (Hoare, 1972). However, Men'Shikov Abuladze and D'Takonov (1975), claimed that T. ninaekohlyakimovi is distinct from T. evansi on grounds of more ribosomes and more prominent polyphosphate granules and rhizoplast it

possesses. More work would be needed to substantiate the placing of T. ninaekohylakimovi as a distinct species from T. evansi.

It is apparent that the seperation of T. evansi from other members of the subgenus Trypanozoon using the criteria of Hoare (1956) is difficult. Absence or rarity of pleomorphism in the blood stream forms or the inability of the trypanosome to develop in Glossina spp are not reliable criteria as they may occur with other trypanosome species. Thirdly, strain differences may also affect these criteria. Some trypanosomes such as T. vivax can be transmitted mechanically and they can also occur outside the tsetse fly belt. Hence, a better method for identification of this parasite is still required.

1:2:3 EPIDEMIOLOGY

T. evansi affects different animal hosts in different parts of the world. In Indochina, epidemics tend to involve horses mainly, followed by camels, bovines and buffaloes, whereas in the Soviet Union and Middle Asia, the main hosts are camels and to a lessor extent horses. In Africa, T. evansi affects mainly camels (Mahmoud and Gray 1980). This means that the disease is mainly confined to North of the line drawn from Northern Nigeria, Lake Chad, Barhel-el-Ghazal and

Northern Kenya (Gatt Rutter, 1967). But Pease (1906) indicated that the parasite also affects dogs. Today numerous literature supports this fact (Mahmoud and Gray, 1980). However, little work has been done on dog trypanosomiasis in Kenya. Perhaps T. evansi is more widespread than it is thought.

In Tripolitania, the disease occurs in the same areas as Malaria and morbidity follows the same seasonal trend as malaria (Alongi and Balboni, 1935). This seems to point to an arthropod as the transmitter of this disease.

Natural infections have also been observed in mules (McKFadyean, 1894) and donkeys (Bennett, 1933). Splenectomy may reveal trypanosomes which are otherwise undetectable in apparently healthy camels (Denning, 1972). Apparently the spleen plays a vital role in the pathogenesis of this disease.

T. evansi obtained from camels, horses, mules and dogs was found to be pathogenic to sheep and goats but the clinical signs in goats and sheep were milder (Khasanov and Ivaniskaya, 1974). Other workers have observed the same findings (Chand and Singh,1971; and Malik and Mahmoud 1978). Evidence obtained from experimental infections confirms that donkeys, cattle,

sheep and goats undergo a protracted course of the disease that results into what has been called "Carrier State". Such hosts may act as reservoir hosts (Chand and Singh, 1971; Ilemobdade, 1971; Khasanov and Ivaniskaya, 1974; and Malik and Mahmoud, 1978).

Cattle reservoirs in South America are known to provide a source of T. evansi for vampire bats. The trypanosome multiplies in the bat which then transmits the organism to the susceptible horses (Mahmoud and Gray, 1980). In most camel rearing areas, camels are kept separately from cattle. However, sometimes camels are in close association with donkeys, goats, sheep and horses. This has been observed in the Sudan (Mahmoud and Gray, 1980) and in most parts of Northern Kenya where camels are reared. The fact that these animals may be danger to camels has already been indicated.

Wild animals may also act as reservoirs of T. evansi. Wells, Angles and Morales (1976) reported that healthy capybara (Hydrochoerus hydrocheeris) can harbour T. evansi and can constitute as a reservoir of infection from horses and dogs in Columbia. Shaw (1977) has reported that Ocelot (Felis pedalis) to play a similar role in the Amazon. Earlier, Marinkelle (1976) incriminated Orang-utan (Pongo pygmaeus) as a reservoir of T. evansi in Sumatra. The same author managed to transmit T. evansi to monkeys

experimentally. Kirmes and Taylor-Lewis (1978) observed that ticks cannot be efficient transmitters of T. evansi. In Kenya, little has been done as regards the reservoir of T. evansi. Despite the fact that Kenya has a rich wild fauna, little is known about their danger as potential reservoirs of T. evansi. However, there is ample evidence that a number of wild fauna harbour other species of trypanosomes (Olubayo, 1978).

1:2:4 TRANSMISSION

T. evansi is transmitted to camels by biting flies (Cross and Patel, 1922 and Nieschulz, 1930). Yutuc (1949) observed that tabanids were the chief transmitters of the disease and he reports of more than 2000 spp. of tabanids in the whole world. The author also noted that incidence occurred after the heaviest rainfall. In British East Africa (East Africa) Leese (1914) observed that spp. of Tabanus were abundant during and after the wet season and its rapid fall as the dry season advanced. Gruvel and Balis (1965) noticed a seasonal incidence of the disease originating each year from a domestic foci. They noted that most outbreaks occurred during the wet season especially at its end. This seems to coincide with the incidence of the tabanid flies. Similar observations were made by Yagi and

Razing (1972a, 1975) in Sudan. Yagi and Razing (1972b, 1975) surveyed the tabanids of the Southern Sudan and studied their seasonal abundance. Their findings coupled with those of Wilson (1978) on the camels of Southern Darfar, Sudan, showed that the prevailing ecological conditions favouring the breeding and prevalence of tabanids also supported the development of suitable camel browsing conditions where Acacia Senegal shrubs grew in abundance. Hence the camels kept in these places are at the risk of being bitten by these flies and therefore of contracting trypanosomiasis. A similar observation has been made at OL-Maisor, Galana, Kulal and Ngurunit in Kenya. (Wilson et al. 1981).

However, the correlation between the seasonal outbreak of T. evansi infections and the incidence of the tabanids has not been studied. A thorough study of tabanids and their correlation with T. evansi outbreaks in camels of Kenya is therefore recommended.

De Jesus (1951) in his study on Surra transmission in rats and dogs showed that trypanosomes can be inoculated through the normal mucosa of the digestive tract and that this mode of transmission possibly operates in nature much more in the carnivora than the omnivora or herbivora. Infections with T. evansi were also effected per conjuctival membrane and

per vagina. The author also managed to effect transmission via fresh and old wounds by the agency of house flies. Under natural conditions on farms and ranches, accidental bruises and wounds as well as numerous insect bites with blood oozing therefrom are quite common. Therefore it is highly probable that Surra transmission through the agency of the ubiquitous house flies also operates in nature, at least to a certain extent (Mahmoud and Gray, 1980).

1:2:5 PATHOGENESIS AND PATHOLOGY OF THE DISEASE CAUSED BY TRYPANOSOMA EVANSI

In camels, the disease due to T. evansi infection generally takes a chronic course (Gatt Rutter, 1967). Pease (1906) noted an extremely variable course of T. evansi infection in camels. The author observed that in some cases the course was rapid, taking only a few months. In others he noted a more chronic form, the camels living and remaining in fair condition for some years. Camel keepers have sometimes given accounts of the disease lasting only a week and seldom exceeding four months in duration. Pease (1906) considered this to be due to failure by the camel owners to discern the early stages of the disease in camels. However, it is apparent that in conditions which impair the host defence mechanisms, for example in treatment

with cortisone, the prepatent period of the disease may be shortened and an acute disease may result (Tongson and Brown, 1970). An acute form of the disease has also been reported with parasites always demonstrable in the peripheral blood and ending fatally after a few weeks (Gatt Rutter, 1967). Thus it is apparent that while T. evansi infection in camels generally takes on a chronic form, the acute or subacute form also occur.

In other domestic animals T. evansi infection expresses itself in all forms. Verma and Gautama (1978) observed a subacute and chronic form of Surra in buffalo and cow calves in which a mortality of 80% was recorded. The authors noted a maximum period of what they called "Carrier-State" of 68 and 190 days respectively. Alongi and Balboni(1935) were able to produce the subacute form of T. evansi infection in camels by a subcutaneous inoculation of the infective blood.

The pathogenesis of the disease due to T. evansi infection in equines and bovines in Nigeria was investigated by Ilemobade (1971) in experimental infections. All the infected horses died of the disease. Six out of the seven donkeys survived.

T. evansi was found to have no effect on oxen. The author concluded that cattle and donkeys could be latent carriers of T. evansi for several months. Lingard (1899), Pease (1906) and Schein (1908) reported that buffaloes and cattle acted as carriers of T. evansi for as long as 786 days and that mortality although rare, might occur in some of the infected animals. But the observations of Roa and Mudaliar (1934) were that cattle and buffaloes were probably not reservoir hosts of T. evansi, although organisms collected from cattle by them could infect horse and were pathogenic, an abservation made earlier by Pease (1906). Furthermore, it has been cited that Verma and Gautama (1978) observed a subacute and chronic form of the disease in buffalo and cow calves.

In goats and sheep the disease is thought not to be very grave. These animals may act as carriers (Pease, 1906; Theiler, 1906; Mahmoud and Gray, 1980). In their study of the pathological changes due to T. evansi infection in pigs, Srivastava and Ahluwalia (1972) observed that following an incubation period of 24-30 days, trypanosomes could hardly be found in the peripheral blood but the blood remained infective to rats. Except for intermittent fever, no other clinical signs were observed. Losos and Ikede (1972) report of this apparent disappearance of trypanosomes from the peripheral circulation in their review of

the pathology of trypanosomiases in domestic and laboratory animals. It is therefore apparent that cursory examination of blood or lymph fluid smears alone may not detect the trypanosomes. Chang, et al. (1976) reported an outbreak of Surra in pigs in epidemic proportions. The onset of this disease was marked by anorexia, depression, weakness and occational vomiting, followed by a storm of abortions in the pregnant sows. They observed no distinct clinical signs pathognomonic to the disease until the temperatures started to fall within 48 hrs preceding death, when the grade of parasitaemia went to its peak, Hudson (1944), and Lewis (1948 and 1954) noted that the largest number of parasites in cattle infected with T. vivax was present towards the end of the first week of elevated temperature but that at the time of death parasitaemia was often too low or not detectable. Theiler (1906) observed that the disappearance and appearance of trypanosomes in the blood varied with the animal species and the species of trypanosome. In the dog, the author observed that the shortest period of incubation in T. evansi infection was 3 days. After which the temperatures raised and the parasites appeared. A fever which persisted for some days and then disappeared was noted. The same phenomena repeated several times. Pease (1906) observed similar findings in camels although the incubation period in the camels was varied. This series of exacerbations

and remissions of the temperature curve is characteristic of trypanosomiasis (Theiler, 1906; Pease, 1906; and Losos and Ikede, 1972).

In the camel, Theiler (1906) noted that parasites were most abundant in the peripheral circulation during the fever stage and less so during the remissions. Towards the end of the disease, irregularities in temperature curve were noted with temperatures falling below normal or remaining higher in the evening and low in the morning. These observations seem to contradict those of Chang et al. (1976) on Surra outbreak in pigs. However, they agree with the views of Theiler (1906) that the course of disease in trypanosome infection varies with the species of the animal and the trypanosome species.

In the camel, Pease (1906) noted five main stages in the course of infection with T. evansi:-

(a) An early stage in which the attack is extremely insidious, the camel presenting no marked external symptoms of the disease although the organisms may be swarming in the blood from time to time. The animal maintains this condition for months. But he also noted that in other cases, fairly marked symptoms soon

followed infection. They included:- occasional dullness and fever, harshness of the coat, petechiated mucous membranes, weakness and loss of condition. The author therefore concluded that symptoms vary considerably according to the severity of the attack (the virulence of the trypanosome) and the individual susceptibility of the animal.

(b) "Kam hog" - second stage - characterized by fugitive attacks of fever, during which the camel is dull, sluggish, and inclined to move. The camel at this stage has lost the brightness of the eye which is so marked in a healthy camel - the organ becomes dull and in some cases watery (an indication of conjuctivities). The urine usually becomes scanty and highly coloured and has a peculiar oduor. The coat looks unthrifty. There may be petechiae on the mucous membranes, but it is not constant. There is in the majority of cases slow but progressive loss of condition. In mild chronic cases the above symptoms evolve exceedingly slowly, the camel at one time picking up a little and again falling off. In this

stage, recovery is possible and the animal maintains appetite throughout.

(c) "Dodia" - Oedematous swellings start to appear in the chest region. This is the third stage.

(d) "Surra" - Characterized by dropsy of the legs and the hind parts. The stage is marked by progressive anaemia. Recovery is almost hopeless. Even if it did occur, the animal becomes useless for two years or more.

(e) "Phipri" - Which means "Lung disease" very often the predominating symptoms when death occurs.

These dian descriptions serve well to illustrate the course of the disease in the camel.

Burke (1891) reports of paralysis having been observed in camels by most veterinary surgeons in most parts of India. Brar and Sharma (1962) described a nervous form of surra in bovines in natural outbreaks. The authors maintained that the symptoms of this form similated those of anthrax, poisoning with organophosphorus compounds and metabolic diseases for example, ketosis, parturient

paresis and hypomagnesaemia. But Verma and Gautama (1978) did not observe any nervous signs in buffalo and cow calves, nor did they observe any correlation between parasitaemia and rise of body temperature unlike in the horse. It appears the finding of the nervous syndrome is not shared by all workers. However, Mahmoud and Osman (1979) describe the course of T. evansi infection in Sudanese camels in a way almost similar to that of Pease (1906). The authors indicate that the disease is manifested by an elevation of body temperature associated with presence of trypanosomes in the peripheral circulation. They point out that the affected camels show progressive anaemia loss of condition, weakness and often rapid death. Milder cases develop relapsing parasitaemia with or without pyrexia. Some camels develop oedema in their dependant parts, and a characteristic sweet smell readily identifiable by native camel owners. Finally, they indicate that some camels may harbour the trypanosomes for two to three years thus constituting a reservoir of infection to susceptible camels and other hosts.

