HUMAN PLAGUE IN SOUTHERN AFRICA DURING THE PERIOD 1965-1969

Margaretha Isaäcson

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THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH
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P.O. BOX 1038
POSSUS

TEL. ADR.
"BACTERIA"

TEL. 724-1701

HOSPITAL STREET,
HOSPITAALSTRAAT.

JOHANNESBURG.

14th August, 1972.

The Registrar,
Faculty of Medicine,
University of the Witwatersrand,
Esselen Street,
Johannesburg.

Dear Sir,

With reference to the enclosed thesis "Human plague in southern Africa during the period 1965-1969" I hereby declare that the work covered is exclusively my own.

The nature of the subject is however such that assistance was required in obtaining specimens when the sheer volume of work under pressure of epidemic conditions surpassed my ability to do this alone. Virtually all the serological work was performed by myself as were the tests characterizing the plague bacillus.

Having been in charge of the S.A.I.M.R. plague laboratory, which is the reference laboratory for southern Africa, since 1965 the routine diagnostic work was done under my constant supervision and control by a qualified medical technologist.

A small part of the routine diagnostic work was performed in branch laboratories and this has been duly acknowledged where relevant in the thesis.

Although it was not possible to visit all the epidemic areas, a large number of the patients were examined by myself.

The planning of the investigations was entirely done by me and so were the follow-up studies, analyses and evaluation of all results.

Although antibiotic sensitivity studies on plague isolates were originally planned to be included, these have been omitted as they are to form the subject of a thesis by one of my technologists.

The Mouse Protection Index (MPI) tests could not be done as an error of judgement resulted in a loss of irreplacable serum specimens.

The original protocol was expanded however by inclusion of the biochemical and *in vitro* virulence studies as well as by the work resulting from the unanticipated occurrence of typhoid.

Yours sincerely,

Dr. M. Isaacson
Dept. of Microbiology.

In Memory of my Parents: Walburga and David J. Isaäcson

ACKNOWLEDGEMENTS

The work which culminated in this thesis was performed during my employment in the Department of Microbiology of the South African Institute for Medical Research, Johannesburg.

My gratitude goes out in the first instance to Dr K.F. Meyer of the George Williams Hooper Foundation in San Francisco, USA, under whom I had the privilege of studying techniques applied in this work and who first suggested plague as the subject of a thesis.

I deeply appreciate the facilities and materials so freely made available by the Director of this Institute, Professor J.H.S. Gear. My thanks are also extended to Professor J.F. Murray, the Deputy Director, who supplied encouragement when it was most needed.

Professor H.J. Koornhof, for his endless patience, for permitting me to go on numerous field trips even when this was not convenient, and with many inspiring discussions earns my very deep gratitude.

I am equally grateful to Dr D.H.S. Davis who, by his profound knowledge of southern African plague in particular and by making available old records, was of great assistance.

Mr. N.J. Richardson and Dr C.G. Anderson who spent many weeks producing the purified plague fraction 1 antigen before my arrival on the plague scene deserve my retrospective recognition. My thanks also go to Dr A.F. Hallett and Miss T.P. Crowngold who accompanied me on several field trips and whose collaboration I valued.

Sincere appreciation is extended to Mrs. S. Hébert and her staff in the library, and also to Mr. M. Ulrich and his staff in the photographic department for their assistance.

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I am very grateful to Miss Joyce Hall for making available the two Anglo-Boer war photographs reproduced on pages 19 and 21 and also to Miss Janet A. MacGill for a wealth of biblical material which she generously put at my disposal.

Last, but by no means least, I wish to give my sincere thanks to the thousands of people who freely consented to donate blood samples. On more than one occasion village chiefs would make the statement: "If it will help our people then we shall not refuse". It is my most sincere wish that this confidence will prove justified.

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1 INTRODUCTION

Pioneer work in the field of plague has been done in South Africa by a number of distinguished scientists. In the nineteen thirties and forties Pirie and Grasset systematically investigated the immunogenic properties of various strains of Yersinia pestis and published their results in several articles [Pirie and Grasset, 1935, 1938 and 1941].

The epidemiology of southern African plague has been worked out to a great extent by Dr D.H.S. Davis whose publications on the subject enjoy international recognition.

Amies, working at the South African Institute for Medical Research [SAIMR], studied the morphology and the antigenic structure of the plague bacillus and was the first to describe [1951] a simple plague haemagglutination test using sheep erythrocytes which he had sensitized with his alcohol precipitated envelope antigen of Y. pestis. He believed this antigen to be a simple protein and Baker et al. [1952] suggested that it might prove to be identical to their plague fraction 1B. Subsequently Chen and Meyer [1954] showed Amies' antigen to be a polysaccharide and published an improved haemagglutination technique using tanned sheep erythrocytes sensitized with the purified plague protein fraction 1B.

Following his original research Amies made the following highly prophetic observations:

The haemagglutination test is unlikely

> to be of much value as a diagnostic procedure because of the slow development of immunity in plague. It may, however, prove useful as a means of confirming retrospectively a diagnosis made on clinical grounds alone. For research purposes it is a useful method of following the development of circulating antibody in animals undergoing active immunization.

Today, twenty years and many plague outbreaks later, Amies' words can hardly be improved upon as an evaluation of the now widely used plague haemagglutination technique.

Meyer [1964], following his visit to Ovamboland during the 1963 plague epidemic, reported the usefulness of serological studies in the retrospective diagnosis of plague and pointed out that diagnosis by isolation of Y. pestis frequently fails in remote regions due to inadequate facilities and delays in transport of specimens.

In several endemic regions of the world [USA, USSR, Vietnam, Madagascar] the use of the plague haemagglutination test in extensive human and animal surveys has provided new knowledge on locally prevailing epidemiological conditions.

Dr K.F. Meyer's findings and his invitation in 1966 for a SAIMR staff member to study the new laboratory techniques under his guidance in San Francisco resulted in an expanded programme of plague research in South Africa. The haemagglutination test was introduced as a routine plague diagnostic procedure at the SAIMR early in 1967. Anticipating this introduction the collection of serum specimens was commenced in 1966 in order to determine subsequently to what extent conclusions reached in other endemic regions

of the world could be applied to southern African conditions.

The following chapters present various observations on human plague in southern Africa in recent years and in particular the results of serological studies.

2 REVIEW OF THE HISTORY OF PLAGUE

2.1 The Philistine Plague

The occurrence of bubonic plague among the Philistines in the year 1320 BC is still subject to controversy. There can be no doubt that an epidemic with a high mortality did occur, but opinions differ on the nature of this disease.

In the Authorized Version of the Old Testament it is recorded how Israel was defeated in battle against the Philistines who captured the Ark of God, an act which they came to regret bitterly for

> ...the hand of the Lord was heavy upon them of Ashdod, and He destroyed them, and smote them with emerods...
> [1 Samuel 5 verse 6]

In a vain effort to rid themselves of God's punishment the Philistines then carried the Ark inland to Gath where

> ...the hand of the Lord was against the city with a very great destruction, and He smote the men of the city, both small and great, and they had emerods in their secret parts.
> [1 Samuel 5 verse 9]

The same happened when the Ark was conveyed from Gath to Ekron. The Philistines then decided to send the Ark to 'its own place' and were advised by their priests to send with it a trespass offering which was to consist of

> ...five golden emerods, and five golden mice according to the numbers of the lords of the Philistines for one plague was on you all, and on your lords. Wherefore ye shall make images of your emerods, and images of your mice that mar the land...
> [1 Samuel 6 verses 4,5]

It is the interpretation of the word 'emerod' around which controversy has erupted and which has given rise to

the belief by some that the Philistine disease was bacillary dysentery accompanied by the appearance of haemorrhoids.

Hirst [1953] described his own experiences during the first world war and stated that he

> ...never heard of haemorrhoids as a complication of the acute phase of the disease, and they are very rarely, if ever, seen as an accompaniment of chronic bacillary dysentery.

Mollaret [1969] put forward the hypothesis that the Philistine disease may have been the acute form of intestinal schistosomiasis and suggested that, if S. mansoni was then already prevalent in Palestine, this would explain the relative resistance of the local inhabitants as compared to the Philistine invaders. This hypothesis is based on the assumption that the lesions described as emerods were in fact haemorrhoids.

It should be mentioned in this connection that in the Dutch bible as authorized by the States General of the United Netherlands the lesions are described as 'spenen' which, literally translated, means nipples.

The Afrikaans bible speaks of 'geswelle' meaning swellings or tumours.

The New English Bible of which the Old Testament was first published in 1970 does not make use of the word emerods. In 1 Samuel 5 verse 6 it states that the Lord

> ...threw them into distress and plagued them with tumours and their territory swarmed with rats. There was death and destruction all through the city.

Chapter 5 verse 9 and chapter 6 verses 4,5 also refer to tumours but then there is a sudden change to the use of

the word haemorrhoid in 1 Samuel verse 17.

In the original Hebrew the term 'ofalim' meaning hills is used [Hirst, 1953].

To summarize then, various versions of the Old Testament make mention of an epidemic disease manifested by the appearance of tumours or swellings with a high mortality and which started in a coastal city [Ashdod] and then spread inland [to Gath and Ekron]. At the same time there was an overabundance of rodents. These features are indeed very suggestive of bubonic plague.

2.2 The Plague of Rufus

The next reference to plague in the pre-christian era was made by Rufus, a physician of Ephesos in about 100 AD, who wrote about a highly fatal bubonic plague in Libya, Egypt and Syria which occurred during and before his time as far back as about the end of the third century BC [Wu Lien-Teh, 1936].

Simpson [1905] quoted the following observations made by Rufus.

> The buboes that one calls pestilential are very acute and often cause death.
> Diocorides and Posidonius have referred to them at length in their treatise on the plague which in their time raged in Libya, and they have said that it was accompanied by an acute fever, intense pain, perturbation of the whole body, delirium, eruption of large buboes hard and without suppuration, developing not only in the usual places but also in the popliteal space and elbow, although in general such inflammations do not form in these places.
> One can foresee a plague which approaches by paying attention to the bad condition which the seasons pre-

> sent; to the manner of living less profitable for health, and to the death of animals which precedes its invasion.