The pathological changes that take place in various organs and tissues of the body as a result of T. evansi infection are mainly those due to degeneration as a result of poor nourishment (Burke, 1891). The

author points out degenerative changes of adipose character take place. When organs and parts so weakened by starvation are distended with anaemic blood which they cannot utilise, sloughs result from gangrene of the imperfectly nourished tissues as evidenced in the ulcerations of the stomach, etc., noted in this disease. The alimentary mucous membrane in general is anaemic with petechial and ulcerated sports, the result of disintegration and atrophy. There is a large deposit of fat everywhere but especially noticeable under the skin giving the tissues a peculiar jaundiced appearance which is shown to be due to the deposition of fat globules when viewed under the microscope. The author indicated that the parasites obstruct blood,a fluid rich in oxygen and this leads to the conversion of albuminoid tissues to fat. The lymphatic glands throughout all the body are more or less swollen and oedematous. There is usually acute dropsy with no other post-mortem lesions save the extreme anaemia and yellowing of the tissues due to fatty degeneration. Burke (1891) seemed to think that the yellowing was not due to bile pigments. Pease (1906) observed nearly similar changes to those seen by Burke. The author observed post-mortem changes which included a generally emaciated carcase with wounds on it. Pallid mucous membranes, yellow

gelatinous exudate under the skin, pallid and ischaemic muscles and depletion of fat in the usual fat depots. Further, he pointed out the possibility of a considerable quantity of fluid in the abdominal pleural and pericardial cavities and oedema of the lungs or broncho-pneumonia. He also noted that normal heart fat gives place to a yellow gelatinous material. Burke (1891) also observed that the heart is the seat of marked changes in T. evansi infection in camels. Pease (1906) observed that heart vessels contain yellowish white clots and frequent subendocardial haemorrhages. He also noted splenomegally and oedema of the lumbar region.

Early symptoms of hepatitis present in surra may be due to congestion of the liver caused by irritation of the parasites in the biliary capillaries, leading to their rupture and subsequent deposits in the liver parenchyma (Burke, 1891). The author observed extravasation and staining of the nerve cells of the grey matter of the spinal cord in these cases which showed paralysis of hind quarters or other nervous symptoms. But he observed no other pathological changes in the brain or the spinal cord.

Misra and Chaudhury (1974) noticed that during infection with T. evansi in buffaloes, the parasite

develops visceral forms including trypomastigotes, amastigotes and sphaeromastigotes in the heart, optic lobe, cerebellum and lungs. They claimed that amastigotes and sphaeromastigotes were not degenerate forms but active visceral phases. However, Pease (1906) considered these forms to be degenerate and not active visceral phases. Another peculiar behaviour of T. evansi is its apparent loss of the kinetoplast under certain conditions (Hoare, 1964; Warton et al, 1973, Killick-Kendrick, 1954).

As concluded by Pease (1906), the post-mortem changes have no diagnostic bearing. Though Burke (1891) was of the view that the pathological changes taking place in the various organs and tissues were due to degeneration because of improper nourishment, today there is enough evidence to show that his view was an over simplification. Many authors have reported complicated changes in the blood chemistry and cellular components during infection of various animals with T. evansi. More recently, Boid et al.(1980) conducted studies on the serum proteins and immuno-globulins during infection with T. evansi in Sudanese camels. The authors observed an increase above normal of the total protein concentration in the experimental and naturally infected camels. However, albumin levels in both cases decreased below normal. Alpha-globulin levels remained unchanged. Beta-globulin levels were

unchanged in experimental infections but decreased in naturally infected camels. Gamma-globulin IgM levels showed significant increase of about 5 times pre-infection while IgG did not show any significant change, apart from minor fluctuations during experimental infection. Luckins (1972) observed increases of 2-9 times the pre-infection levels of IgM, two weeks after cattle were introduced into an enzootic area. But serum IgG levels however, showed only a two fold increase during the same period. IgM elevation during trypanosomal infection has been observed by many workers and IgM elevation has at times been used for the diagnosis of trypanosomal infection. However, it is not a reliable method since there are other conditions apart from trypanosomal infection which lead to IgM elevation.

Verma and Gautama (1977) observed no increase in the serum proteins in buffaloes infected with T. evansi. However, they observed an increase in beta and gamma-globulins and decrease in albumin and alpha-globulin. Jatkar, et al. (1973) showed that protein levels fluctuated during T. evansi infection in Indian camels. In acute infection, albumin remained unchanged, but fell below normal during the chronic disease. Beta-globulin increased in the acute disease, but, remained unchanged during the chronic infection.

On the other hand, gamma-globulin increased during both the acute and chronic diseases. Jatkar and Singh (1974) found that in infected camels, glucose concentration showed a wide variation, but in general, they found it to be inversely proportional to trypanosome count. Hypoglycaemia in trypanosomal infection has been reported by many authors in various animal species and in man (Losos and Ikede, 1972).

Dwivedi et al. (1977) and Boid et al. (1980) conducted studies on changes in serum enzyme levels during infection with T. evansi. Dwivedi et al. (1977) found that in the dog serum glutamic oxalotransaminase (SGOT) did not change, while serum glutamic pyruvic transaminase (SGPT) showed a slight temporary rise in serum between the 6th and 15th day of infection with T. evansi. Boid et al. (1980) observed elevated levels of sorbital dehydrogenase, SGPT and SGOT above pre-infection levels whereas alkaline-phosphatase decreased during the period of patent parasitaemia. However, after treatment with quinapyramine sulphate B.Vet.C. (Antrycide) the enzyme levels returned to normal.

Increased level of globulins and decreased level of albumin were seen by Anonym (1951-52) in

camels infected with T. evansi. Sammadar (1962) observed a gradual fall in total serum protein in T. evansi infection in the goat. Clarkson (1968) reported a decrease in albumin and beta-globulins with an increase in gamma-globulin in sheep infected with T. vivax. Verma and Gautama (1977) found that the most consistent change in T. evansi infection in the buffalo calf was the gradual increase in gamma-globulin. They found that the level was higher in chronic cases than in acute cases and explained this in the light of paroxyms of parasitaemia which continued to stimulate the antibody forming system. It was found that the animals which succumbed to infection in 20-21 days had very low levels of gamma-globulins. Those surving for 35-96 days had large quantities of gamma-globulins. Goodwin (1970) observed that most of the immunoglobulins (IgM and IgG) in T. brucei infection in rabbits were non-specific. This can be explained from the high degree of antigenic variation shown by trypanosomes during the course of infection (Gray, 1965). Because of the non-specific nature of the gamma-globulins the animals which suffer chronic infections also succumb to infection.

Castillo and Joaquin (1955) observed that in rats infected with T. evansi the blood sugar level

dropped with increase in parasitaemia. Hypoglycaemia was noted on the 3rd day post-infection which progressed to an all time low level. The authors failed to prolong the lives of rats by injection of glucose intravenously. It was debatable as to whether hypoglycaemia is the cause of death in trypanosomal infections (Castillo and Joaquin, 1955).

Burke (1891) noted a marked leucocytosis during T. evansi infection in the horse. Assoku (1975) observed a severe anaemia together with reticulocytosis and hepatomegally in rats infected with T. evansi. Histological examination of the liver, spleen and bone marrow confirmed increased erythropoietic activity and concluded that the anaemia observed was due to extra-vascular destruction of erythrocytes rather than inhibition of haemopoietic activity. All the infected rats showed significant immune responses to infecting trypanosomes, peak agglutination titres occurring 10-12 days after infection coincidentally with maximum destruction of RBC. While this suggests an auto-immune reaction, serological examination of sera and erythrocytes from all infected and control rats did not reveal the presence of either circulating and or absorbed erythrocyte auto-antibodies. Furthermore, there was no in vivo trypanosomal antigen coating of erythrocytes from either infected or multiple antigen

infected rats. Repeated intravenous injection into rats of more than 100 microgrammes per gramme body weight of soluble T. evansi antigen resulted in moderately severe probably antibody mediated anaemia. It was considered that an immunologically mediated mechanism may be responsible for the development of anaemia accompanying T. evansi infection. The aetiology of anaemia in trypanosomal infection has been attributed to a number of factors. Kaaya (1975) observed that in goats infected with T. congolense, the anaemia which developed was mainly due to increased red blood cell destruction from the peripheral blood and medullary inhibition. Earlier, Omuse (1972) had observed that in T. congolense infection in cattle there was development of an intense anaemia. The author admitted that the aetiology of the anaemia was difficult to determine but suggested that medullary depression, red blood cell haemolysis and red blood cell phagocytosis (which suggested auto-immune reaction) were some of the possible causes of the anaemia. The pathogenesis of anaemia in T. evansi infection in camels needs further investigation as this may add more light to understanding of the aetiology of anaemia in other trypanosomiases.

In conclusion, Surra in camels and in equines is manifested by an elevation of body temperature which

is directly associated with parasitaemia and progressive development of anaemia, loss of condition and weakness. Recurrent episodes of fever occur during the course of the disease. Oedema, particularly in the dependant parts of the body, urticarial plaques and petechial haemorrhages in serous membranes may be observed. The disease may be fatal within months or may last for a few years. Spontaneous recovery is rare (Hornby, 1952; Stephen, 1970). The disease is often rapidly fatal for camels, dogs and horses but it can be mild and cryptic in bovines, donkeys, goats and sheep. The pathogenesis of anaemia still needs further investigation.

1:2:6 DIAGNOSIS OF CAMEL SURRA

Camels infected with T. evansi tend to show emaciation and anaemia. Tentantive diagnosis would be possible on these grounds. However, there are other conditions affecting camels which manifest emaciation and anaemia such as: worm infestations, over work, under nourishment and many others. It is difficult to diagnose camel trypanosmiasis from the clinical picture alone.

Diagnosis of infection by examination of peripheral blood and lymph fluid is satisfactory in

animals with acute infection, but is more difficult in chronic or latent disease when parasitaemia may be very low (Pease, 1906; Theiler, 1906 and Luckins, et al. 1979).

Inoculation of camel blood samples into rodents is of some diagnostic value (Bennett, 1933 and Godfrey and Killick-Kendtrick, 1962). However, laboratory animals are not always available in endemic areas and this technique is therefore not used widely in the field.

A number of chemical tests for T. evansi infection which depend on increased serum globulin content have also been described. These tests include the mercuric chloride test (Bennett, 1929, 1933), the formal gel test (Plantureux, 1923; Knowles, 1924, 1927) and the thymol turbidity test (Abd-el-Ghaffar, 1960). However, these procedures are not specific for trypanosomiasis and they are not sufficiently reliable for accurate diagnosis (Pegram and Scott, 1976).

Serological diagnosis is complicated in trypanosome infections, as the organisms show continous antigenic variation during the course of infection.

Gray (1965) pointed out that there was no limit to the antigenic variation in T. brucei in in vitro culture as well as in monthly passage in rats. Cunningham and Vickerman (1961) were able to demonstrate variation in a relapse strain of T. cruzi. To-date, much work has been done on antigenic variation in trypanosomes (Cross, 1978, Barbet et al. 1978, Doyle, et al. 1978, Barry and Hajduk, 1978).

More specific serodiagnostic tests based on the detection of levels of common circulating antibodies including complement fixation test (Shoening, 1924, Sabanshiev, 1973), indirect haemagglutination test (Jatkar and Singh, 1971) and precipitin test (Bansal and Pathak, 1971) are also available. These procedures have not been widely applied in the field on camels. More recently, fluorescent antibody test and enzyme immuno assay for the serodiagnosis of infection might be of use in the diagnosis of T. evansi infections (Sabanshiev, 1972, 1973; Luckins, Gray and Rae, 1978).

In the present work, an Indirect haemagglunation (IHA) and mercuric chloride tests were developed and used for the detection of circulating antibodies to T. evansi infection in camels.

1:2:6:1 INDIRECT HAEMAGGLUTINATION TEST (IHA)

Red blood cells coupled with antigens are currently employed for the detection of minute amounts of antibodies. Most often the coupling of the antigen to the erythrocytes is achieved by bis-diazotized benzidine (Pressman, et al., 1942) or tannic acid (Boyden, 1951). Based on these two linking agents, several modifications of the indirect haemagglutination test have been carried out (Kabat, 1961 and Stavisky, 1964). Avrameas, et al., (1967) found gluteraldehyde, cyanuric chloride and tetra azotized 0-dianisidine to be efficient coupling agents. Since then, gluteraldehyde has been employed widely by many workers in the Indirect haemagglutination test.

Gill (1964) used a modification of Boyden's technique (1951) in an attempt to develop Indirect haemagglutination test for the diagnosis of T.evansi infection in rabbits. He found the test to be specific, sensitive, easy to perform and gave results which were reproducible. Gill (1966) modified this test by using formalin as a fixing agent, but the results he got were not reliable. However, he was still of the opinion that IHA was

more sensitive than the complement fixation test.

Verma and Gautama (1977) compared three serological tests for the diagnosis of T. evansi infection in bovines, namely the Indirect haemagglutination test, Gel-diffusion and Indirect Fluorescent antibody tests. The authors found that IHA was more reliable and more sensitive and detected haemagglutinating antibodies against T. evansi between the 4th and the 8th day of infection, much earlier than the other two tests gave any positive results. The test gave no false positives. Using the Indirect haemagglutination test, Jatkar and Singh (1971) found that titres of 1:20 and above were positive for camels suffering from T. evansi infection. The test detected all animals which were positive by the animal inoculation method. Shien (1974) found the Indirect haemagglutination test to be specific and sensitive for the detection of anti-trypanosome antibodies in the blood of ruminants. The author got antibody titres of 1:20 and above for the positive cases and below 1:10 for the negative cases among the goats he screened. He recommended the test as a good method for epidemiological survey for Surra.

1:2:6:2 MERCURIC CHLORIDE TEST

This test was first developed by Bennett and Kenny (1928). Like the formal-gel test, this test detects an increase in serum protein concentration. Bennett (1933) recommended this test as a replacement for the formal-gel test. Abdel-Latif (1958) and Leach (1961) confirmed the diagnostic value of the mercuric chloride test. A number of workers have however doubted the degree of accuracy claimed for this test (Mahmoud and Gray 1980). However the mercuric chloride test is still used as a diagnosing tool in the diagnosis of Surra in camels.

The advantage of this test is that it uses small amounts of serum and and results can be obtained in a short time (Bennett and Kenny, 1928). The fact that it depends upon raised serum immunoglobulin levels makes it less precise because there are many conditions in the tropics which could lead to raised immunoglobulin levels in camels. (Cunningham et al, 1967)

CHAPTER 2

2. MATERIALS AND METHODS

2:1 Location of the areas at which camel serum samples were collected.

The map of the Republic of Kenya showing the location of four sites at which camel serum samples were collected is shown in Fig. 2. These areas are KULAL, OL MAISOR, NGURUNIT and GALANA. A brief description of these areas is given below.