It is generally accepted that what Rufus described was the occurence of bubonic plague.

2.3 The Pandemic of Justinian

The first authenticated pandemic of plague occurred during the reign of the emperor Justinian. It is said to have started in Pelusium, a port city in lower Egypt, in the year 542 AD. It is however probable that Pelusium played a role in the evolution of the Justinian pandemic similar to that played by Canton and Hongkong at the start of the third pandemic in that plague had rather insiduously and over a number of years approached these busy commercial ports. From these the disease was transmitted explosively to distant countries by shipping and via the caravan routes. To support the view that Pelusium was not the origin of the first pandemic we have the assertion by Evagrius, a citizen of Antioch, that the disease had come from Ethiopia [Roberts, 1933].

Although the Justinian plague pandemic was predominantly bubonic in nature other forms of the disease were seen as well.

Simpson [1905] presents a detailed description of the plague of Constantinople as written by Procopius of Caesarea in his history of the Persian war. Procopius spoke with some disdain of rather unproductive speculations by others about the cause of the scourge.

> Let the sophist discuss the matter,
> let the meteorologist take his view

> each in his own way, but I am going to relate where this pestilence began and in what manner it destroyed mortals.

This he then proceeds to do in a graphic and enlightening manner. Certain extracts are well worth quoting.

> It arose in Egypt, with the inhabitants of Pelusium, then dividing, it spread one way through Alexandria and the rest of Egypt, the other into Palestine which borders on Egypt, and then travelled over the world, always advancing with a progress marked by certain definite spaces of time.

Procopius accurately observed the development of post infective immunity.

> If it passed over any place, only slightly or mildly touching the inhabitants, it returned there afterwards, leaving untouched the neighbours against whom it had spent its rage before, and it did not depart from there before it made up the full measure of the dead in proportion to the amount of destruction which it had brought on its neighbours.

He also suggested that the disease was imported via the sea routes.

> Always beginning at the sea coast it spread into the interior.

When describing the clinical course of plague Procopius started by giving the typical features of the bubonic form.

> On a sudden they became feverish, some immediately on awakening, others while walking, others while doing one thing, others another.
> ...but from morning until evening the fever was so mild that neither the patient nor the physician who felt the pulse had any suspicion of danger; and none of those who caught the plague thought of death. But, in

> some cases on the same day, in others on the next, in others in a few days after there arose a bubo, not merely on what is called the groin, but under the armpit; in some cases the bubo appeared behind the ears and in other parts.

He then related how some people became delirious, while others sank into a coma and he made observations which appear to indicate septicaemic plague.

> The malignant violence of the disease killed some at once, others after many days; with some, all over the body black pustules, as large as a bean broke out. These could not survive even for a single day, but in the same hour as the pustules appeared they breathed their last. Many dropped down dead from a sudden vomiting of blood.

Procopius p tured the scenes occurring at the height of the outbreak in Byzantium in 544 AD where the number of the dead reached 5000 a day and subsequently 10000 and even more than that.

These daily scenes of death, horror, the accumulation of the corpses and the stench which hung over the streets, all of these equalled or surpassed those so well known during the Black Death of the middle ages.

Agathius described the second outbreak of plague in Constantinople which occurred in 588AD and gave a clear description of the fulminating septicaemic form of plague [Simpson, 1905].

> Some people without any feverishness or any pain, going about their daily work, sometimes at home and sometimes abroad fell down, and at once became lifeless, as if they had taken death as a chance turn up.

The spread of the Justinian pandemic was extensive, embracing countries in the near and middle East while

there is evidence of plague occurring in European countries such as France, Germany, Italy, Spain and Ireland.

By the end of the sixth century the pandemic nature of plague had ceased though epidemics continued to occur here and there at long intervals.

It has been estimated that the first pandemic claimed the lives of one hundred million people.

Hirst [1953] suggested the ancient central Asiatic plague focus as having been the origin for this pandemic but there is convincing evidence presented by Wu Lien-Teh [1936], Pollitzer [1954] and especially Roberts [1935] which points to the central African focus as being the probable source.

2.4 The Second Pandemic

The Crimean ports are generally considered as constituting the starting point for this pandemic in 1346. The tremendous impact made by this scourge in Europe and the vast amount of literature covering all its aspects and focussing attention on this particular period of time in history tend to mask the fact that plague had continued to occur in scattered fashion since the end of the first pandemic.

Simpson [1905] referred to a plague which affected India in 1032 and which spread West as far as Constantinople and was probably responsible for its occurrence in Europe in 1034. There was a steady increase in the incidence of plague from the eleventh century onwards. In the year 1346 the disease was disseminated from the Crimean ports to Europe on board ships. Once in Europe it spread in a devastating manner.

There is, however, ample evidence that plague was rampant in central Asia immediately prior to 1346. Pollitzer [1954] made mention of the existence of memorial stones dating back to 1338/1339 in old Nestorian graveyards situated in the Semirechinsk district which lies in the central Asiatic plague focus. The inscriptions on these stones indicated plague to be the cause of death of the persons concerned.

Constantinople was once again reached in 1347 and England in the year 1348.

A prominent feature of this pandemic was the high incidence of pneumonic plague. The possibly frequent cyanotic appearance of the patients may have been responsible for the subsequent designation 'Black Death'. Alternatively this name may be the result of the observation of skin haemorrhages which we now know to be a common occurrence in patients with consumptive coagulopathy associated with fulminating infections.

It was noticed that individual outbreaks often started with a pneumonic phase and that bubonic cases only appeared later [Wu Lien-Teh, 1936]. This feature suggests that the infection was spread from one locality to another by patients or carriers who, by airborne transmission, gave rise to a primary pneumonic plague outbreak. The subsequent bubonic phase may have been mediated by human fleas and even lice. This view is supported by the work of Blanc and Baltazard [1945] who drew attention to the importance of these vectors in the inter-human transmission of plague in Morocco in recent times.

Following the initial devastating wave of plague during the latter half of the fourteenth century the disease maintained a firm grip on Europe for almost three hundred years. Many cities were repeatedly affected by serious epidemics. It was only during the last third of the seventeenth century that plague regressed in Europe in an easterly direction. The last cases in Europe were reported in the year 1841. Although a marked decrease in plague was observed also in Asia it did not disappear entirely from that continent.

2.5 The Third Pandemic

This pandemic also originated in the central Asiatic plague focus. The Chinese province of Yunnan is often stated to have been the starting point but Wu Lien-Teh [1936] emphasized that Yunnan was not an endemic area and that plague was imported there from regions further west. According to Pollitzer [1954] plague outbreaks became frequent towards the end of the eighteenth century and the infection appeared to spread into adjacent Yunnan without however prevailing epidemically

It is generally agreed that the Mohammedan Rebellion of 1855 upset the ecological balance in Yunnan and caused the dissemination of plague towards the East.

Emile Rocher in 1871 was an eye witness of the plague in Yunnan [Simpson, 1905]. He found the country to be ravaged by warfare, the population massacred while famine and plague contributed to the general misery. In his 'Notes sur la Peste au Yun-nan' Rocher asserted that the belief that the epidemic was due to evil miasmas emanati

from the soil was supported by the observation that small animals, for example rats, which lived in drains or underground were the first to be stricken. He gave an accurate description of the clinical course of bubonic plague and the social effects of the disease. In addition Rocher observed that the start of the epidemics in the plains coincided with the commencement of the harvest, but that plague occurred in the mountains later in the year and concluded that the disease was spread into the mountains by mountain dwellers on their return home after having worked as harvesters in the plains.

Plague reached the capital of Yunnan in the year 1866 and the southern Chinese town of Pakhoi in 1867. Simpson [1905] based this rapid spread to Pakhoi on the simple fact that the town was the home of troops returning from Yunnan.

Canton, the capital of Kwantung, was reached only in 1894 when Dr Mary Niles saw her first plague patient in January of that year. A raging epidemic followed. Plague appeared in Hongkong in May 1894 when the Canton epidemic was at its height. The annual entry of some twelve thousand vessels, mostly from Canton and its neighbourhood, into the port of Hongkong made this rapid spread inevitable. From Hongkong plague was conveyed via the shipping routes to Japan, Australia, India, Africa and the Americas.

It was during the Hongkong epidemic that Kitasato and Yersin independantly observed the causative organism, Y. pestis, and Yersin gave the first accurate morphological description of this bacillus in 1894.

Bombay, free of plague for nearly two hundred years, fell victim to the disease in 1896 and a crippling epidemic ensued after an initial slow progress. The situation there was aggravated by the violent opposition put up by the population to preventive measures taken by the authorities.

By land and by sea other parts of India became affected and the annual mortality eventually exceeded one million. The heavily affected Indian ports also became major sources for the dissemination of plague to other parts of the world.

This pandemic was instrumental in producing the first significant scientific discoveries made in respect to the diagnosis, epidemiology, treatment and prevention of plague. The discovery of the causative agent was soon followed by the production of an antiserum by Yersin, Rowe, Calmette and Borrel. On the whole, the results of serotherapy varied considerably.

In 1896 Haffkine prepared the first plague vaccine which was a heat killed suspension of bacteria preserved with carbolic acid.

It was established that the disease in rats was the same as that in man and in 1897 Ogata suggested that the flea might play a role in the transmission of plague. Paul Louis Simond, working in Bombay, set down the main facts about transmission, epidemiology and control. A graph prepared by Dr Clark, MOH for Hongkong, shows the similarity between the rise and fall of the rat epizootic and those of the human epidemic in 1900 [Fig.1].