2:1:1 KULAL AREA

This area lies at an altitude of 700-2000m above sea level. It receives a rainfall of 100-600mm per annum. The vegetation of this area comprises evergreen forest; perennial grassland which is composed of bushland plus dwarf shrubland.

2:1:2 NGURUNIT

Ngurunit lies at an altitude of 700m above sea level. This area receives a rainfall of about 300-700mm per annum. The vegetation comprises bushland plus dwarf shrubland.

2:1:3 OL MAISOR

Ol Maisor lies just to the north of the equator in Kenya's Laikipia District, at an altitude of about 1730-1890m above sea level. This site comprises an area of about 12,140 hectres and and receives an annual rainfall of about 580mm. The vegetation comprises of Themeda, Seteria, Hyparrhenia, Londetia, Cynondon grasslands, scattered widely with Acacia spp. Bush and thicket containing Euclea, Plums, Grevia and Acacia brevispica are to be found. This area carries Boran cattle stocked at 4-6 hectres to each beast, Merino or Merino/Dorper sheep at 1-3 per head of cattle and indigenous goats crossed with a variety of exotic male introductions. Camels were introduced in this area in 1974.

2:1:4 GALANA

Galana ranch is more than 400,000 hectres. It is found south of the equator in the hinterland of the Kenya Coast. It lies in the fringe of the Coastal rain belt, in a semi-arid savannah, at an altitude of 270m above sea level. It receives an average annual rainfall of about 550mm. Tsetse-flies occur in parts of the ranch. To the extreme east, the vegetation consists of thick coastal bush

plus forest containing Afzelia quanzensis, Brachysteria spiciformis and Bombax rhodognaphaloin. This merges into light Despyros mespiliformis parkland which in turn gives way to Acacia-Commiphora woodland. In the west and lowest rainfall zone, Commiphora dominate. In all zones there is extensive grassland characterized by Chloris spp., Schoenfeldia transiens and Aristida spp. among a wide range of other species.

2:2 COLLECTION OF SERUM FROM CAMELS

Serial samples of sera were collected from camels at the sites indicated on the map (Figure 2). Samples were collected monthly where possible. The camels were bled from the jugular vein and blood was collected into sterile universal bottles. Inoculation of mice with camel blood was always done immediately after bleeding. For each blood sample, a pair of mice were inoculated with 0.5mℓ of the blood. Blood for serum separation was flown to Wilson Airport (Nairobi) from where it was transported to Muguga (Kikuyu) for processing. Routinely, the blood was left overnight at +4^{o}C. This allowed the serum to separate from the clot. The serum was then separated into centrifuge tubes and

Fig.2. Map of Kenya showing the Location of areas at which Camel serum samples were collected (CS)

centrifuged at 1000g for 10 minutes. Lastly, 2 drops of Sodium azide (0.02% NaN_3) were added as a preservative to the serum. The serum was then stored as 2mℓ amounts in bijour bottles at -20°C till required. In addition, blood was always preserved for routine haemotological examination. Such blood was preserved with disodium EDTA.

2:3 ANTIGEN

The antigen for the Indirect haemogglutination test was prepared from Trypanosoma evansi, KETRI 2429. This strain was isolated from a camel at Ukunda, Coast Province of Kenya, in 1978. It was passeged nine times in mice and then kept in the trypanosome bank using E.S.G. buffer containing 10% glycerol. Before use, this strain was first inoculated into mice. The mice were bled at peak parasitaemia and the parasitised blood diluted with E.S.G. to give a final concentration of six trypanosomes per field. This blood was inoculated into white rats. Each rat was injected with 0.5mℓ of the blood mixture. The parasitaemia of the rats was then monitored daily. At peak parasitaemia (when the number of trypanosomes per field was beyond counting) the rats were bled by cardiac puncture. The parasites were seperated from blood cells elements on a column of DEAE-52 cellulose (Lanham and Godfrey,

1970). The separated trypanosomes were washed three times with 0.1M Phosphate-buffered saline (PBS) pH 7.4 and stored at -79°C until required. Several batches of this trypanosome material were prepared this way. Later, the batches were pooled and diluted one in four (1:4) with ice-cold PBS and then subjected to twenty seconds ultrasonication at maximum amplitude on an MSE ultrasonic Disintegrator (Safam Electrical Instrument Co. Ltd. England). The resulting trypanosome suspension was centrifuged at 10,000g for 20min. at +4°C. 0.5mℓ amounts of the supernatant were frozen at -79°C as stock antigen. Before the use of this antigen material, its protein concentration was determined by the method of Lowry et al. (1951).

2:4 THE INDIRECT HAEMAGGLUTINATION TEST (IHA)

For the purpose of this project IHA was performed on a micro-scale (Fig. 3 and Fig. 4). This was done in order to reduce the amount of the reagents and the time taken to carry out the procedure. The method employed was a modification of Gill (1964) and Jatkar and Singh (1971). The equipment for such a procedure was described by Sever (1962) and is available commercially

(Titretek Microtitration Equipment, Flow Laboratories Ltd., Irvine, Scotland). It consists of small plastic disposable plates each with 96 wells (Titretek/Linbro Plates with V-shaped wells, Flow Laboratories Ltd.) which will hold up to 0.3mℓ, droppers calibrated to deliver 0.05mℓ of solution and microdiluters which are calibrated to pick-up 0.05mℓ volumes.

2:4:1 Preparation of red blood cells and serum for the IHA test.

Sheep red blood cells were used in this test. Preparation and sensitisation (coating with antigen) of the sheep RBCs is described below:-

2:4:1:1 Collection of blood

Materials:

Universal bottles with 5mm hole in cap and containing 10mℓ of Alsever's solution, (RGI) autoclaved for 15min. at 15 lb/sq in.

10mℓ sterile syringes

Several sterile suitable gauge needles (12-16G x 16G x about 1")

70% alcohol

Cotton Wool

Donor Sheep.

Method:

1. Cotton wool soaked in alcohol was used to clean the jugular area of the donor sheep.

2. 10mℓ of blood was collected from the jugular and injected through the cap of the bottle into the Alsever's solution. The blood and Alsever's solution were mixed immediately by inverting the bottle several times.

3. Step (2) above, was repeated with another universal bottle until enough blood had been collected.

4. After the collection of blood, the jugular area was cleaned again with alcohol.

5. The filled universal bottles were placed in the fridge ($4^{o}C$) until ready for use (maximum one week).

2:4:1:2 Stabilising (fixing) of cells

Sheep red blood cells were stabilised using 1% buffered glutaraldehyde solution. Fixing of red blood cells using glutaraldehyde instead of formalin

was described by Bing, et al., (1967), Smith and Harvey (1977) and by Walther and Grossklaus (1972).

Materials:

Blood in Alsever's solution,

Graduated centrifuge tubes,

Centrifuge,

PBS pH 7.2 (RG 2),

Glutaraldehyde solution (RG 3),

Beaker (250mℓ size for 10mℓ of blood) with cover,

Stirrer and followers,

2mℓ vials for storage of cells,

Liquid nitrogen container.

Method:

1. The red blood cells in Alsever's solution were centrifuged in graduated tubes (700g for 5 mins).

2. Supernatant was sucked off and the tubes refilled with PBS PH 7.2 to same volume. The cells were resuspended.

3. Centrifugation was repeated at 700g for 5 mins.

4. Steps 2-3 were repeated four times.

5. The volume of the packed cells was noted and the supernatant sucked off.

6. The cells were then resuspended in a little glutaraldehyde solution. The cell suspension was then transferred to a beaker of suitable size.

7. Glutaraldehyde solution was then added to the beaker until there were 50 volumes of glutaraldehyde solution to each volume of packed red cells (eg. 4mℓ of RBCS needed 200mℓ of glutaraldehyde solution).

8. The above cell suspension was stirred gently for 60mins, in a covered beaker at room temperature.

9. Thereafter, the cell suspension was centrifuged at 700 g for 5 mins.

10. As (2)

11. (9) and (10) above were repeated four times.

12. A small drop of the cell suspension was taken on a glass slide, with a cover-slip and examined under the microscope. In

cases where the cells were observed to be sticking together, the whole material was discarded and the process repeated with a new blood sample. Only cell suspensions which appeared separated under the microscope were used in the test.

13. The volume of cell suspension was adjusted with PBS to make a final suspension of 25% of RBCS.

14. The above suspension was transferred to clean beaker and stirring continuously, 0.9mℓ (900 microlitres) aliquots were taken into small plastic vials and stoppered tightly.

15. The vials were placed into suitable containers and put into the gas phase of liquid nitrogen for at least one hour, or preferably overnight.

16. The vials were stored at -20°C until required. Generally the following volumes were observed in the test (see table of volumes - Table 1).

Table 1: Volumes of blood and cells used in the IHA test.

10mℓ of blood needed 10mℓ of Alsever's solution

10mℓ of blood gave 3-4mℓ of packed RBC

1mℓ of packed RBC gave 4mℓ of 25% RBC (for storage)

4mℓ of 25% RBC gave 40mℓ of 2.5% RBC (for tanning/sensitising)

40mℓ of 2.5% RBC gave 133mℓ of 0.75% RBC (for use)

Each plate needed about 5mℓ of RBC (0.75%)

∴ 133mℓ of 0.75% RBC was enough for 25 plates

∴ 10mℓ of blood was enough for 75 plates

2:4:1:3 Inactivation and Absorption of the Sera used in the IHA Technique

Before use the serum samples were routinely inactivated by incubating in a water bath at 56°C for 30 minutes to destroy the complement. Smith and Harvey (1977) found this treatment to give clearer results. They were then absorbed by adding equal volume of washed packed sheep erythrocytes suspended in diluent PBS pH 7.2, for 30 minutes at room temperature to remove antisheep antibodies present in the camel serum. After the incubation, the sera were centrifuged at 700 g for 5 minutes. The supernatant was

retained and the precipitate discarded. Such sera were denoted inactivated and absorbed (1 + A). The (1 + A) sera were stored at -20°C until required for use. Due to the addition of the RBC solution the sera were now diluted 1:2.

Normal rabbit serum at a dilution of 1% in PBS pH 7.2, was used as the diluent. Before use, normal rabbit serum was also inactivated and adsorbed in the same way as the camel sera. The presence of 1 + A rabbit serum prevented pan-agglutination.

2:4:2 Preparation of Microdiluters and Microtitre Plates

As stated earlier, a micro system was used for this technique. The microdiluters picked up 0.05mℓ solution and were prepared according to the manufacturers' instructions. Each microdiluter was flamed to dull red luminescence, then quenched in distilled water. Directly prior to use, each one was checked for delivery by dipping it in 0.85 per cent saline then placing it on the maker's delivery testing card (marked blotting paper). If a microdiluter was functioning correctly, the solution it carried quickly came off and onto the card, marking the standard area.

0.05mℓ of diluent was placed into each well of a microtitre tray using a calibrated 0.05mℓ dropper from the microtitre system. 0.05mℓ quantity of the (1 + A) serum to be tested was picked up by a prepared microdiluter which was then placed in the 1st well in the plate. The microdiluter was gently rotated in the well to mix the serum with the diluent, then transferred into the next well of the row. The solutions were also mixed and by repeating this process, a number of serial dilution was made. The last well of each row was always left with no test serum in it as a diluent control. Next, 0.05mℓ drop of sensitized RBCs at a concentration of 0.75 per cent was added to each well and the plate gently rotated to mix the serum and the cells. The plates were left for three hours at room temperature for the pattern to develop. This produced by the settled cells, remained stable for several days if the plates were kept at $4^{o}C$.

2:4:3 Titration of antigen

Materials:

Conical centrifuge tubes (15mℓ, graduated)

Cylindrical centrifuge tubes (15mℓ, marked

at 10mℓ and 3.7mℓ)

Centrifuge

Microtitre plates (V or U-wells) + lid

Diluters (50 microlitres)

Dropping pipettes (25 or 50 microlitres).

Automatic adjustable sampler and tips.

Vials (900 microlitres) of 25% suspension of fixed sheep RBCs.

Tannic acid solution 1:20,000	25mℓ
PBS PH 6.8 (RG2)	30mℓ
PBS PH 7.2 (RG2)	235mℓ (enough to make diluent)
Haemagglutination diluent (RG5)	100mℓ

Known T.evansi +ve serum. 50 microlitres.

RG5, +ve and -ve sera were 1 + A.

Method:

1. The vial of frozen RBC was thawed. Into one conical centrifuge tube labelled "A" RBCs were put and topped up to 9.0mℓ with PBS PH 7.2 into the other centrifuge tube labelled "B" was put 9.01mℓ of PBS PH 7.2

2. The tubes were centrifuged opposite each other at 700g for 5 minutes.

3. Supernatant from "A" was decanted and PBS from "B" added to "A". The cells were resuspended and 1.0mℓ removed from "A" transferred to a cylindrical centrifuge tube labelled "untanned RBCs".

4. 4.0mℓ were transferred from "A" into tube "B". 4.0mℓ of 1:30,000 tannic acid were added to each tube. After thorough mixing, the cells were left at room temperature for 15 minutes.

5. Tubes "A" and "B" were centrifuged against each other at 700g for 5 minutes. The supernatant was decanted and a little of PBS PH 6.8 was added to each tube. The cells were resuspended and the contents of "B" were added to tube "A", washing out the tube with a little PBS PH 6.8 so as not to lose any cells.

6. 8.0mℓ of PBS PH 6.8 was added to tube "A". Tube "A" was filled to 8.0mℓ with PBS PH 6.8 and centrifuged against B containing 8.0mℓ of water only, at 700g for 5 minutes.

7. Eight Cylindrical centrifuge tubes were labelled: $^{1}/_{20}$, $^{1}/_{40}$, $^{1}/_{80}$, $^{1}/_{160}$, $^{1}/_{320}$, $^{1}/_{640}$, $^{1}/_{1280}$

and "Saline". Into the first tube was put 1.5mℓ of PBS PH 6.8. Into the other seven tubes was put 1.0mℓ PBS PH 6.8.

8. Using the automatic sampler, 0.5mℓ of antigen was added to 4.5mℓ of PBS PH 6.8 and mixed (This gave $^{1}/_{10}$ dilution). Into the first tube was put($^{1}/_{20}$) 1.5mℓ of $^{1}/_{10}$ antigen solution. The contents of this tube were thoroughly mixed, and then 1.0mℓ removed and added to the tube labelled "untanned RBCS". Another 1.0mℓ aliquot was taken from the $^{1}/_{20}$ tube and transferred to the next ($^{1}/_{40}$) tube. The contents of this tube were thoroughly mixed.