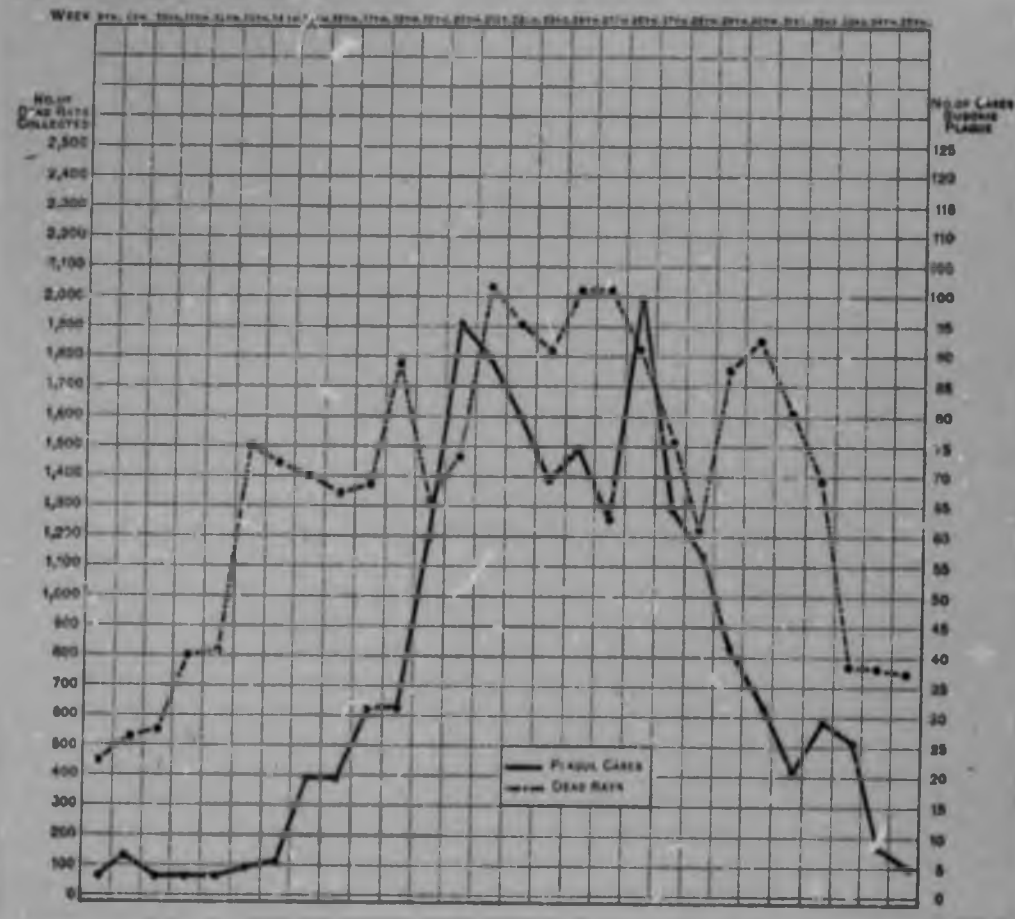

Fig.1 Graph showing the similarity between the rise and fall of the rat epizootic and those of the human epidemic in Hongkong in 1900.
[Reproduced from 'A Treatise on Plague' by W.J.R. Simpson, 1905]

Of great interest are also the results of many animal experiments carried out by Simpson in Hongkong in the year 1902. Fig.2 and Fig.3 show some of his findings.

North Africa became involved by the third pandemic in 1899 when plague appeared in Egyptian and Algerian sea ports. Outbreaks of plague occurred however in Tanzania [then Tanganyika] in the years 1886-1889, i.e. some years before the onset of the third pandemic. As Tanzania is

CHART VIII.

Temperature of a monkey placed in the same cage as a rat dead of plague and which has been opened and examined. Rat was quite free of fleas.

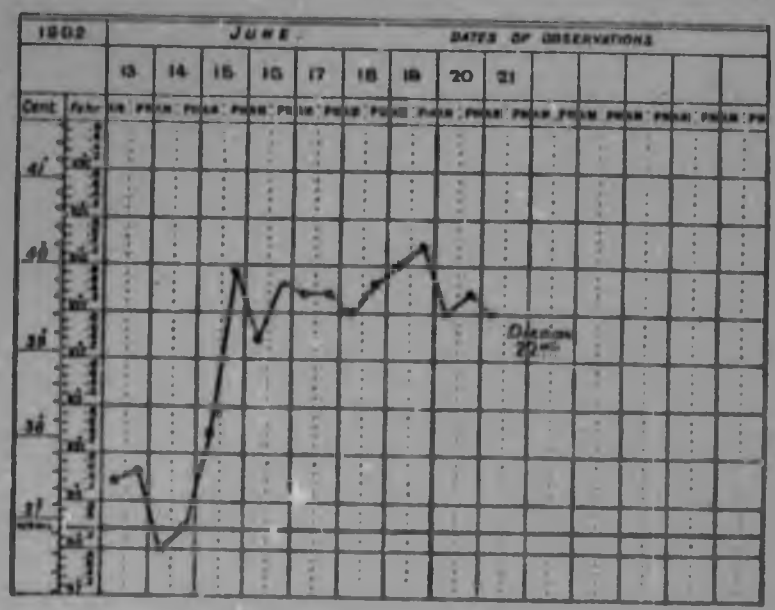

Fig.2 Results of Simpson's animal experiments. [Reproduced from 'A Treatise on Plague' by W.J.R. Simpson, 1905]

traversed by the old trade route from Uganda to the Indian Ocean coast [Pollitzer, 1954] and Uganda is believed to constitute the ancient central African plague focus it seems reasonable to assume that the occurrence of plague in central and east Africa at this time was of local origin and not a manifestation of the spread from the central Asiatic focus. This view is strengthened by the occurrence of plague in Nairobi, the inland capital of Kenya, in 1902 while Mombasa which is the only sea port in this region remained free of plague until 1912 [Roberts, 1935].

Madagascar was invaded by the disease in the year

CHART IX.

Temperature of monkey placed in cage, having a rat dead of plague in adjoining cage, but with impossibility of contact. Rat was covered with fleas.

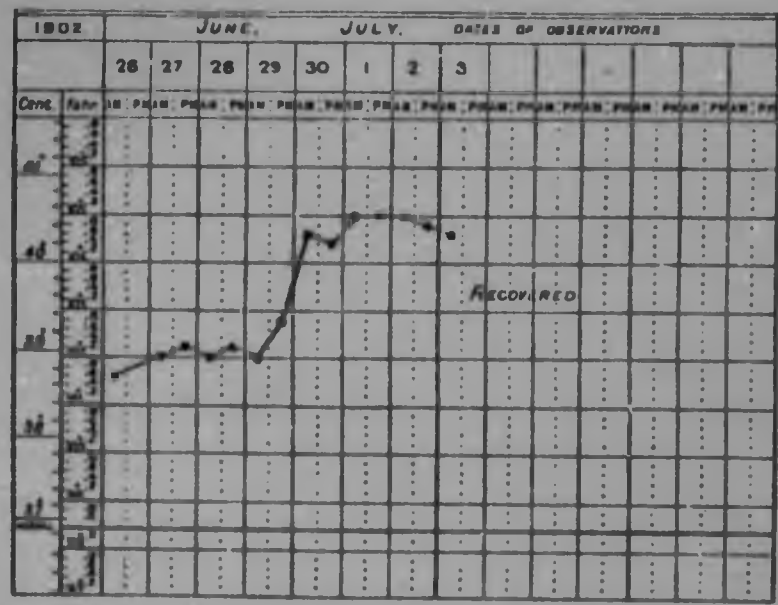

Fig.3 Results of Simpson's animal experiments. [Reproduced from 'A Treatise on Plague' by W.J.R. Simpson, 1905]

1898 via a cargo ship from India.

Southern Africa experienced plague for the first time during the third pandemic as the disease had not been known to exist here prior to the year 1899.

The South American plague situation at this time is of interest as it directly affected subsequent events in South Africa.

In 1899 a Dutch ship arrived in Montevideo from India. Dead rats had been noticed on board during the journey and some sailors became ill and died of an undiagnosed condition. On arrival in Montevideo the ship's cargo was transferred to an Argentinian coastal vessel,

the 'CENTAURO', which entered a number of South American ports. Plague was thus transmitted to several South American states [Pozzo, 1945]. Argentina was invaded through its port of Rosario in September 1899 and this centre became a major source for the introduction of plague into South Africa.

Mitchell [1905] told of the arrival of the S.S. 'KILBURN' in Cape Town in March 1900 from Rosario. The ship carried a cargo of forage and during the journey several crew and the captain became ill with plague. The latter died at sea. Within a few days of arrival of the ship in Table Bay five more cases of bubonic plague developed on board but all of these recovered.

South Africa at that time was the scene of the second Anglo-Boer war [1899-1902]. Though it would be rash to state that plague would not have become endemic in this country in the absence of hostilities there is no doubt that the war produced conditions which favoured the spread of plague inland from coastal centres. Throughout history there are numerous examples of the association between war and plague, starting with the war of the Philistines and ending in our time with the Vietnam war.

Large shipments of grain, fodder and other military supplies were imported from the plague stricken ports in South America and India and stockpiled in Cape Town, Port Elizabeth and other centres by the British military authorities [Fig.4].

Within three years all the major South African ports were affected by plague, either through intercommunication by rail and shipping or by fresh introduction from South

Tons upon tons of Oats for Tommy's faithful friends, at De Aar, S. Africa

Fig.4 Copy of an original Anglo-Boer war photograph showing the ideal conditions for rodent harbourage provided by the haphazard stockpiling of fodder.

America and India.

Always rats in the harbour areas or near railway sheds were stricken first, to be followed by a human plague outbreak. Fortunately human plague in South Africa never reached the proportions of outbreaks in India and China. The largest outbreak in this country

occurred in 1901/1902 when a total 766 cases was recorded with 371 deaths in Cape Town [Mitchell, 1905].

During these early years plague was introduced into some inland centres by means of infected fleas and rodents carried by rail or ox wagons transporting goods from the coast.

The first reported case of plague contracted in the interior concerned an African labourer who was employed by the military authorities at the Modder River Remount Station [Fig.5].

Occasionally, people harbouring plague became ill after having travelled inland from the coast. An example of this is the first recorded case of human plague in South Africa. The patient was an Indian who had arrived in Lourenco Marques, capital of Portuguese East Africa, from Bombay and proceeded to travel by rail to Heidelberg in the Transvaal. He became ill on the 31st of January, 1899, and was removed from the train at Middelburg in the Transvaal [Mitchell, 1927].