9. 1.0mℓ was taken from the $^{1}/_{40}$ tube and added to the next ($^{1}/_{80}$) tube. It was repeated with the next tube $^{1}/_{80}$ tube. It was repeated with the next tube $^{1}/_{160}$ and so on, up to the $^{1}/_{1280}$ tube. 1.0mℓ was removed from the $^{1}/_{1280}$ and discarded. Nothing was added to the tube labelled "saline". At the end there was 1mℓ of diluted antigen in each of the seven labelled tubes, plus one containing 1.0mℓ of saline only. Also there was one tube containing 1.0mℓ of untanned RBCS plus 1.0mℓ of $^{1}/_{20}$ of antigen solution

10. Centrifuge tube "A" (from step 6) was then taken and supernatant decanted and PBS PH 6.8 was added. The cells were then suspended. 1.0mℓ cell suspensions of this tube were added to tubes labelled $^{1}/_{20}$ up to $^{1}/_{1280}$ including the one labelled "Saline". Nothing was added to tube labelled "untanned RBCs".

11. The tubes were shaken gently to mix the contents and then left at room temperature for one hour. The tubes were shaken every 15 minutes to mix the contents.

12. All the tubes were centrifuged at 700g for 5 minutes thereafter. The supernatant was decanted from each tube and 10.0mℓ of PBS PH 7.2 added to each tube. The cells were resuspended. The cells were again centrifuged at 700g for 5 minutes.

13. The supernatant was decanted from each tube then haemagglutination diluent added to each tube to the 3.7mℓ mark. Centrifugation was continued again at 700g for 5 minutes.

14. Step 13 above was repeated.

15. The supernatant was decanted again. Haemagglutination diluent was again added to each tube. The cells were resuspended. These cells were now ready for use in the IHA (0.75%).

16. 50 microlitres of the haemagglutination diluent were put into each well of the microtitre plate excluding column 11 (see Fig. 3).

17. Into a small tube or vial 450 microlitres of haemagglutination diluent were put. Using a 50 microlitre diluter 50 microlitres of +ve serum were transferred into the vial or tube above. The contents were mixed well. This gave $^{1}/_{10}$ dilution of the serum. The same process was repeated for the -ve serum, but instead 1.0mℓ of the diluent was used (This gave a ½ dilution).

18. Using diluters doubling dilutions of the +ve serum were made in the plate as in (Fig. 3). For each row dilution was stopped at column 9. Another doubling dilution of the +ve serum was made down column 12- and stopped at well G 12.

19. 50 microlitres of $^{1}/_{20}$ -ve-serum was added from a dropping pipette to each well in column 11.

20. 50 microlitres of $^{1}/_{20}$ sensitised RBCS were added (using a dropping pipette) to each well of row A up to and including A 11. 50 microlitres of $^{1}/_{40}$ sensitised RBCs were added to row B up to and including B 11, and so on down to row H (saline).

21. 50 microlitres of untanned RBCs were added to each well of column 12.

22. The plate was agitated gently and covered. The plates was left undisturbed for three hours at room temperature or at 4^{o}C over night. Results were scored as in Table 2. The optimum antigen concentration for sensitization was taken to be the concentration which gave maximum activity with the standard +ve-serum but no activity against standard -ve-serum at $^{1}/_{20}$.

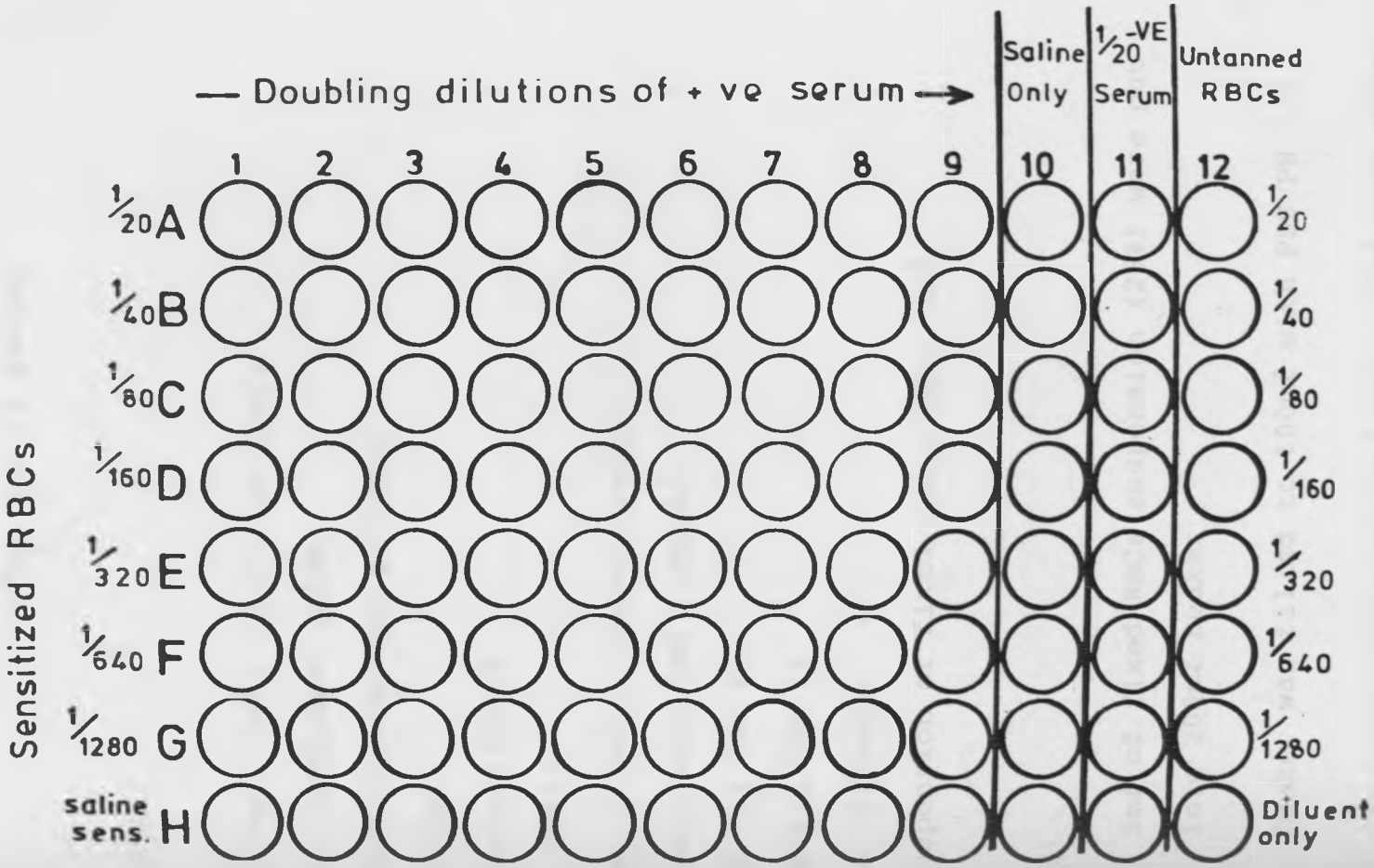

Fig 3. Antigen titration

2:4:4 Bulk sensitisation of cells

(enough for 25 plates)

Materials:

Centrifuge tubes (graduated 50mℓ)

Beaker (graduated 200mℓ)

Magnetic stirrer and follower

Centrifuge

Dispensor (1.8mℓ)

Vials (2mℓ)

Antigen solution (pre-titrated)

1% tannic acid (RG 4 stock)

PBS PH 7.2 (RG 2)

PBS PH 6.8 (RG 2)

Liquid nitrogen

25% suspension of fixed sheep RBSc (5mℓ)

Method:

1. 5mℓ of fixed RBCs suspension (25%) were put into 200mℓ beaker

2. Beaker was filled to 100mℓ with PBS PH 7.2

3. 250 microlitres of 1% tannic acid were added, and mixture stirred for 15 minutes at room temperature.

4. The cells were transferred to two 50mℓ centrifuge tubes and centrifuged at 700g for 5 mins.

5. Supernatant was decanted and PBS PH 6.8 added to a 50mℓ mark in each tube. The cells were resuspended and the contents of each tube transferred to a 200mℓ beaker.

6. The antigen was added as follows:
Vol. of antigen added = dilution required
x 50 x 1000 - microlitres.

7. Stirring was continued slowly at room temperature for one hour.

8. The cells were transferred to two centrifuge tubes and centrifuged at 700g for 5 mins. The supernatant was decanted and the tube refilled to a 25mℓ mark with PBS PH 7.2

9. Step 4 was repeated three times.

10. The contents of both tubes were transferred to a beaker or dispenser. While stirring continuously, 1.8mℓ aliquots were dispensed into 25 vials.

11. The vials were put into a suitable container and placed in the gas phase of liquid nitrogen over-night.

12. Thereafter, the cells were removed and stored at -20^{o}C until required.

2.4.5 Testing of Unknown Sera

Materials:

Microlitre plates (V or U-shaped wells)
+ lids
Pre-sensitized cells
Haemagglutination diluent
Conical centrifuge tubes (15mℓ, graduated)
Centrifuge
Diluters (50 microlitres)
Dropping pipettes (25 microlitres)
Distilled water and normal saline for diluters
Standard +ve serum
Standard -ve serum
Unknown sera
PBS PH 7.2 (RG 2).

Method:

1. Each vial of presensitised cells was washed into a centrifuge tube. Each centrifuge tube was then topped up to 6.0mℓ with haemagglutination diluent. The tubes were then centrifuged at 700 g for 5 minutes. The supernatant was tipped off from each tube and the tubes refilled to 6.0mℓ. The cells were resuspended. These cells were then used in the test.

2. 50 microlitres of the haemagglutination diluent were put into each well of the microtitre "test" plate.

3. A microtitre "pre-dilution" plate was used in all cases. For each serum (both standards as well as test sera) 75 microlitres were put into the first of a series of three wells, and then 50 microlitres were put into the remaining two. (This ultimately gave a pre-dilution of 1/10 before transferring to the test plate).

4. A 50 microlitre diluter was used to transfer 50 microlitre of serum into the first well of the pre-dilution series (this puts 50 microlitres with 75 microlitres thereby making 50/125 dilution which is equivalent to 2/5). The diluter was left in the well and the process repeated for the next and remaining sera, each into the first well of a series of three pre-dilution wells. The diluter was left in the well in each case.

5. Doubling dilutions were made by transferring and mixing each diluter into the

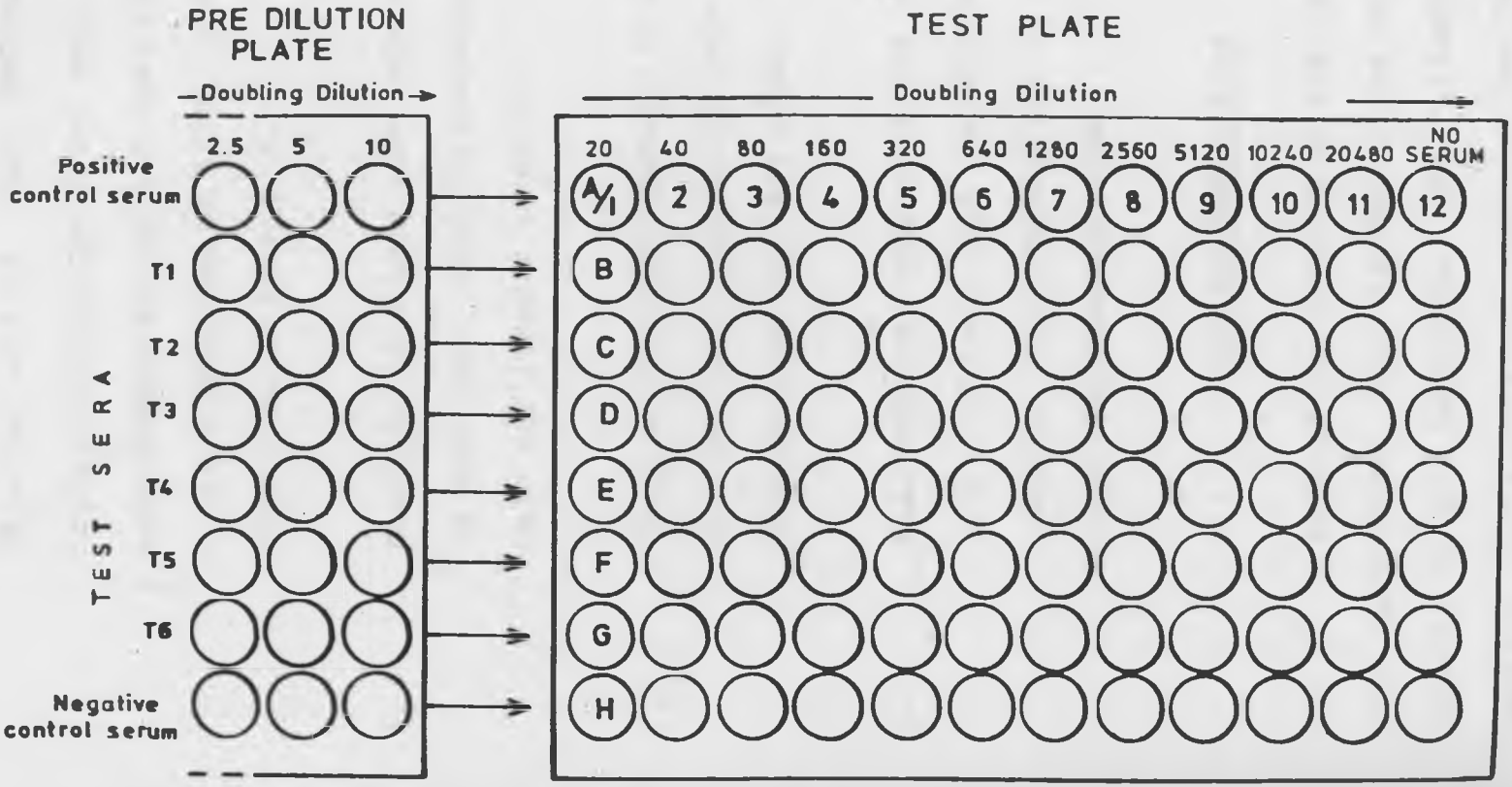

Fig. 4. Testing Unknown Sera

next well and next to give a final pre-dilution of 1/10. Each diluter was then transferred to the first well of the appropriate row in the test plate. This gave a first dilution of 1/20. A suggested layout is as in Fig. 4.