Johannesburg experienced a major human plague outbreak in 1904 when 112 cases with 82 deaths were notified for that year [Porter, 1903-1906]. The infection was probably introduced with goods transported from the coast. This epidemic was largely confined to the Indian location where socio-economic conditions were deplorable.
Dr Charles Porter, the then MOH for Johannesburg was deeply disturbed by conditions in the location and informed the local authority on several occasions that the area constituted a health hazard to the entire city. As early

Getting a Transport Wagon up the North Bank of the Modder (Feb. 18.), S. Africa.
Copyright 1900 by Underwood & Underwood

Fig.5 Copy of an original Anglo-Boer war photograph illustrating the conveyance of military supplies from plague affected coastal centres to inland depots. Modder River was the source of the first South African case of human plague contracted in the interior.

as November, 1902, Dr Porter testified before the Insanitary Area Commission as follows.

> Well, I wish to say most seriously and advisedly that, as Medical Officer of Health for Johannesburg I consider the existence and continuance of that location in the most emphatic manner fraught with

> danger to Johannesburg. I am not saying this merely as a witness before this Commission, but I shudder to think what would occur if plague or cholera broke out in that place.

It was only after repeated warnings of this kind and in the face of considerable opposition that steps were finally taken to evacuate the Indian location on the 17th of March, 1904. On the 19th of March a pneumonic plague outbreak was recognized in the location.

It must be emphasized that the opposition encountered in Johannesburg by the health authorities was not unique in the history of the third pandemic. Simpson [1905] quoted the Health Officer of Bombay who faced Hindu religious beliefs.

> It was often pathetic to see the anxiety of some people to save an insect from disinfecting fluid.

Active hostility developed in Bombay and the health authorities were faced by opposition from the public on the one hand and, as in Johannesburg, by lack of insight displayed by non-medical officialdom on the other hand.

San Francisco fared little better in the matter of cooperation with control measures during the plague outbreak in 1900. The health authorities attempted to investigate the situation but according to Smith [1941]

> The governor went to such length to discredit the investigation that the Federal Treasury sent an impartial commission - three men of unimpeachable authority - to establish the facts.

These three examples characterize the transitional period in the history of plague between the Black Death of the Middle Ages when little or nothing was known

about the disease and the present time when sufficient is known to control plague efficiently. In the early years of the third pandemic tremendous contributions were made towards diagnosis, treatment and control but the new methods were as yet unproven and, above all, unknown to the general public. These methods were therefore often regarded as unwarranted interference in their lives, religious beliefs and their means of earning a living without any established precedent of beneficial results.

In South Africa the murine phase of plague [domestic rodent plague in urban areas] came to an end in 1912 [Davis, 1948] after a reintroduction by sea into Durban. This resulted in a human outbreak of 32 cases with 26 deaths.

Mitchell [1927] stated that:

> After the eradication of the infection in Port Elizabeth, East London, Durban and Johannesburg in 1905, it was hoped that the disease had been completely eradicated from the country, and as the years went on without any further developements save for the recrudescence in Kingwilliamstown in 1907 and the small outbreak in Durban in 1912 in both of which the infection was localized and quickly eradicated - this hope almost crystallised into certainty. The year 1914 brought a rude awakening and opened a new chapter in the history of plague in South Africa.

Davis [1948] described the commencement of the sylvatic plague phase in the year 1914. This was characterized by the establishment of the disease in wild rodent reservoirs and the occurrence of human plague in a

scattered fashion, usually on remote farms.

Consequent to the transport of plague infected rats and fleas from coastal centres three primary sylvatic foci became established inland. These were located in the southwestern Transvaal/northwestern Orange Free State, the Cape midlands and the Uitenhage district in the Cape Province.

Between the years 1919 and 1931 these foci extended and spread occurred into South West Africa, Botswana [then Bechuanaland] and Lesotho [then Basutuland].

During the murine phase of plague the affected rodents were the domestic species Rattus rattus [Fig.6], Rattus norvegicus and Mus musculus [Hallett. 1967].

In 1903, before the onset of the sylvatic phase, an isolated outbreak of plague did occur in wild rodents of the species Rhabdomys pumilio within a radius of eighteen miles around Knysna in the Cape Province [Mitchell, 1927].

This epizootic was a transient phenomenon and had no apparent sequelae. It was only in 1921 that the existence of plague in wild rodents, suspected since 1914, was confirmed. The main reservoir animals were, and still are, gerbils of the species Tatera brantsii [Fig.7], Tatera leucogaster and Desmodillus auricularis [Fig.8]. Plague is most commonly transmitted from the wild to the domestic rodent species via Praomys [Mastomys] natalensis [Fig.9], the multimammate mouse [Davis. 1948], a semidomestic rodent living in close proximity to man but venturing out into the veld at night where it commonly makes temporary use of gerbil burrows.

Fig.6 Rattus rattus, an important plague-susceptible domestic rodent.

Fig.7 Tatera brantsii, the principal wild rodent plague reservoir animal in South Africa.

Fig.8 Desmodillus auricularis, a relatively plague resistant wild rodent which plays an important role in the sylvatic plague cycle.

Fig.9 Praomys [Mastomys] natalensis, a semi-domestic rodent which serves as an intermediary in the transmission of plague from wild to domestic rodents.

Since 1912 virtually all plague in southern Africa has occurred in rural areas where plague is enzootic.

While improved methods of surveillance, prevention and control has resulted in a markedly decreased incidence, the firm hold of plague on our wild rodent population makes its total eradication highly improbable within the foreseeable future.

3 MATERIALS AND METHODS

Much of the work was done in the field where conditions varied considerably and it was sometimes necessary to improvise and modify techniques to suit the circumstances. Deviations from conventional methods will be described where applicable in the following chapters.

The occasional need to take food, drinking water, motor fuel and camping gear severely limited the amount of laboratory equipment that could be transported. Consequently a streamlined field routine was developed which now enables the performance of normal plague diagnostic work under adverse conditions and with a minimum amount of equipment.

3.1 Collection of Blood Specimens and Separation, Storage and Transport of Sera

Two-millilitre disposable syringes which however permit the withdrawal of 3 ml blood, and disposable needles of size 21 x 1,5, were used. During mass surveys approximately 2,5 ml blood were taken by venepuncture of the antecubital vein. The blood was put into sterile glass tubes 75 mm long with an internal diameter of 7 mm and with a capacity of 3 ml. After clot retraction had occurred the specimens were centrifuged and the sera decanted into similar tubes and frozen at the nearest hospital or in a portable freezing apparatus.

The use of the narrow tubes as specified has several advantages not the least of which is that they require little space. In addition they permit the rapid decant-

ing of sera thus obviating the need for transporting space occupying, fragile pasteur pipettes which are required for the time consuming transfer of sera from tubes with larger diameters. Lastly, these tubes are ideal for the subsequent steps in the performance of the haemagglutination tests. On completing a field investigation the serum specimens are packed in commercially available coolbags for transport to the laboratory.

3.2 Collection of Throatswabs

These specimens were collected with charcoal tipped swabs on wooden sticks. The throatswabs were stored in Stuart's transport medium at ambient temperature until they could be processed in the laboratory. Processing took place within two weeks of collection.

3.3 Collection of Rodents and Fleas

Rodents and fleas were collected by the technical field staff of the South African State Department of Health and the Lesotho Ministry of Health.

Rodents were found dead, dug up from burrows or trapped. Dead rodents were examined for the presence of Y. pestis either in the field or they were stored in salt according to the method described by Amies [1952] for subsequent examination in the laboratory.

Fleas were collected by using the techniques of de Meillon et al. [1961].

3.4 The Plague Haemagglutination [PHA] and Haemagglutination Inhibition [PHAI] Tests

Tanned sheep erythrocytes sensitized with Y. pestis Fraction 1 antigen were used for the PHA and PHAI tests.

Both protein fractions 1A and 1B were prepared at the SAIMR according to the methods of Baker et al. [1952].

Our first attempts at preparing the sensitized sheep erythrocytes according to the technique developed by Chen and Meyer [1954] were unsuccessful in that unexpected and inconsistent results were obtained. It was then found that erythrocytes originating from some sheep were quite unsuitable while those from other sheep gave highly satisfactory results. Since the use of a selected source of erythrocytes in 1967 no further major problems were experienced.

Some minor modifications were introduced in the preparation and use of the reagents. Chen and Meyer [1954] recommended the use of a 2,5 per cent suspension of erythrocytes in saline for the tanning and sensitizing procedures. It was found that the results were not affected by the use of a 5 per cent suspension tanned with an equal volume of a 1: 20000 tannic acid solution and sensitized with an equal volume of a 25 microgram per ml solution of Y. pestis fraction 1B. The final 5 per cent suspension of tanned and sensitized cells in 1:250 inactivated and absorbed normal rabbit serum was kept as a stock solution at a temperature of 4° C to 10° C for up to 4 weeks. The amount of haemolysis which occurred during this period was not found to affect the red cell concentration to any significant extent. Instead of diluting the cells to a 0,5 per cent working suspension a 0,7 per cent suspension was routinely used as this was found to make for easier reading of the results.

The PHA and PHAI tests were performed by means of the microtechnique described for virological work by Sever [1962] and subsequently adapted to plague serology by Cavanaugh et al. [1965].

The sera were first inactivated for 30 minutes at 56°C and then absorbed with washed and packed sheep erythrocytes from the same source and collected at the same time as those used for tanning and sensitizing.

When large numbers of sera had to be tested these were first subjected to a screening procedure in which the only controls used were a positive serum with a known PHA titre and a negative serum obtained from a laboratory reared rabbit.

The sera were serially diluted in 1:100 inactivated and absorbed normal rabbit serum to give final dilutions after addition of the sensitized erythrocytes ranging from 1:4 to 1:128. This enabled the screening of 16 sera per plexiglass plate but did not prevent detection of prozone phenomena which in our experience has not exceeded titres of 1:64.

After the screening procedure the tests were repeated on those sera which showed haemagglutination. The sera were diluted to a final titre of 1:8192 and this was done in duplicate. To the second set were added tanned erythrocytes not sensitized with Y. pestis fraction 1B antigen in order to detect those sera showing nonspecific haemagglutination due to heterologous antibodies. When such a serum originated from a patient suspected of having plague a repeat serum was always requested after an inter-

val of at least a week. If specific plague antibodies were present but masked by heterologous antibodies in the first specimen they can in most instances be expected to have reached a titre exceeding that of the heterologous antibodies in the second specimen. The latter type of antibody has only rarely exceeded a titre of 1:64 in the sera tested in our laboratory.

Following the advice of Dr K.F. Meyer in 1969 [personal communication] the PHAI test has been incorporated as an additional control on all sera yielding positive PHA results. The PHAI tests were carried out in the same manner as the PHA tests but the serum dilutions were performed in 1:100 normal rabbit serum containing 25 microgram Y. pestis fraction 1B per ml of serum. This additional control is of value in eliminating positive results due to cross reacting components in the plague antigen. Those of our sera which yielded positive PHA results prior to 1969 have been retested in this manner.

3.5 The Plague Fluorescent Antibody Staining Technique

This method was not employed as a routine diagnostic procedure as the availability of only small amounts of the special reagents necessitated selectivity in its application. The test was therefore restricted to the examination of specimens obtained from isolated patients [possible index cases] or animals suspected of suffering from plague or of having died of the disease.

The reagents were kindly supplied by Dr Leo Kartman, then of the Communicable Diseases Center, San Francisco.

The tests were performed according to the method described by Moody and Winter [1959].

3.6 Characterization of Y. pestis Isolates

The plague isolates obtained during the period under discussion were tested for the presence of certain virulence factors by means of the assay methods summarized by Surgalla et al. [1970]. They were also tested biochemically for their ability to ferment lactose, glucose, mannite, sucrose, maltose, salicin, rhamnose, melibiose and glycerol, to reduce nitrate and to split urea. The methods recommended by Baltazard et al. [1956] were used for these tests. For the orthonitrophenyl-beta-D-galactopyranoside test the method described by Cowan and Steel [1970] was used.

3.7 Locus Identification and Mapping Method

The system of mapping as described by Davis [1948] was used on maps of scale 1:5 000 000. The maps, printed in black, have a superimposed blue grid divided into quarter degree squares. The grid is not reproduced in black-and-white photography. A code is used to indicate in which quarter degree square a particular locality is situated. For example, the map reference for the farm Alettasrus in the Vryburg district of the northern Cape Province is 26.24.Ad. Using this example Fig.10 illustrates Davis' map reference method. The hatched area [26.24.Ad] is the quarter degree square enclosed by latitudes $26^{o}15'$ and $26^{o}30'$ south, and longitudes $24^{o}15'$ and $24^{o}30'$ east.

Appendix I consists of such a map and shows the loci where human plague occurred during 1965-1969.

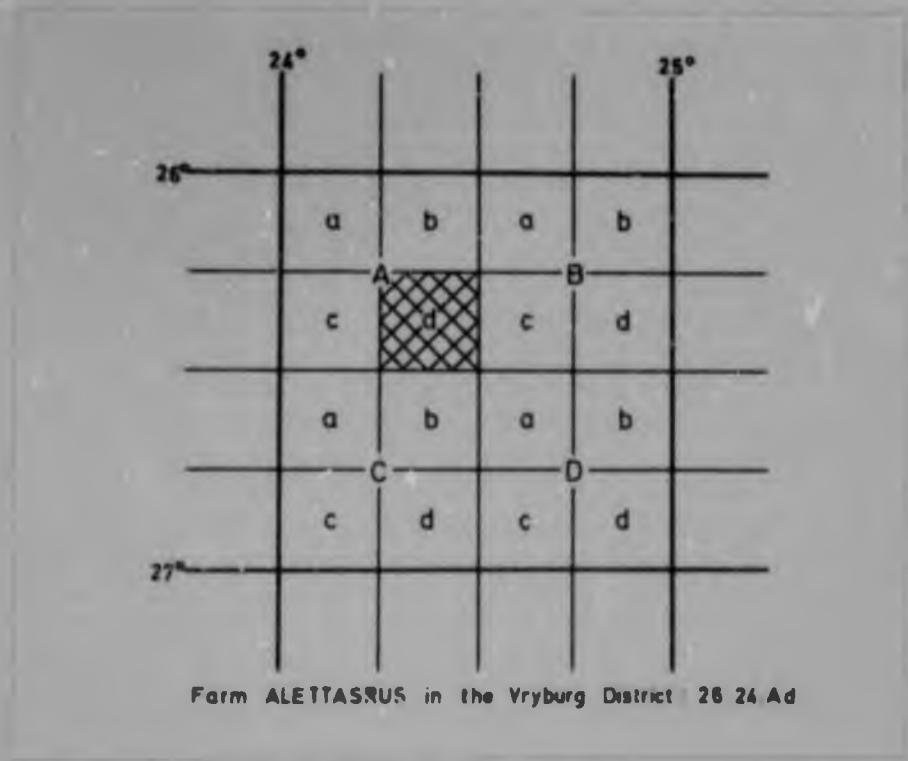

Fig.10 Illustrating Davis' map reference method used in this study [see text].

3.8 Vaccination

To the best of our knowledge none of the persons included in this study have ever received plague vaccine as a prophylactic measure. Consequently this factor did not need to be considered in the interpretation of our PHA results.

4 ECOLOGICAL CONDITIONS DURING THE PERIOD 1965-1969

Southern Africa is that part of Africa south of the Zambesi, Okavango and Kunene rivers. It embraces South Africa, South West Africa, Lesotho, Botswana, Swaziland, Rhodesia and the southern part of Portuguese East Africa,

Wild rodent plague is known to occur only in the first four of these countries and during the period 1965-1969 human plague occurred in South Africa, South West Africa and Lesotho. This period was marked by a transient increase in the incidence of plague with a peak during the plague year 1967/68[1]. In other parts of the world a similar trend was shown. Fig.11 illustrates this by means of a comparative annual plague incidence for several enzootic regions for the period 1960-1971. Other foci however did not show this tendency.

A cyclical fluctuation in southern Africa is by no means unusual. Davis [1948] mentioned a periodicity of 5-6 years in human plague and attributed this to a general periodicity in the fluctuation of the wild rodent population density. He furthermore drew attention to the distribution of a small number of outbreaks over 3-4 years [1940-1943] and contrasted this to an earlier tendency towards a concentration of plague in one or two years. Of great relevance to our recent plague situation

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1. The concept of a 'plague year', taken from one southern mid-winter [July 1st] to the next [June 30th] was first introduced by Davis [1948] as the incidence of human plague is highest in the summer.

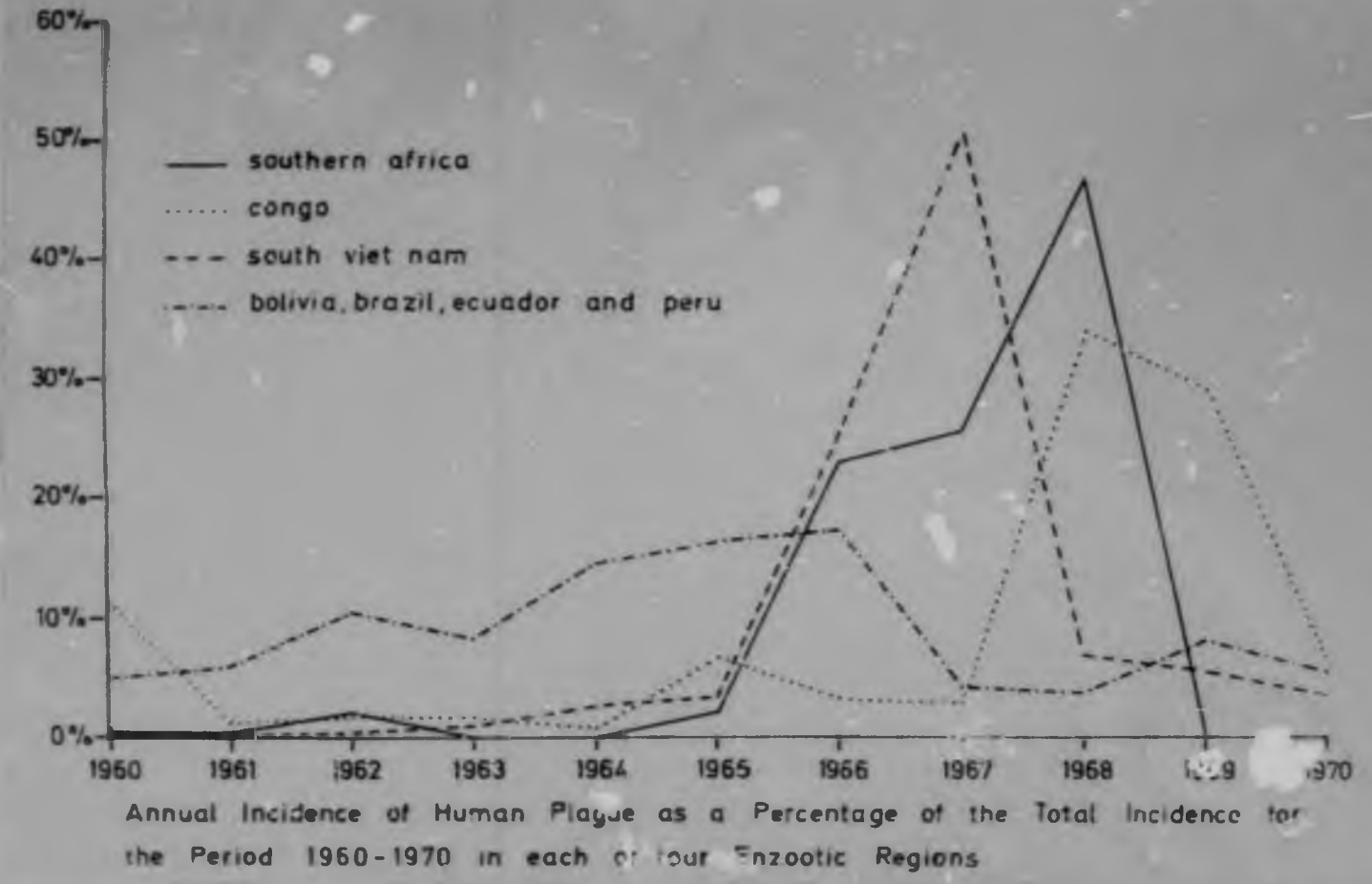

Fig.11 Annual incidence of human plague shown as a percentage of total incidence for the 11-year period 1960-1971 in each of the enzootic regions. Total notifications during 1960-1971 were: Vietnam 11063 cases; Bolivia, Brazil, Ecuador and Peru had 5107 cases, Congo 233 and southern Africa 331. [Compiled from notifications published in the WHO Epidemiological and Vital Statistics Reports, by the South African State Department of Health and from SAIMR records]

is Davis' observation on events in 1935/36 concerning the then prevalent massive increase in rodent density following a period of abnormal rainfall. This was followed by a marked recrudescence of plague.