6. Doubling dilutions were made up to well 11 in each row. Well 12 contained diluent only (as reagent control).

7. Using a dropping pipette, 50 microlitres of mixed cell suspension were put into each well on the test plate.

8. The plate was then agitated gently, covered with a lid and left undisturbed for three hours at room temperature or at $4^{o}C$ overnight and read as below.

2.4.6 Reading the Plates

Reading the results of IHA is always somewhat subjective. This is one of the major disadvantages of this technique. The following method of scoring the plates was employed (see Table 2).

Table 2: Reading the Plates

+++	positive	A smooth mat of cells with the edges collapsed to varying degrees
++	positive	A smooth mat of cells at the bottom of the well.
+	positive	Most cells agglutinated but a small button of cells can be seen in the bottom of the well.
±	negative	Most of the cells forming a button at the bottom of the well but still some haemagglutination.
-	negative	All the cells forming a button at the bottom of the well - no haemagglutination

The particular point that is taken as the end point or titre for the titration is quite arbitrary and may vary from worker to worker. In this study, the serum dilution in the last well with ++ reading was taken as the titre for that serum. It was considered that this would lead to less variation from day to day.

It is inevitable that in a technique such as this, with many stages, there will be minor differences occurring from day to day in the details of the

technique and this can lead to a variation in the titre as much as +ve or -ve one doubling dilution. The variation in the ++ve reading will be less than that using the lower +ve or -ve end point because of the relatively large amount of antiserum required for a ++ve reading.

Weak serum samples from the field were sometimes not strong enough to give a ++ve reading. However, the presence of antibody could still be detected by noting the last well with any haemagglutination at all.

2:4:7 The haemagglutination inhibition (HI) technique

Boyden (1951) pointed out that the addition of a given antigen to its homologous antiserum resulted in the inhibition of the effect of the latter in agglutinating erythrocytes sensitised with this antigen and that this effect was specific. The degree to which a given antigen will inhibit a homologous antiserum from agglutinating erythrocytes sensitised with this antigen can be used as a measure of concentration of the antigen in solution.

Briefly, an aliquot of a solution which may or may not contain antigen is added to an equal aliquot

of antiserum, the latter being at a concentration greater than at its end point in the IHA technique. To the mixture is then added tanned, antigen coated (sensitised) RBCs. If there was no or very little homologous antigen in the first solution, haemagglutination will occur. However, if there was sufficient antigen present to neutralise all the antibody in the antiserum, no haemagglutination. This test was performed using serum from a camel which was known to be suffering from chronic surra (Kulal No. 6), from which results were positive with mouse inoculation on several occasions and had given a high titre in the IHA technique. The T. evansi KETRI 2429 protein extract (described earlier) was used as the antigen (inhibitor). Bovine serum albumin was used as the control.

2:4:8 Quantities

In the table of quantities below +ve and -ve control sera were put only in one plate in a series. This necessitated bulking the sensitised cells after making up to volume and using all the other reagents (e.g. haemagglutination diluent) from the same stock. Separate serum control had to be put up, otherwise, for each batch of reagent used. In each case provision was made for a little more than was actually required.

Table 3: Quantities used in the IHA

UNKNOWN Sera	TEST PLATES	DILUTING PLATES	0.75% RBC		DILUENT			Centrifuge Tubes
			Vol (mℓ)	Vials	Vol (mℓ)	Rabbit Serum 1mℓ vial	Rabbit Serum 2mℓ vials	
6	1	1	6	1	20	-	1	1(+1)
14	2	1	12	2	40	-	2	2
22	3	1	18	3	60	-	3	3(+1)
30	4	1	24	4	80	-	4	4
38	5	2	30	5	100	-	-	5(+1)
46	6	2	36	6	120	1	1	6
54	7	2	42	7	140	1	2	7(+1)
62	8	2	48	8	160	1	3	8
70	9	3	54	9	180	1	4	9(+1)
78	10	3	60	10	200	2	-	10

2:4:9 The controls for the Indirect haemagglutination test

The controls used for the haemagglutination test are summarised in Table 4. Only the TAg+ cells with positive serum should haemagglutinate, all the other control titrations should be negative, as should diluent control included in each row. However, as these were almost always negative, only two of the controls were set up routinely, these being TAg+ cells with the standard positive and negative control sera. In

addition, each row included a diluent control. The inclusion of these controls with each test also allowed a day to day check to be made in the titres.

Table 4: Controls for use with the IHA test

Type of cells added	Serum in serial dilution in plates	
* TAg+	Positive Serum	Test
TAg-	"	Control
NTAg+	"	"
NTAg-	"	"
* TAg+	Normal Control Serum	Control
TAg-	"	"
NTAg+	"	"
NTAg-	"	"

T - tanned rbc's

NT - not tanned rbc's

Ag+ - cells coated in antigen

Ag- - cells not coated in antigen, i.e. TAg+ - tanned coated in antigen

* - controls used in all studies

2:5 Haematological Examination

Camel blood for haematological examination was preserved in disodium EDTA. Where possible, haematological examination was always done on the same day after bleeding the camels. However, because of transport problems, sometimes the examination was delayed. This resulted into some samples getting spoilt or lost.

Packed cell volume (PCV) or haematocrit, was determined by the Hawksley micro-centrifuge technique (Boddie, 1969). This technique was chosen because of its simplicity and the ease with which it could be applied on many blood samples at ago. Secondly, this method is very accurate and produces reproducible PCV values because there is less trapping of serum (Schalm, Jain and Carroll, 1975). The packed cell volume results were expressed as percentage.

Red blood cell (RBC) and white blood cell (WBC) counts were done using a Coulter Counter, Model ZBI (Coulter Counter Electronics, Inc. Hialeah, Florida). Haemoglobin determination was done using a haemaglobinometer (Coulter Counter Electronics Inc. Hialeah, Florida).

Only the results of PCV and WBC were employed for this study. It was deemed necessary to compare the PCV

and WBC volume and the serological results on one hand, and the mouse inoculation results on the other.

2:6 Mercuric Chloride Test

This test was carried out as described by Bennet (1933). One drop of undiluted camel serum was added to 1mℓ of freshly prepared 1:20,000 solution of mercuric chloride. The test was carried out in glass test-tubes (19 x 75mm). The contents of the tubes were mixed well and examined after 15 minutes. Only sera with distinct opalescence were considered as positive for trypanosome infection.

2:7 Mouse Inoculation Test

Mouse inoculation was done in the field after the bleeding of the camels. Inoculation was done in pairs for each blood sample. Inoculated mice were placed in clean cages and given food and water ad-libitum. Parasitaemia of such mice was always checked daily from tail blood by microscopic examination of thick and thin blood smears. Parasitaemic mice were always noted and trypanosome species from such mice identified by microscopic observation on grounds of morphology, motility and by Isoenzyme Starch gel eletrophoresis (Gibson, et al., 1978)

CHAPTER 3

RESULTS

3:1 The Indirect haemagglutination (IHA), mercuric chloride (MC) and mouse inoculation (MI) tests

The results of the IHA test using known positive and negative sera were always reproducible. The positive control serum (from KULAL camel No. 6) for the IHA test gave a maximum IHA titre of 1:1280. This control serum was also strongly positive with the MC test and the same camel had shown patency with the MI test on several occasions. The negative control serum used (from a camel which had been kept at KETRI, MUGUGA) gave a maximum IHA titre of 1:20, was MC negative and this camel was also negative on mouse inoculation. Serum from a newly born camel calf (before suckling) gave a maximum IHA titre of 1:10. Since the test sera in the IHA test were diluted in a doubling manner, a titre of 1:40 was taken as being positive for *T. evansi* infection in camels. This has been recommended earlier by Jatkar and Singh (1971) and by Verma and Gautama (1977).

According to the method of Lowry *et al*. (1951) the crude extract from *T. evansi* (Strain 2429) used in the IHA test, had a protein concentration of

1.8mg/mℓ. After titration of this stock antigen, a dilution of 1:1280 gave a maximum IHA activity with the standard positive serum, but no IHA activity the standard negative serum at 1:20 dilution. This was taken as the optimum antigen concentration (i.e. 14.7µg/mℓ) and was always used to sensitise the sheep RBCs for the IHA test.

The results of the haemagglutination inhibition test were satisfactory. Thus the heterologous protein, bovine albumin used at a concentration of 500µg/mℓ had no effect on the maximum haemagglutination titre. On the contrary, homologous antigen (T. evansi extract) at a concentration of 73.5µg/mℓ completely inhibited the reaction proving that the reaction was specific.

A total of 2100 samples were collected during the study period. Of these, the MI test detected 133 patent samples (6.3%). The IHA test detected 1005 positive samples (47.9%). Of the 133 MI positive samples, IHA test detected 126 (94.7%) and the MC test detected 88 (66.4%) positive samples. In this study, the IHA test detected more positive samples than the MC test. The MI test detected the least number of positive samples. The IHA test detected a higher percentage (94.7%) of the patent samples than the MC test (66.4%).

The IHA was therefore by far more sensitive in detecting patent cases than the MC test.

3:2 Point Prevalence Rates (PPR)

Tables 5-8 show the PPR of trypanosomiasis for each camel herd as judged by the MC and MI tests and by the presence of the IHA (haemagglutinin) antibody. The PPR in this case is defined after Schwabe et al. (1977) as below.

$$\text{PPR} = \frac{\text{No. of disease cases existing in a population at a point in time.}}{\text{No. of animals in that population at that same point in time.}}$$

A graphic presentation of PPR for the 4 herds is given in figures 5-8. The PPR for KULAL herd was much higher than any of the other herds over most of the study period, as judged by the MI test and to a considerable extent by the IHA and MC tests. However, the PPR steadily dropped and then stabilised up to the end of the study period. As judged by the IHA and MC tests, the PPR shows a November-December peak and a May-June peak, seeming to coincide with the two main wet seasons of this area. In NGURUNIT and GALANA herds, the PPR was fairly low throughout the

study period as judged by the MI. However, though the PPR for NGURUNIT herd, as judged by the IHA and MC tests is also fairly low, two small June and January peaks can still be seen. In OLMAISOR herd the PPR showed a dramatic rise over the period of June to October, 1980. However, thereafter, the PPR stabilised over the rest of the study period. Even then, two small peaks can also be seen, one in October and the second in March (as judged by the IHA and MC tests).

The sampling time intervals for NGURUNIT, GALANA and OLMAISOR herds were sometimes fairly long and not consistent. For this matter, the PPR-results of these herds must be examined with caution.

Table 5: Point Prevalence Rates (PPR) for Kulal herd over the indicated study period, as judged by the IHA, MC and MI tests.

Date of serum collection	No. of animals sampled	No. of Positive cases			PPR (%)		
		IHA	MC	MI	IHA	MC	MI
NOV. 79	21	17	8	8	81.0	38.1	38.1
DEC. 79	20	14	9	9	70.0	45.0	45.0
MAR. 80	21	10	8	9	47.6	38.1	42.9
APR. 80	20	14	7	9	70	35	45.0
MAY 80	21	13	10	8	61.9	47.6	38.1
JUL. 80	21	16	8	2	76.2	38.1	9.5
AUG. 80	21	10	7	-	47.6	33.3	-
SEPT.80	21	9	7	2	42.9	33.3	9.5
OCT. 80	21	11	9	2	51.4	42.4	9.5
NOV. 80	20	14	10	-	70.0	50.0	-
JAN. 81	21	12	11	2	57.1	52.4	9.5

Table 6: Point Prevalence Rates (PPR) for NGURUNIT herd over the indicated study periods judged by the IHA, MC and MI tests.

Date of serum collection	No. of animals sampled	No. of Positive cases			PPR (%)		
		IHA	MC	MI	IHA	MC	MI
DEC. 79	42	31	21	6	73.8	50.0	14.3
FEB. 80	26	19	13	4	73.0	50.0	15.4
MAR. 80	22	15	8	NIL	68.2	36.4	NIL
MAY 80	107	46	42	6	43	39.3	5.6
JUN. 80	41	31	26	-	75.6	63.4	-
JUL. 80	107	30	21	3	28.0	19.6	2.8
SEPT.80	115	48	38	-	41.7	33.0	-
NOV. 80	118	24	16	4	20.3	13.6	3.4
JAN. 81	120	32	27	3	26.7	22.5	2.5

Table 7: Point Prevalence Rates (PPR) for OLMAISOR herd over the indicated study periods judged by the IHA, MC and MI tests.

Date of serum collection	No. of animals sampled	No. of positive cases			PPR (%)		
		IHA	MC	MI	IHA	MC	MI
JUN. 80	91	51	28	7	56.0	30.8	7.7
OCT. 80	69	50	20	19	72.5	29.0	27.5
NOV. 80	142	66	51	9	46.5	35.9	6.3
FEB. 81	136	67	57	5	49.3	41.9	3.7
MAR. 81	145	90	68	6	62.1	46.9	4.1

Table 8: Point Prevalence Rates (PPR) for GALANA herd over the indicated study periods judged by the IHA, MC and MI tests.