Fig.11 also shows that, after a period during which the incidence of human plague in southern Africa was very low, the year 1965 ushered in a four-year period of increasing prevalence.

The year 1966 was marked by abundant rains which temporarily relieved drought conditions which had existed over large areas for some five years. Table I shows the rainfall data for the period July 1965 to July 1968 for some of the plague enzootic regions. Each region has a number of rainfall measuring stations and the two figures in each column of the table represent the lowest and the highest measurements obtained in the region concerned. These data, showing an annual rainfall during 1966/67 which generally exceeded a hundred per cent of the long term average, confirm the subjective impression of the rainfall situation gained at the time.

These unusual climatic conditions were associated with a generalized domestic and wild rodent population explosion. In some regions the long awaited rains were however too late to save drought stricken crops. Consequently a below-average food supply only was available to an above-average rodent population. It was therefore not surprising that reports appeared in the lay press of complaints by the public about a noticeable presence of domestic rodents in urban areas. The author observed

Table I. Rainfall data for the period 1.7.1965 - 1.7.1968*

district and map reference	plague-year	rainfall in mm	number of rainy days	percentage of long term average	altitude in meters
Vryburg [26.24.D]	1965/66	248 - 317	12 - 64	55 - 67	1189 - 1325
	1966/67	587 - 708	40 - 88	128 - 163	
	1967/68	326 - 480	30 - 67	72 - 103	
Senekal [28.27.B]	1965/66	321 - 465	23 - 51	51 - 68	1433 - 1676
	1966/67	690 - 890	37 - 75	92 - 144	
	1967/68	352 - 524	26 - 74	55 - 87	
Mohaleshoek [30.27.B]	1965/66	531 - 721	46 - 74	67 - no data	1370
	1966/67	848 - 866	64 - 77	108 - " "	
	1967/68	433 - 444	54 - 57	56 - " "	
Uitenhage [33.25.D]	1965/66	426 - 744	72 - 123	86 - 126	15 - 290
	1966/67	397 - 705	36 - 118	106 - 136	
	1967/68	274 - 881	71 - 110	73 - 113	
Transkei [33.27.A]	1965/66	322 - 654	33 - 102	68 - 94	610 - 1219
	1966/67	378 - 885	37 - 100	81 - 133	
	1967/68	202 - 500	18 - 74	36 - 68	
Transkei [31.27.C]	1965/66	443 - 489	54 - 68	75 - 95	834 - 1036
	1966/67	575 - 602	81 - 89	102 - 112	
	1967/68	258 - 288	65 - 84	49 - 50	

*Compiled from 'Report on Meteorological Data' for the years 1966, 1967 and 1968 of the RSA Department of Transport.

the unusual presence of dead wild rodents, victims of motor traffic, on national roads.

Tatera brantsii, the chief plague reservoir animal in most of southern Africa's enzootic regions, is a wild rodent which normally does not appear in the close vicinity of human habitats. In 1968, however, burrows of this species were detected within a few meters of the entrances to human dwellings in Lesotho.

The combination of climatic and ecological conditions as described above favoured a recrudescence of plague and made this a predictable event in southern Africa.

5 PLAGUE IN OVAMBOLAND

5.1 Description of the Area

Ovamboland is in the northernmost part of South West Africa and is situated approximately between latitudes $17^{o}25'$ and $18^{o}30'$ south, and longitudes $14^{o}00'$ and $17^{o}30'$ east. The territory is undulating and consists mostly of a sandy soil with a noticeable absence of rocks and stones. In the south there are huge grass covered plains on which game may still be observed in large numbers. In the northern parts subtropical vegetation makes an appearance [Fig.12].

Fig.12 Typical vegetation in northern Ovamboland. Note the large termite nest in the centre of the photograph.

An interesting feature are the numerous 'oshanas', shallow water courses with clay beds which run from

north to south and are dry for most of the year [Fig.13].

Fig.13 An 'oshana', a dry shallow water course which becomes flooded during the summer rainfall season.

However, during the summer rainfall season the oshanas are flooded by run-off water originating in the highlands of Angola [Portuguese West Africa] which is responsible for the inundation of large parts of Ovamboland.

The Ovambo villages are built on higher ground between the oshanas. They are composed of a complex system of grass roofed huts which are separated from each other by narrow passages formed by high pole fences [Fig.14]. Adjoining the huts are large food storage baskets which are slightly raised off the ground by wooden frames on short legs [Fig.15]. Huts, passages and food baskets are in their turn enclosed by a pole

Fig.14 A corner of an Ovambo village.

Fig.15 Ovambo food storage baskets showing ideal rodent harbourage conditions.

fence around which are lands cultivated mainly with 'Mahango', a type of millet which forms the staple diet of the population. The lands are fenced in by dense hedges interspersed with prickly pears [Opuntia species].

Clearly, this entire complex provides ideal conditions for rodent harbourage.

Climatic conditions are such that most children are clad in a loincloth or little skirt only.

A favoured occupation of children is hunting, with the aid of bows and arrows, of small animals such as wild rodents and birds which are then eaten [Fig.16].

Fig.16 Three Ovambo herd boys dressed in loincloths and armed with bows and arrows used for killing wild rodents and birds.

The territory is served by a large, modern and well equipped State Hospital which is centrally situated at Oshakati [map reference 17.15.Dc]. This hospital meets the general medical requirements of the population in its immediate vicinity. Scattered throughout Ovamboland are a number of mission hospitals and clinics. Many of these are run by experienced resident nursing sisters who conduct several out-patient sessions per week during which they diagnose and treat a wide range of common minor ailments. Patients with more serious conditions are admitted to the mission hospital to await attendance by a medical officer who regularly makes visits. Patients requiring urgent treatment or specialized facilities which are not available locally are transferred to the State Hospital at Oshakati. Many of the hospitals are linked by radio communication.

As elsewhere, shortage of qualified staff is a problem in Ovamboland but the system of supplying medical services to the population as outlined above has proved to be efficient.

Preventive health services, e.g. malaria control, are supplied by the South West African health authorities in collaboration with the hospitals.

5.2 The Situation prior to 1965

The first known outbreak of human plague in Ovamboland occurred in 1932. Fourie [1932] concluded that the disease had its origin in the northern Cape Province from where he believed it to have spread

across the Kalahari Desert to Ovamboland. From 1932 plague was reported virtually every year until 1948/49 after which a twelve-year period of inactivity set in. Since a number of these early reported outbreaks were not confirmed by laboratory methods their identification with plague should be treated with reserve. Peak incidences occurred in 1934/35 and 1942/43. From the year 1961/62 the disease occurred annually with peaks during the plague-years 1962/63 and 1967/68.

The outbreaks of 1961/62 and 1962/63 were investigated thoroughly by Geldenhuys [1963] and bacteriologically confirmed by the SAIMR laboratories in Windhoek and Johannesburg.

Dr K.F. Meyer of the George Williams Hooper Foundation in San Francisco visited the plague affected area in 1963 and obtained sera from patients for haemagglutination studies, the results of which have been published [Meyer, 1964].

5.3 The 1965/66 Epidemic

Six cases of bubonic plague were notified during the period March-July 1966. There were no deaths. Information could be obtained on five of the patients and the available data are presented in Table II. The patients' sera were submitted to Dr K.F. Meyer for the haemagglutination studies as these were not performed in South Africa on a routine basis until 1967.

Table II. Clinical and laboratory data on five suspected plague patients in Ovamboland during the plague year 1965/66

Patient	sex	age in years	site of bubo	Y. pestis isolation from bubo	PHA titres and hospital day in brackets
M.N.	F	18	left inguinal	+	1:4[1]; 0[14]; 1:4[19]
A.M.	F	11	right inguinal	-	1:16[16]; 0[19]
H.J.	F	3	left inguinal	-	1:16[6]
A.P.	F	2	left inguinal	-	1:4[?]
P.S.	F	5	bilateral cervical and right cubital	not done	1:4096[1]

5.4 The 1966/67 Epidemic

In this plague-year seventysix cases of suspected bubonic plague with one death were notified. The area was visited and investigations were carried out into several aspects of the outbreak. The details of the associated rodent epizootic have been reported by Hallett [1967].

The earliest plague patients lived in the Ongandjera district [map reference 17.15.Dc] and spread occurred from there in a northwesterly direction. Signs of extensive rodent mortality were found in the form of inactive gerbil [Tatera] burrows [Fig.17].

Fig.17 Inactive gerbil burrows as indicated by the absence of freshly excavated soil and by the obstruction of the entrances by grass.

A school teacher reported the finding of dead rats at her home and investigation revealed the presence of dead Praomys [Mastomys] natalensis inside dwellings while gerbil colonies in the vicinity were found to be abandoned.

Xenopsylla philoxera were recovered from rodent burrows and two pools of these fleas yielded Y. pestis on culture and animal inoculation.

The monthly incidence of suspected plague is shown in fig.18.

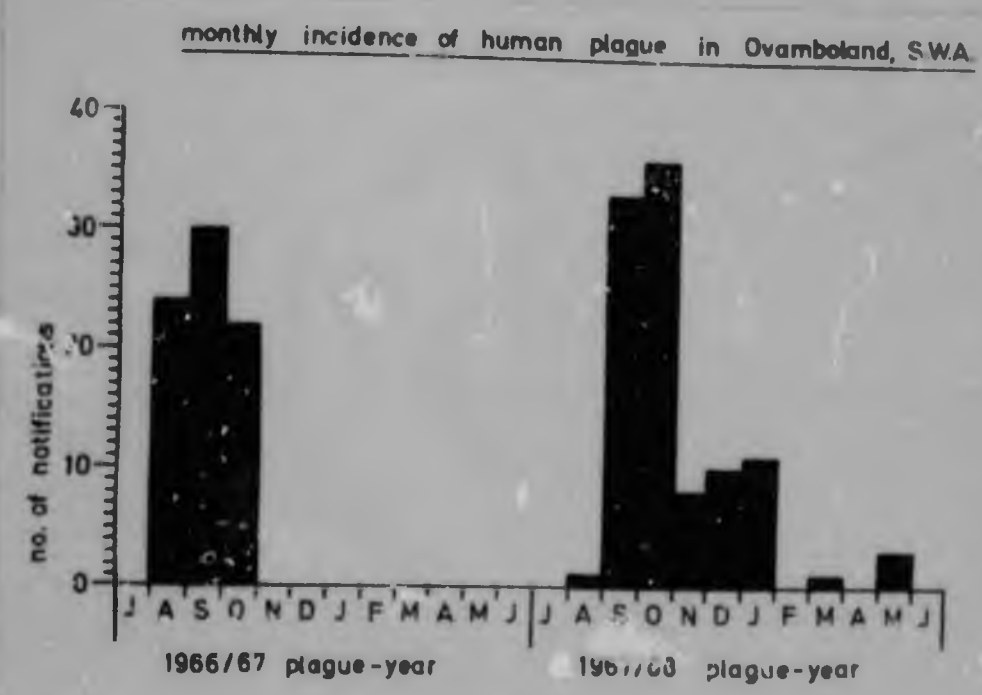

Fig.18 Monthly incidence of human plague in Ovamboland during the period 1966/67/68.

Table III summarizes such clinical and laboratory data as could be obtained on each patient.

Table III. Available clinical and laboratory data on 76 notified cases of suspected plague during the 1966/67 epidemic in Ovamboland. [For abbreviations see page 55]

case number	sex	age in years	temperature on admission	site of bubo	Y. pestis isolation	PHA titre on admission	subsequent PHA titres and hospital day in brackets	miscellaneous observations
1/66	M	12	normal	R.I.	-blood	1:128	1:4096[23]	Bubo still present on day 23
2/66	M	5			+bubo	1:512	1:2048[26] 1:8192[28]	
3/66	F	24		L.I.	- „	0		
4/66	F	24	raised	L.I.	- „	0		
5/66	F	24	raised	L.I.	- „	0	0 [26]	
6/66	F	55		L.I.	-blood	0		
7/66	M	10		B.I. L.A.	- „	0		
8/66	F	20	38,6°C	L.I. R.A.	- „	1:32	0 [24]	Splenomegaly

Table III continued. For headings see page 49.

9/66	M	16	raised	R.I.	-blood	0	0	[31]	Splenomegaly
10/66	F	17	normal	I.	- „	0	0 0	[5] [19]	No bubo on day 19
11/66	F	45	normal	R.I.	- „	0	0	[19]	
12/66	F	28	39,5°C	R.I.	- „	1:32	1:256	[21]	
13/66	F	13	normal	B.I.	- „	0	0 0	[5] [19]	
14/66	F	14	raised	B.I.	+ „	0	0	[22]	
15/66	M	43	normal	R.I.	- „	0			
16/66	F	36	raised	R.A.	+ „	1:512	1:512	[31]	
17/66	M	6	raised	B.I.	- „	0			
18/66	F	23	normal	L.I.	- „	0	0	[19]	
19/66	F	65	38,3°C	R.I. R.F.	+ „	1:256	1:32	[19]	
20/66	M	7	normal	L.I.	- „	1:256			No bubo on day 16
21/66	M	16	normal	R.I.	- „	0	1:64 1:128	[5] [19]	Epistaxis on day 9 Bubo still present on day 16
22/66	F	25		R.I.	- „	0	1:64 1:32	[5] [24]	No bubo on day 16

Table III continued. For headings see page 49.

23/66	F	28		L.I.	-blood	0	0	[17]	Bubo still present on day 17
24/66	F	45		L.I.	- "	0	1:32	[24]	Bubo still present on day 12
25/66	F	12		R.I. R.A.	- "	0	1:64 1:64	[5] [19]	
26/66	F	25	raised	L.I.	- "	0			
27/66	F	1		L.F.	- "	0	0	[31]	Small gland still present on day 12
28/66	F	21		L.I.	- "	0	1:32	[25]	No bubo on day 12
29/66	F	19		R.I.	- "	0	0	[25]	
30/66	M	14	39,2°C	R.A.	- "	1:512	1:32	[25]	
31/66	F	5			- "	0			
32/66	F	23		L.I.	- "	0	0	[25]	No bubo on day 11
33/66	M	5	39,5°C	B.A.	- "	0	0	[31]	Buboes still present on day 9
34/66	M	6	38,4°C	L.C.	- "	1:512	1:512 1:64	[12] [31]	Presented also with diarrhoea and vomiting
35/66	M	11		B.I. B.F.	- "	0	0	[31]	
36/66	M	4		R.C.	- "	0	1:1024 1:1024	[14] [31]	Had a membrane on right tonsil and 'bullneck' appearance resulted in a provisional diagnosis of diphtheria

Table III continued. For headings see page 49.

37/66	F	7				0		
38/66	F	4				0		
39/66	F	26	normal	I.	-blood	0	1:64 [24]	
40/66	F	5	40,0°C	R.I.	- „	0	1:128 [14] 1:128 [31]	Splenomegaly
41/66	M	11	40,0°C	R.I.	+ „			Died on day of admission, no serum available
42/66	F	40	raised	R.I.	-bubo	1:1024	1:128 [21]	
43/66	F	17	normal	R.I.	-blood	0		
44/66	F	28		R.I.	- „	0	1:32 [10] C [31]	Splenomegaly
45/66	F	17	38,9°C	L.I.	- „	0	1:256 [15] 1:256 [29]	
46/66	F	26	38,3°C	L.I.	- „	0	1:512 [15] 1:1024[29]	
47/66	F	6	raised	L.I.		0		
48/66	F	20				0	0 0	
49/66	F	14			+	0	1:1024[14] 1:64 [28]	

Table III continued. For headings see page 49

50/66	M	2				0	1:512 [14] 1:512 [31]	
51/66	?	?				0		Not included in Fig.19
52/66	F	40				0	0 0	
53/66	F	?				0	0 0	
54/66	F	18				0	0 0	
55/66	F	45			+blood	0	1:512 [25] 1:32 [41]	
56/66	F	50				0	1:512 [18] 1:64 [34]	
57/66	F	45				0	0 0	
58/66	?	?				0	0 [10]	Not included in Fig.19
59/66	M	36	raised	L.I. L.F.		1:2048	1:1024[39]	
60/66	F	35				1:16	1:1024[21] 1:128 [36]	
61/66	F	18	38,3°C	L.I.		0	1:128 [15] 1:64 [30]	

Table III continued. For headings see page 49.

62/66	F	17	38,9°C	L.I.		0		
63/66	F	14	37,8°C	L.I.		0		
64/66	F	17	37,8°C	R.I.		0		
65/66	F	3	38,6°C	L.I.		0		
66/66	M	5	37,8°C	B.I.		0		
67/66	M	3		I. F.				No specimens received
68/66	F	15		L.F.		0		
69/66	F	1		L.F.				No specimens received
70/66	M	16	raised	L.I.	+blood	0	1:128 [14] 1:16 [36]	Presented also with a cough
71/66	F	65	raised	R.A.	+blood	0	1:512 [14] 1:256 [30]	Presented also with a cough
72/66	F	45	raised	R.I.	+blood	0	1:4096 [15] 1:256 [31]	
73/66	F	40	40,6°C	L.I.		0	1:512 [18] 1:64 [34]	
74/66	F	40	raised	L.I.		0	1:128 [14] 1:16 [101]	

Table III continued. For headings see page 49.

75/66	F	37	raised	R.I.	0	1:64 [14] 1:16 [30]	
76/66	M	29					Patient absconded on day of admission

Abbreviations

A. Axillary
B. Bilateral
C. Cervical
F. Femoral
I. Inguinal
L. Left
R. Right

Fig.19 shows the PHA results of seventy patients separated according to age and sex. No blood was received from four patients, and neither blood nor information was obtained from two more patients. These are therefore excluded from Fig.19.

During the investigation 337 sera were obtained from apparently healthy people living in the plague affected area. Their PHA results are shown in Table IV.

Table IV. PHA results of 337 apparently healthy people living in the plague affected area during the Ovamboland epidemic of 1966/67.

Number of sera	PHA titre
332	0
2	1:4
2	1:256
1	1:512

5.5 The 1967/68 Epidemic

Following a quiescence of nine months an exacerbation of plague occurred in August 1967. Between then and June 1968 a total of 103 suspect cases was notified. There were no deaths. The monthly incidence of plague during this outbreak is also shown in Fig.18.

Blood was received from seventythree patients but only seventeen cases could be serologically tested on more than one occasion. Table V presents the scanty clinical and laboratory data available on each patient and in Fig.20 the initial PHA results of the patients are shown for each sex and age group.