Date of serum collection	No. of animals sampled	No. of positive cases			PPR (%)		
		IHA	MC	MI	IHA	MC	MI
JUN. 80	133	65	54	3	48.9	40.6	2.3
AUGU.80	143	52	44	2	36.4	30.8	1.4
NOV. 80	151	69	34	1	45.7	22.5	0.67
JAN. 81	164	79	55	4	48.2	33.5	2.4

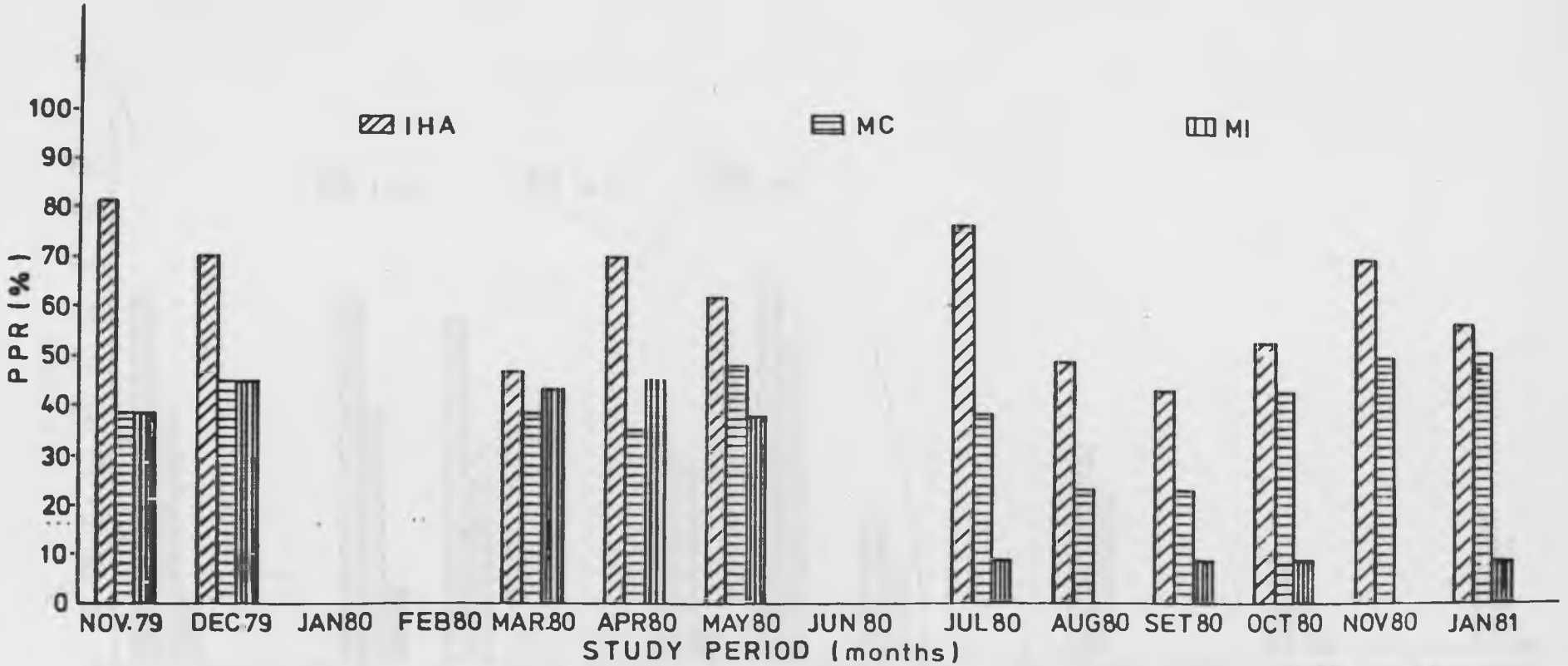

Fig.5. Point Prevalence Rates (PPR) for Kulal herd judged by IHA, MC, and MI tests

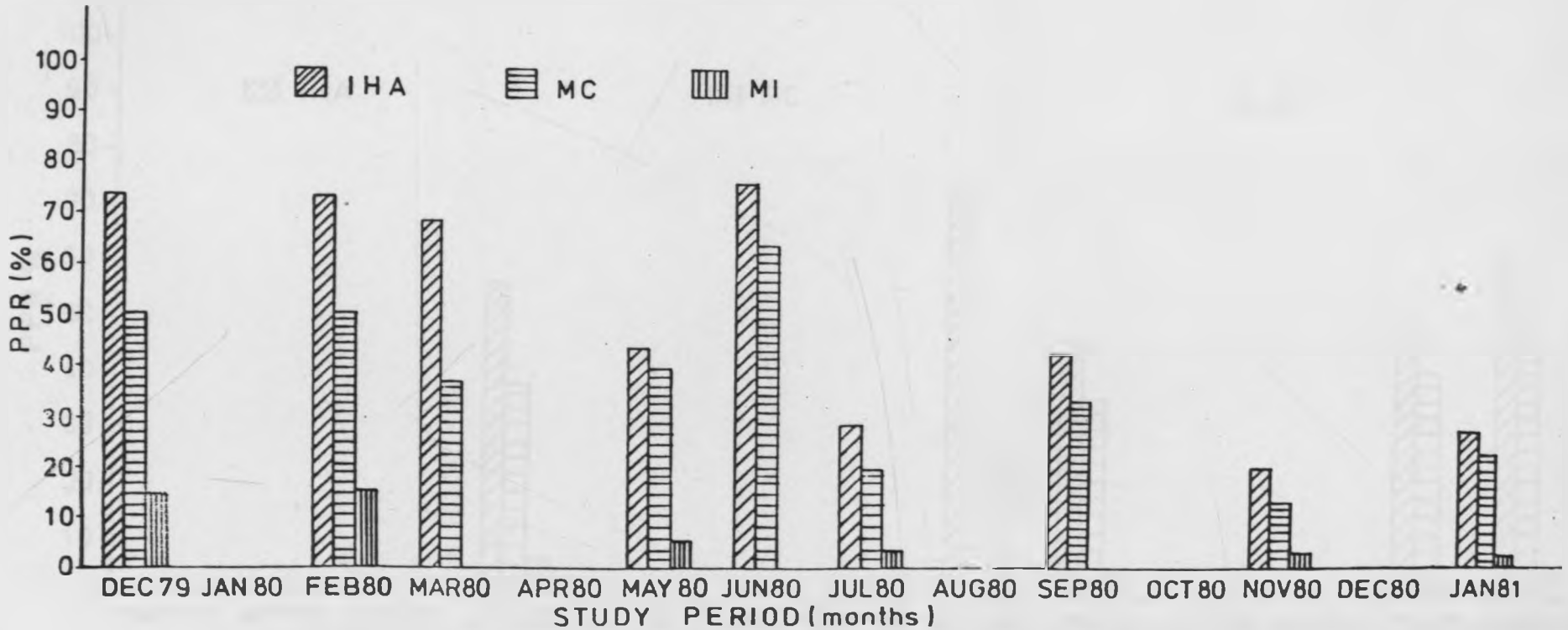

Fig.6. PPR (%) for Ngurunit herd as judged by IHA, MC, and MI tests.

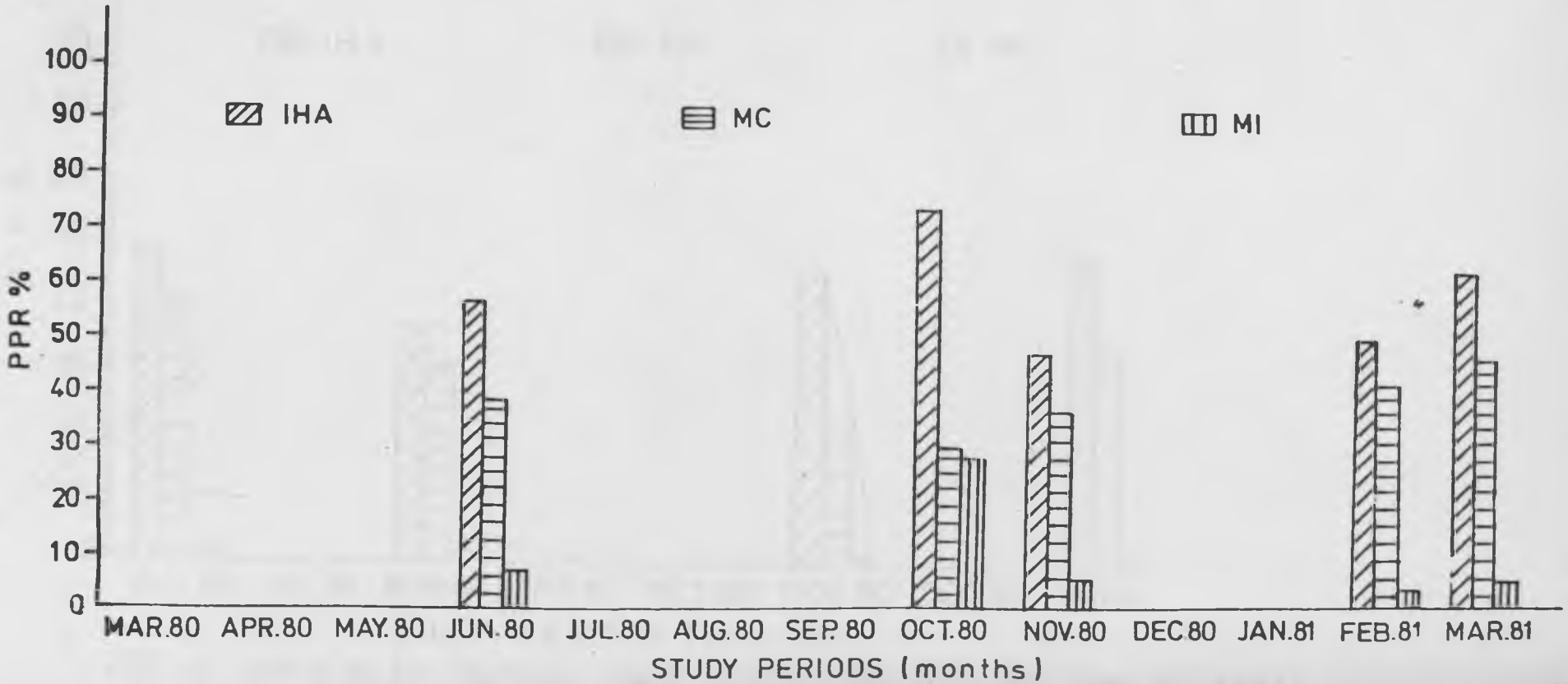

FIG. 7. PPR % Olmaisor herd judged by IHA, MC, and MI tests.

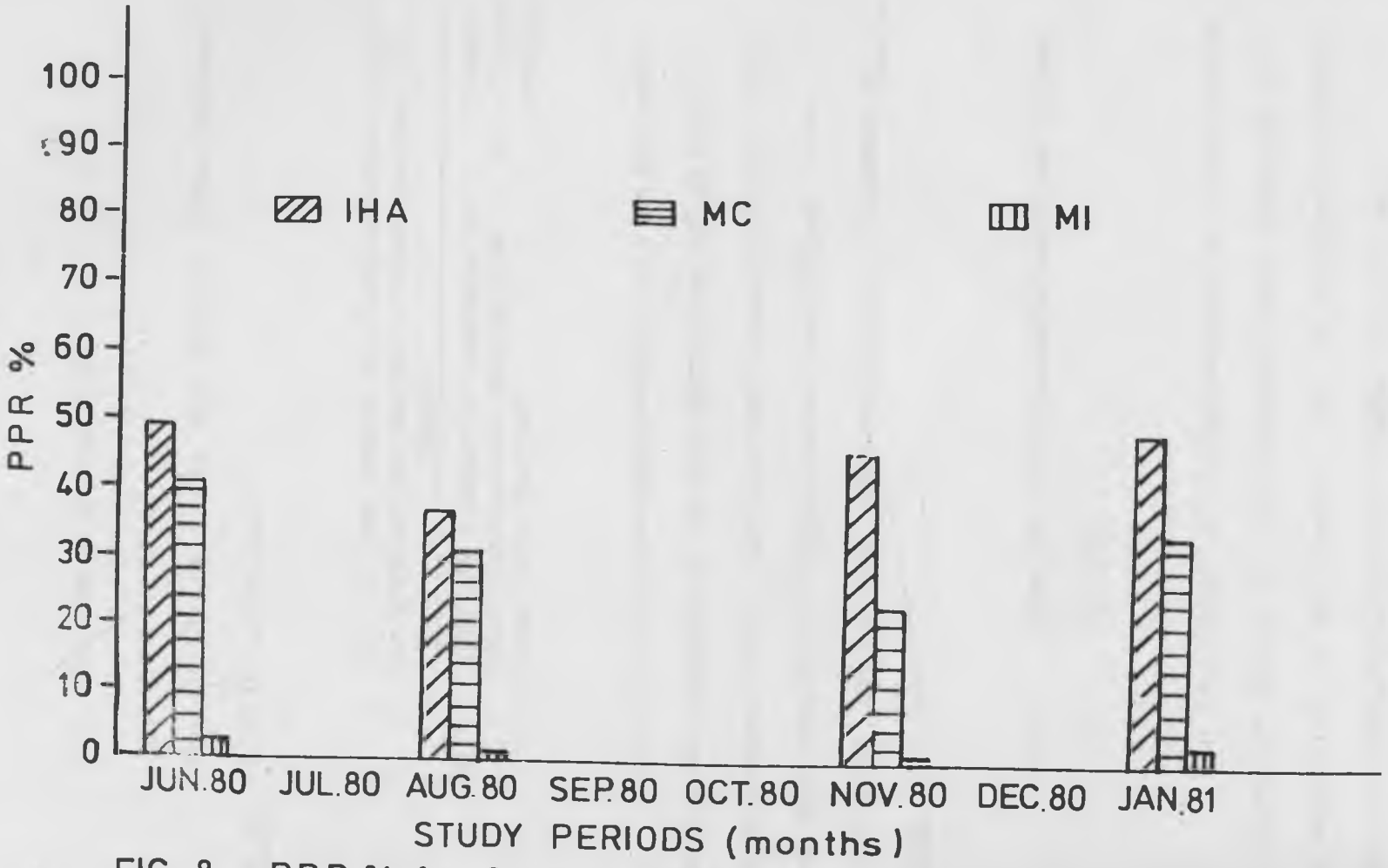

FIG. 8. PPR % for Galana herd judged by IHA, MC, and MI tests.

3:3 Incidence Rates (IR).

The incidence rates are defined after Schwabe et al. (1977) as below:-

$$IR = \frac{\text{No. of new cases of disease which occur in population during stated period of time}}{\text{Average number of animals in that population during the same time period}} \times \text{Length of time period.}$$

The IR of trypanosomiasis in all herds were determined according to the detection of patent parasitaemia (MI), circulating haemagglutinin (IHA) and the species of trypanosome identified i.e. Isoenzyme starch gel electrophoresis (Gibson et al 1978).

3:3:1 The IR of trypanosomiasis as judged by IHA.

The incidence of trypanosomiasis in all herds is given in table 9. The results show that the IR is very high in all herds. It is highest in KULAL (95.5%) herd, followed by GALANA (80.6%) and NGURUNIT (79.4%) and lastly, OL MAISOR (73.2%).

3:3:2 The IR of trypanosomiasis as judged by patent parasitaemia (MI)

The IR for all herds as judged by MI is given in table 9. The IR is again highest in KULAL herd (71.4%). However, OLMAISOR herd is second (21.7%) followed by NGURUNIT (9.7%) and lastly, GALANA herd (5.9%).

3:3:3 The IR as judged by the species of trypanosome identified

The type of trypanosome infecting camels in all herds belonged to the Trypanosoma brucei sub-group (see table 10) and had similar isoenzyme patterns as the South American T. evansi (Gibson, 1981; Wilson et al., 1981). Though transmission experiments (in tsetse flies) on these isolates are not yet completed, these isolates are considered to belong to T. evansi. T. evansi was isolated in 100% of infected mice inoculated with camel blood from KULAL and NGURUNIT camel herds. However, the dangerous T. congolense was isolated from both OLMAISOR and GALANA camel herds (2.6 and 16.6% respectively). Of the T. congolense patent cases, 5 were not positive with IHA and the rest gave weak titres.

3:3:4 IR with age

Table 11 shows the results of IR of trypanosomiasis in different age groups (calves, immatures and adults) as judged by the MI, and the IHA antibody over the study period. In KULAL herd, all segments of the herd show a very high IHA incidence. However, there is evidence of MI incidence in all segments of the herd. The immatures and the calves show a higher MI incidence than the adults. Because of the small number of camels in this herd, the results of this herd should be interpreted with caution.

In NGURUNIT herd, all segments of the herd show a high IHA incidence. However, there is evidence of MI incidence in the adults and immature segments of the herd only. The calves show a nil MI incidence.

The results of OLMAISOR herd show a higher IHA incidence among the adults than the immatures and calves. Furthermore, like in KULAL herd, there is evidence of MI incidence in all segments of the herd.

The results of GALANA herd are similar to those of NGURUNIT herd. Thus there is a high IHA incidence in all segments of the herd, being highest among the calves.

However, like in NGURUNIT herd, only the adults and immature segments of the herd show evidence of MI incidence. The calves show a nil MI incidence.

3:4 IHA titre with age

In this study, the IHA test was employed to measure the amount of trypanosome antibodies (haemagglutinin) present in the serum. The higher the IHA titre, the higher the trypanosome antibodies in the serum. Table 12 shows the frequency IHA titre with age in all herds over the study period. Both KULAL and NGURUNIT herds had very small numbers of calves. For this reason, the results for calves of these herds should be examined with caution. Nevertheless, there appears to be a higher frequency of IHA titre in the calves and immatures that in the adults, in all the four herds. The results of table 11, with the exception of OLMAISOR herd, also confirm these observations.

3:5 Possible categorisation of camel trypanosomiasis (T. evansi) into types with reference to KULAL herd

The KULAL herd had the greatest sampling frequency. For this reason, it was used to attempt to categorise camel trypanosomiasis into types named below. The 21 camels were typed according to the clinical picture, percentage of samples positive on MI, mean PCV and WBC Count and the presence of the IHA antibody over the entire period of the study. Table 13 shows these results. The animals were divided into 4 distinct types namely:-

(a) Type 1 (9.5%) - defined as acute trypanosomiasis followed by death and is characterised by a high patency rate on MI, a low PCV and marked leucocytosis.

(c) Type 2 (47.6%) - defined as chronic trypanosomiasis, showing some clinical signs (emaciation) and characterised by a high patency rate on MI, a marked leucocytosis and low PCV.

(c) Type 3 (14.3%) - defined as chronic

trypanosomiasis, showing no clinical signs and characterised by a low patency rate on MI, a normal PCV and some leucocytosis

(d) Type 4 (28.6%) - defined as chronic trypanosomiasis, showing no clinical signs and characterised by a nil patency rate on MI and a normal PCV and WBC count values.

The frequency of IHA titre in the four types of camel trypanosomiasis are shown in Table 14.

All types show the presence of IHA antibody. Types 1 and 2 have higher and more frequent levels of IHA antibody than those of types 3 and 4.

3:6 Variance of PCV and total WBC counts between the 4 herds over the whole study period.

Table 15 shows the analysis of variance of PCV and total WBC counts between 4 herds. The mean PCV for GALANA herd was normal. The PCV means for NGURUNIT and OLMAISOR herds were below their normal values. However, the difference between their values is not statistically significant. The PCV mean for KULAL herd was below normal. Statistical significance exists between the differences of mean PCV for KULAL herd and the other 3 herds and between the differences of mean PCV for GALANA herd and the other 3 herds.

The mean WBC counts of KULAL and NGURUNIT herds show moderate leucocytosis. However, the difference between their mean WBC counts is not statistically significant. The differences between the means of OLMAISOR and the other 3 herds or between GALANA and the other 3 herds are statistically significant. The mean WBC counts for OLMAISOR and GALANA herds show leucocytosis and slight leucocytosis, respectively.

Table 9: The Incidence of camel trypanosomiasis in all herds over their respective study periods, as judged by mouse inoculation and the presence of IHA antibody.

Herd	Incidence of trypanosomiasis (%)		Period of study
	Mouse inoculation	IHA antibody	
KULAL	71.4	95.2	Nov. 79 - Jan. 81
NGURUNIT	9.7	79.4	Dec. 79 - Jan. 81
OLMAISOR	21.7	73.2	June 80 - Mar. 81
GALANA	5.9	80.6	June 80 - Jan. 81

Table 10: A summary of trypanosome identification on all infected mice obtained from all herds over the study period

Herd	Percentage of isolates detected as	
	Trypanosoma evansi (Trypanosoma brucei Sub-group)	Trypanosoma congolense
KULAL	100%	NIL
NGURUNIT	100%	NIL
OLMAISOR	83.4%	16.6%
GALANA	97.4%	2.6%
	$\bar{x}$ 95.2%	$\bar{x}$ 4.8%

Table 11: The incidence rates (%) of trypanosomiasis with age in all herds over their respective study periods, as judged by MI and the presence of IHA antibody

Herd	ADULTS			IMMATURES			CALVES		
	No.	MI	IHA	No.	MI	IHA	No.	MI	IHA
KULAL	12	58.3	91.6	4	100	100	5	80.0	100
NGURUNIT	121	11.6	78.5	46	6.5	78.3	7	NIL	100
OLMAISOR	69	17.4	84.1	41	39.0	68.3	28	7.1	57.2
GALANA	82	8.5	70.8	35	8.6	85.7	53	NIL	92.5

Table 12: A summary of the frequency of appearance of the different IHA titres with age in the 4 herd over the study period

Herd	Herd structure	No. Animals	Frequency of IHA titre								
			<40	40	80	160	320	640	1280	2560	5120
KULAL	Calves (3m)	0									
	Calves (3-6m)	2	0.09	0.18	0.18	0.05	0.32	0.05			
	Calves (>6m)	3	0.30	0.27	0.21	0.12	0.03	0.06			
	Immatures (>1 yr < 4 yrs)	4	0.07	0.19	0.26	0.07	0.14	0.09	0.09	0.07	0.02
	Adults (≥4 yrs)	12	0.54	0.17	0.16	0.06	0.03	0.02	0.02		
NGURUNIT	Calves (3m)	1	0.20	-	0.40	0.2	-	0.20	-	-	-
	Calves (3-6m)	2	0.60	-	-	0.40	-	-	-	-	-
	Calves (>6m)	4	0.20	0.10	0.30	0.40					
	Immatures (>1 yr<4 yrs)	46	0.58	0.11	0.11	0.16	0.03	0.01			
	Adults (≥4 yrs)	121	0.69	0.08	0.10	0.08	0.03	0.01			
OLMAISOR	Calves (3m)	18	0.17	0.28	0.11	0.11	0.22	0.06	0.05	-	-
	Calves (3-6m)	21	0.56	0.24	0.05	-	0.05	0.05	0.05	-	-
	Calves (>6m)	28	0.62	0.21	0.14	-	-	-	-	-	0.03
	Immatures (>1 yr < 4 yrs)	40	0.30	0.15	0.20	0.03	0.20	0.03	0.06	0.03	-
	Adults (≥4 yrs)	67	0.38	0.15	0.15	0.06	0.16	0.06	0.03	0.01	-
GALANA	Calves (3m))		0.18	0.14	0.21	0.43	0.04				
	Calves (3-6m)	53	0.38	0.44	0.09	0.09					
	Calves (>6m)		0.40	0.27	0.07	0.04					
	Immatures (1 yr < 4 yrs)	35	0.56	0.14	0.19	0.10	0.01	0.01			
	Adults (≥4 yrs)	82	0.66	0.18	0.08	0.06	0.02				

Table 13: The categorization of type of camel trypanosomiasis in KULAL herd (1) over the study period and judged by clinical picture, presence of circulating trypanosomes, and haematology.

Type	Clinical picture	No. of animals (%)	Percentage of samples positive on mouse inoculation	Mean PCV(%) ± SE	Mean WBC ± SE (10^3/cumm)	IHA antibody
1	Acute - death	2 (9.5)	50.0	24.13±0.62	25,350±1256	+
2	Chronic showing some emaciation	10 (47.6)	39.0	24.79±0.58	21,860±1334	+
3	Chronic showing no symptoms	3 (14.3)	21.2	30.56±0.22	17,033±3621	+
4	Chronic showing no symptoms	6 (28.6)	NIL	27.48±1.84	13,050±784	+

Table 14: Summary of the frequency of appearance of the different IHA titres in the four categories of camel trypanosomiasis in Herd 1 over the year November 1979 - November 1980.

Group	Type of disease	No. of animals	IHA TITRES									No. obs
			<40	40	80	160	320	640	1280	2560	5120	
1	Acute - death	2 (9.5)	0.09	0.18	0.18	0.05	0.32	0.14	0.05	-	-	22
2	Chronic-positive MI Low mean PCV	10 (47.6)	0.18	0.21	0.24	0.10	0.10	0.07	0.06	0.01	0.03	110
3	Chronic-positive MI Normal mean PCV	3 (14.3)	0.71	0.03	0.23	0.03	-	-	-	-	-	31
4	Chronic-no positive MI Normal mean PCV	6 (28.6)	0.56	0.25	0.11	0.06	0.02					64

Table 15: An analysis of variance of PCV and total WBC counts between herds over the whole study period.

		Source	df	MS		
(A)	PCV	Between herds	3	1406.2	F^3	75.0 ($P < 0.001$)
		Within herds	1470	18.8	1470	

Herd Means

GALANA	NGURUNIT	OLMAISOR	KULAL
30.89	27.80	27.71	26.43

		Source	df	MS		
(B)	WBC Total Count	Between herds	3	552.3	F^3	42.81 ($P < 0.001$)
		Within herds	1213	12.9	1213	

Herd Means

GALANA	NGURUNIT	OLMAISOR	KULAL
22.14	19.14	19.01	17.60

CHAPTER 4

4 DISCUSSION AND CONCLUSION

4:1 DISCUSSION

According to Gill (1964), Jatkar and Singh (1971), Clarkson et al (1971) and Verma and Gautama (1977), the indirect haemagglutination (IHA) test appears to be a promising test for the serodiagnosis of animal trypanosomiasis. These workers, except Clarkson et al (1971), recommended the use of IHA for the seroepidemiological diagnosis of Surra in camels. Recently, Luckins et al (1978) recommended micro-ELISA and IFAT as being ideal for the diagnosis of camel Surra. However, these tests are more expensive to run than the IHA test. In this study, a micro-scale IHA test was developed and successfully used for the serodiagnosis of camel trypanosomiasis on a large scale (2,100 samples were screened). The test showed a high degree of sensitivity (detected 94.7% of the patent cases) and specificity as observed by Gill (1964), Jatkar and Singh (1971) and Verma and Gautama (1977). Furthermore, the results obtained were always reproducible. The fixing of sheep RBCs with 1% glutaraldehyde was an added advantage as the sensitised cells could be stored at -20°C for long periods and still remain sensitive

(Walther and Grossklauss, 1972; Smith and Harvey, 1977). The test was easy to perform and required no expensive equipment. This is an added advantage for large scale screening in field surveys.

Compared with the MC test, the IHA test detected more positives and more patent samples (94.7 as opposed to 66.4%), proving to be a better tool for the serodiagnosis of camel Surra. However, where other trypanosome infections occur as at GALANA and OLMAISOR, sonicated T. evansi antigen may not be ideal for detecting T. congolense and perhaps other trypanosome infections in camels. Luckins (1977) in his experiment on the detection of antibodies in trypanosome infected cattle using micro-ELISA, observed that T. congolense antigen was the poorest of the trypanosome antigens he used in detecting trypanosome antibodies in bovine test sera. With reference to this study, it therefore means that though the crude T. evansi antigen used is able to detect other trypanosome infections, because of cross-immunity (Luckins, 1977),it may fail to detect, or give very low titre in T. congolense and perhaps other trypanosome infections in camels. However, where the infection is purely T. evansi in nature, it is better to use T. evansi antigen.

The incidence rates as shown in Table 9 indicate that trypanosomiasis is endemic in all the four herds. The isolation of T. congolense at GALANA and OLMAISOR shows that the infection at these places could be mixed, since T. evansi was also isolated (83.4% and 97.4% of the isolates, respectively). At KULAL and NGURUNIT, 100% of the isolates were T. evansi. These results show that the infection is Surra in type and points to mechanical transmission. At GALANA and OLMAISOR, a higher percentage of the infection is Surra (see Table 10) and therefore mechanical transmission is implicated. However, the occurrence of T. congolense points to the fact that tsetse flies may be important in the transmission of camel trypanosomiasis in these two areas since T. congolense is normally tsetse dependant where biological development occurs. From Table 10, it can be seen that the infection in camels is generally of Surra type in all the herds (mean for the four herds is 95.2% for T. evansi, as opposed to 4.8% for T. congolense).

In neighbouring Sudan and Ethiopia, Surra is endemic in camels (Sudan Ministry of Animal Resources, 1960; Pegram and Scott, 1976). This perhaps explains why the incidence rates are highest at KULAL and NGURUNIT.

T. congolense causes an acute disease in camels which is very difficult to cure (Bennet, 1933). The fact that this trypanosome was isolated from some camels in this study, points to its potential danger for the camels in tsetse infested areas of Kenya. As recommended by Bennet (1933) "If deliberate exposure of camels is ever contemplated, an antecendent study of T. congolense infection will be necessary". Such a study had not been carried out in Kenya although camels have already been introduced into tsetse-fly infested areas of this country.

The results of incidence rates (%) of trypanosomiasis with age (Table 11) seem to indicate that a calfhood resistance to trypanosomiasis occurs in NGURUNIT and GALANA herds, as evidenced by a nil MI incidence, coupled with a high IHA incidence among the calves of both herds. This would seem to indicate that maternal trypanosome antibodies play a major role in the observed calfhood resistance in these two herds. In KULAL and OLMAISOR herds, there is evidence of MI incidence in all segments of the herds. In the calves and immature segments of these two herds, the high MI incidence rules out a possibility of calfhood immunity despite the evidence of a high IHA incidence.

The PPR in both NGURUNIT and GALANA herds were low throughout the study period as judged by MI. This indicates that the disease had stabilised at a low level patency. The dramatic rise of PPR over the period of June in OLMAISOR herd indicates that an epidemic was occurring. Though the PPR was high over most of the study period in all herds (as judged by the IHA antibody), a large fall was observed in KULAL, NGURUNIT and GALANA camel herds over the period of May to September, 1980 (Fig. 5, 6 and 8). In OLMAISOR herd, the PPR rose at the same time. The fall of PPR of the IHA antibody could be related to the dry season and the reason that it did not occur in OLMAISOR herd was probably due to the epidemic that was occurring at this time. In Kenya, the annual rainfall regime shows two main peaks; one around March to May, the second one around October and November. The PPR for all herds seem to show elevation around these periods or just after (Fig. 5-8). This elevated PPR may be related to the fly prevalence rates during the same periods. Yutuc (1949) observed that incidence of horse-flies (Tabanus spp.) in the Phillipines was highest after the heaviest rainfall. In British East Africa (East Africa), Leese (1914) observed that Tabanus spp. are abundant during and after the rain season and that their incidence falls rapidly as the dry season advances. Since Surra is transmitted (as cited earlier)

by Tabanus spp., it is logical to expect elevated PPR during the periods when the fly population is elevated (since this study has established that the disease is endemic in the camels of Kenya).

This study has helped to establish that camel trypanosomiasis can be categorised into 4 seemingly distinct types (see Table 13). All these four types showed the presence of IHA antibody and except the fourth type, they all showed the presence of trypanosomes on MI. However, the fact that the fourth type showed no patency on MI test does not rule out infection in these camels. It is possible for the parasites to be scanty or absent from the peripheral circulation in latent infection (Losos and Ikede, 1972). Secondly, it is possible for mice being used in the MI test not to develop parasitaemia or the parasitaemia to be too low to be detected by microscopic examination of the peripheral blood (Losos and Ikede, 1972; Luckins et al 1979).

This study has further proved that camel Surra in Kenya is mainly of the chronic type (3 types, of the chronic form of the disease are given in Table 13, constituting 90.5% of the observed cases in KULAL herd). However, the acute form of the disease was also observed in this herd (9.5%). These results support

the observations of Burke (1891), Pease (1906), Theiler (1906), Gatt Rutter (1967) and Mahmoud and Osman (1979). Of the three chronic types of the disease, type 2 was the commonest (47.6%) and is characterised by emaciation, low PCV(anaemia) and high WBC count (leucocytosis). This type perhaps belongs to the "Surra" stage in the disease description of Pease (1906). The 3rd and 4th types seem to be latent forms of the disease.

From the epidemiological information given in this study, it is possible to assess the stability of the disease in the four herds over the whole study period. The disease appears to be stable at NGURUNIT and GALANA. These two herds show low PPR and the disease in these herds show a low patency. There is little monthly variation in the PPR in these two herds. Calfhood resistance occurs in both herds and there is also evidence of the presence of a high incidence of IHA antibody in all segments of the two herds. Furthermore, analysis of the variance of the PCV and total WBC counts (Table 15), show that GALANA herd had normal mean PCV (30-39). Although the WBC counts for GALANA show slight leucocytosis (17.60), the widespread antibody levels in the calves show that the disease is stable in this herd. In NGURUNIT herd the mean PCV is slightly below normal (27.80) and the mean WBC count shows moderate leucocytosis (19.01).

However, because there is a widespread high antibody level in all age groups of this herd and the PPR do not fluctuate alot, the disease is stable in this herd too.

The disease is unstable in the animals of KULAL and OLMAISOR herds. This is seen from the fact that point prevalence rates fluctuate from month to month (figures 5 and 7). Secondly, both herds show a high patency. Although KULAL herd shows the highest incidence of the IHA antibody, the high patency of the disease and the low PCV (anaemia) suggest that the disease is unstable in this herd. In OLMAISOR herd, the mean PCV is slightly below normal (27.71) and the mean WBC counts elevated (Leucocytosis). However, since the IHA antibody levels of the calves of this herd are low, maternal antibodies do not play a role in the epidemiology of the disease. Therefore the disease is unstable in this herd too.

It is hard to tell why the disease is stable in some herds and unstable in others. Management differences, fly incidence, trypanosome species and strain differences may be some of the reasons. Secondly, poor drug treatment regimens may destablise the disease stability by interfering with premunity and by precipitating the occurrence of drug resistant strains.

4:2 CONCLUSION

Verma and Gautama (1977) recommended the use of IHA for seroepidemiological survey of camel Surra. In this study IHA has been successfully employed on large scale survey of camel trypanosomiasis in Kenya and hence emphasising again its potential value. The results of this study have confirmed that Surra in the camels of Kenya is endemic supporting the earlier report of its occurrence by Fazil (1977). Furthermore, the more dangerous Trypanosoma congolense was also detected confirming the early reports of Bennett (1933). In the camels which are kept in tsetsefly free areas (KULAL and NGURUNIT) the disease was 100% Surra (by isoenzyme characterisation). However, in the camels kept in tsetse-fly infested areas (GALANA and OLMAISOR) both T. congolense and T. evansi were isolated. T. evansi constituted 95.2% of all the isolates and T. congolense only 4.8%. Though this study did not identify any other trypanosomes apart from the two species mentioned above, it is still possible that some of the species mentioned in the review of literature may be affecting camels of Kenya, especially the camels kept in the tsetse-fly infested areas.

Surra in the camels of Kenya (basing on the results of KULAL herd) manifests itself in two forms. The acute form of the disease, constituted (9.5% of all the cases and the chronic form constituting 90.5% of all the cases. The chronic form could still be subdivided into three other types. However, these findings seem to agree with those of Pease (1906), Gatt Rutter (1967),and Mahmoud and Osman (1979).

The disease point prevalence rates seem to correspond to the rain season peaks at the respective sites of the study, supporting the observations of Lease (1914) and Yutuc (1949).

The disease is stable at NGURUNIT and GALANA. In these two herds a strong calfhood immunity is evident showing that maternal antibodies play an important role in the epidemiology of the disease in these two herds. However, it was also observed that at KULAL and OLMAISOR, the disease is unstable. Some of the reasons for this apparent difference in the stability of the disease have been given in the discussion.

The control of camel trypanosomiasis in Kenya faces many of the challenges facing developing countries. There is no available method to control tabanids because of their ubiquitous nature and

inaccessability of their developmental stages. For camels kept in tsetse-fly infested areas, the control of tsetse-flies is far from completion. Furthermore, the isolation of T. congolense from camels at GALANA and OLMAISOR points to the potential danger of this trypanosome and calls for a thorough study of the pathology, pathogenesis and chemotherapy of this trypanosome in the dromedary camels of Kenya. The use of vaccines in the control of trypanosomiasis is far from reality because the problems of antigenic variation remain to be solved. Chemotherapy has produced promising results in the Sudan (Mahmoud and Osman, 1979). However, the use of Naganol (Antrypol) has been complicated by resistant strains that fail to respond to the drug. Though Antrycide methyl sulphate (at 2g/camel) was useful in treating naganol resistant cases, some strains have been isolated in the Sudan which are resistant to both drugs (Mahmoud and Osman, 1979). Furthermore, Antrycide sulphate has been withdrawn from the market because of economic problems encountered by the manufucturers. Berenil (diminazene aceturate) would be a good remedy, but it is found to be toxic to camels at levels curative to similar infections in other hosts (Leach, 1961). It therefore means that the problem of resistant strains of T. evansi which may be in existence already, or which may arise later, and that due to T. congolense

in camels, will remain for long unsolved till better drugs are found.

Nevertheless, chemotherapy seems to be the only effective solution to the problem of camel trypanosomiasis today. To reduce the occurrence of drug resistant strains, the control of trypanocides in circulation by the Government of Kenya, and the use of correct dosages by the veterinarians, must be given strong emphasis. Trypanosomiasis surveillance in camels must continue so that areas with drug resistance strains are mapped. Secondly, such surveillance studies will establish the areas that show stability of the disease. Disease stability is important in the sense that in areas where the disease is stable as in NGURUNIT and GALANA, treatment should be restricted to clinical cases only and not to the whole herd. However, in areas where the disease is unstable as in OLMAISOR and KULAL, periodic whole herd treatment is recommended, especially during and after the rain seasons.

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A P P E N D I X

PREPARATION OF REAGENTS

1. ALSERVER'S SOLUTION (RG 1)

A.	0.1m citric acid 21.02g/ℓ (H_2O)	6.5mℓ
B.	x 10 Normal saline	12.4mℓ
C.	Glucose	5.1g
D.	Trisodium Citrate $2H_2O$ ..	2.0g
E.	Distilled water (to 250 mℓ) ..	150 mℓ

1. Dissolve C and D in E; Add A and B
2. Adjust pH to 6.1 with HCl or NaOH.
3. Make up to 250mℓ (with distilled water)
4. Filter
5. Dispense 10mℓ aliquots into universal bottles
6. Autoclave at 15lb/in^2 for 15 minutes
7. Allow to stabilise for one week in the fridge.

Unopened bottles remain suitable for use for a long time at 4°C.

2. PHOSPHATE BUFFERED SALINE (PBS) (RG 2)

		pH 6.8	pH 7.2
A.	0.2m Sodium dihydrogen Phosphate	142.5mℓ	45 mℓ
B.	0.2m Disodium hydrogen Phosphate	103 mℓ	84.5mℓ
C.	x 10 Normal saline ..	25 mℓ	25 mℓ
D.	Distilled water (to 500mℓ)	200 mℓ	200 mℓ

1. Mix A, B, C, D well. Check pH
2. Adjust pH to 7.2 or 6.8
   (whichever required)
3. Make up to 500mℓ (with distilled water).

3. 1% BUFFERED GLUTARALDEHYDE SOLUTION (RG 3)

For: Fixing red blood cells for IHA

For use: (10mℓ blood)

A.	25% Glutaraldehyde	..	8mℓ
B.	PBS pH 7.2 (RG 2)	..	128mℓ
C.	Distilled water	..	64mℓ

Mix A, B, C. Handle glutaraldehyde with care!

4. TANNIC ACID SOLUTION (RG 4)

1 Stock (1%)

A.	Tannic acid	..	0.1g
B.	Distilled water (to 10mℓ)		9.0mℓ

1. Dissolve A in B
2. Make up to 10mℓ
3. Filter

Store at 4°C. Discard after 1 month.

II 1/20,000 Tannic acid for tanning RBC

A. Tannic acid stock (RG 4 I) 500µℓ

B. PBS pH 7.2 (RG 2) (to 100mℓ) 95mℓ

1. Mix A and B
2. Make up to 100mℓ

Use immediately then discard.

5. HAEMAGGLUTINATION DILUENT (RG 5)

For IHA test.

A. Deactivated normal Rabbit serum 1mℓ vial

B. PBS pH 7.2 (RG 2) .. 99mℓ

Mix well A and B.

Use immediately. Discard after use.

6. SERUM (RG 6)

Whole blood (without anticoagulant)

1. Leave 2-3 hrs. at room temperature
2. Ensure that the clot is detached from the walls of the bottle if not, use a wire or glass rod to detach it.
3. Leave overnight at $4^{o}C$.
4. Remove the serum and centrifuge it at 1000 g for 10 minutes.
5. Use a Pasteur pipette to remove the serum carefully
6. Store at $-20^{o}C$ till required.

7. SERUM - DEACTIVATED (RG 7)

To destroy complement etc. in normal serum Serum (RG 6)

1. Put serum into universal bottles and place in a water bath at 56°C for 30 minutes.
2. Dispense into aliquots.

For IHA test, aliquots of 1.0mℓ and 200μℓ. If larger volumes of serum are to be deactivated, mix well to give even heating and increase the time proportionately.

8. NORMAL SALINE (with 0.01% Azide) (RG 8)

For use.

A.	x 10 Normal Saline (RG 10 stock)	900mℓ
B.	10% Sodium azide ..	9mℓ
C.	Distilled water (to 9L)	8L

1. Put C into aspirator.
2. Add A and B.
3. Put on a lid and mix well by shaking.
4. Make up to 9L mark with distilled water.
5. Mix again.

9. SALINE (RG 9)

Stock Solution (x 10 normal saline)

A.	Sodium Chloride ..	340g
B.	Distilled water (to 4L)	1500mℓ

1. Weigh 2 x 170g of A on a "rough" balance - 9250mℓ plastic beaker will easily hold 170g.
2. Put 1500mℓ of distilled water onto a stirrer and commence stirring. Add the NaCl - use a 2L beaker.
3. When dissolved transfer to aspirator marked at 4L and top up to 4L with distilled water. Filter.

10. 1/20,000 MERCURIC CHLORIDE SOLUTION (RG 10)

A.	Mercuric Chloride	0.01g
B.	Distilled water	200 mℓ

1. Put B into a 250mℓ beaker
2. Put into a stirrer.
3. Add A.
4. Dispense into a 250 reagent bottle.

Use immediately and discard after use. (Mercuric Chloride is poisonous!)

11. E.S.G.

A.	Potassium Dihydrogen Phosphate (KH_2PO_4)	0.3g
B.	Sodium Chloride (NaCl) ..	8.0g
C.	Glucose	2.0g
D.	EDTA (disodium salt) ..	3.0g
E.	Phenol Red (1:5000) ..	5.0mℓ

12. PHENOL RED (1:5000)

Dissolve 0.02g of Phenol Red in 100mℓ of saline pH 7.5, autoclave at 10 lb/in² for 20 minutes.