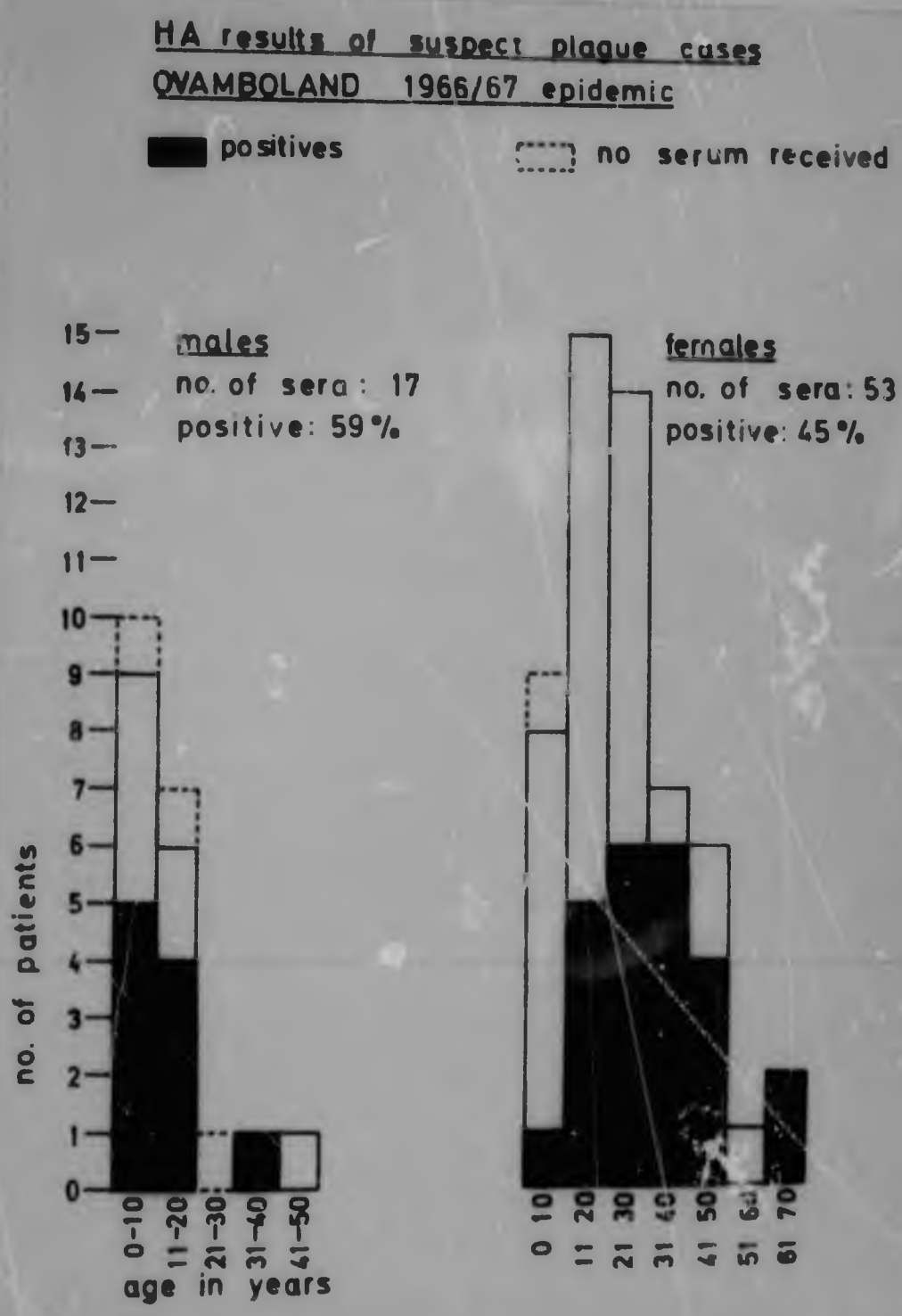

Fig.19 PHA results of 70 patients during the 1966/67 epidemic in Ovamboland. Sera with titres of 1:16 or higher are included in the positives.

Table V. Available clinical and laboratory data on 103 notified cases of suspected plague during the 1967/68 epidemic in Ovamboland. [For abbreviations see page 65.]

case number	sex	age in years	temperature on admission	site of bubo	Y. pestis isolation	PHA titre on admission	subsequent PHA titres and hospital day in brackets	miscellaneous observations
1/67	F	16		R.A.	-blood	0		
2/67	F	1[illegible]		R.A.	- "	0		
3/67	M	27	36,7°C	L.I.				Patient absconded before specimens were taken
4/67	F	17	normal	R.I.	-blood	0		
5/67	M	4/12		Sma.	-bubo			No serum received
6/67	M	1		L.I.	-blood	0		
7/67	F	12		L.I.	- "	0		Temperature 40,0°C on 17th day in hospital
8/67	M	6		L.I.	- "	0		

Table V continued. For headings see page 58.

9/67	F	6		R.I.	-blood	0		
10/67	F	0		L.Sli	- „	0		
11/67	F	17		R.I. R.P.	- „	0		
12/67	F	50		B.I.	- „	1:64		
13/67	F	23		L.I.				No specimens were received
14/67	F	15		L.I.	- „	0	0	[9]
15/67	M	56		O.				No specimens were received
16/67	F	13		R.I.	- „	0	0	[15] Temperature 37,8°C on 13th day in hospital
17/67	F	10		L.I.	- „			
18/67	F	39	37,4°C	R.I.	- „	0	0	[14]
19/67	F	13		L.I.				No specimens were received
20/67	M	8		L.P.				„ „ „ „
21/67	M	23		L.I.				„ „ „ „
22/67	F	22		L.I.				„ „ „ „
23/67	M	12		L.I.	- „	0	0	[6]

Table V continued. For headings see page 58.

24/67	M	25	38,6°C	L.I.	-blood	C		
25/67	M	8	39,5°C	L.I.	- "	0		
26/67	M	46		R.I.	- "	0		
27/67	F	24		L.I.	- "	0		
28/67	F	19	36,7°C	R.I.	- "	0		
29/67	F	6		L.C.			No specimens were received	
30/67	M	36		L.C.			" " " "	
31/67	M	28		R.I.	-blood	1:64		
32/67	M	26		L.I.			" " " "	
33/67	M	12		R.I.	-blood	0		
34/67	F	14		R.I.	+ "	1:64	1:1024[3]	
35/67	M	7		Sme	- "	1:16		
36/67	F	6		Cli	- "	1:16		
37/67	?	13		Sme	- "	0		
38/67	M	12		Sli			No specimens were received	
39/67	F	26		R.A.	- "	0		

Table V continued. For headings see page 58.

40/67	F	10		R.I.	-blood	0		
41/67	M	4		R.O.	- "	0		
42/67	M	4		B.O.	- "	0		
43/67	F	15		P.I.	- "	0		
44/67	F	15		R.A.	- "	0		
45/67	F	27		B.I. L.C.				No specimens were received
46/67	M	9		L.I.	-blood	1:16		
47/67	F	11		Sme	- "	0		
48/67	M	14		R.A.	- "	0		
49/67	F	15	37,8°C	?	- "	1:16	1:16 [23] 1:16 [40]	
50/67	F	14		Abs.		0		Presented with pain in the right side of the neck
51/67	F	15		Abs.	-blood	0		Presented with pain in the left side of the neck
52/67	F	15		Abs.	- "	0		Presented with pain in the left side of the neck

Table V continued. For headings see page 58.

53/67	M	7		Abs.	-blood	0			Presented with a 5-months history of a left sided swelling in the neck
54/67	M	17		Abs.					Complained of a stiff neck. No specimens were received
55/67	F	6		L.A.					No specimens were received
56/67	F	57	37,8°C	Abs.		0			Presented with tenderness in the left groin
57/67	M	9	37,4°C	L.I.	-blood	0	0 0	[10] [26]	
58/67	M	7		C.	- "	0	1:16	[11]	
59/67	F	16		C.	- "	1:16			
60/67	M	1½		B.I.	- "	1:16			
61/67	F	14		L.I.	- "	1:32			
62/67	F	13		L.C.	- "	0			
63/67	F	14		R.C.					No specimens were received
64/67	F	30	raised	Abs.	- "	0			Presented with pyrexia only
65/67	F	15		R.C.					No specimens were received
66/67	F	12		R.C.					No specimens were received

67/67	F	13		R.C.	-blood	0		
68/67	F	10		L.Sli				No specimens were received
69/67	F	16		R.C.				No specimens were received
70/67	F	18		L.C.				No specimens were received
71/67	M	39		R.I.	-blood	1:128		
72/67	F	10	40,2°C	R.I.	- "	1:2048	1:512 [35] 1:2048 [53] 1:2048 [74] 1:512 [120] 1:512 [180]	
73/67	F	6		R.I.	- "	0		
74/67	M	4		L.C.	- "	0		
75/67	M	31	37,8°C	R.I.	- "	1:32	0 [23] 0 [27]	
76/67	F	1½	normal	L.C.				No specimens were received
77/67	F	18		B.I.	- "	1:128	1:16 [40]	Buboes were fluctuant
78/67	M	20		R.F.				Had been treated for 2 months for a right tibial osteomyelitis. No specimens were received
79/67	M	5	40,0°C	R.I.	-bubo			No blood was received

Table V continued. For headings see page 58.

80/67	M	50		R.I.				No specimens were received
81/67	M	13		R.I.				No specimens were received
82/67	M	30		R.I.	-blood	1:8		
83/67	M	12		R.I.	- „	0		
84/67	M	7		L.I.	- „	0		
1/68	M	60	36,7°C	R.I.				No specimens were received
2/68	M	15	37,8°C	R.C.				No specimens were received
3/68	M	27		L.I.	-blood	0		
4/68	F	12						No specimens were received
5/68	F	29	37,8°C	R.I.	-blood	0		
6/68	M	44		R.I.	- „	0		
7/68	F	23	37,8°C	L.I.	- „	0		
8/68	F	12	36,7°C	L.I.	- „	0	1:1024	[41]
9/68	F	35	36,7°C	L.I.	- „	0		
10/68	F	17		R.I.	- „	1:16	0	[29]
11/68	F	19	37,8°C	L.I.	- „	1:16	1:64	[29]
12/68	F	60		R.I.	- „	0	1:512	[20]

Table V continued. For headings see page 58.

13/68	M	12		L.I.	-blood			
14/68	M	14		R.I.	+ „		1:256 [42] 1:512 [56]	
15/68	M	13	36,1°C	R.I.	- „	0		
16/68	M	12		R.I. R.P.	- „	1:64		
17/68	F	13		R.I.	- „	0	1:16 [7]	
18/68	M	10	36,1°C	R.I. R.C.				No specimens were received
19/68	F	13		L.I.	- „	1:64		

Abbreviations

A.	Axillary	O.	Occipital
Abs.	Absent	P.	Popliteal
B.	Bilateral	R.	Right
C.	Cervical	Sli	Sublingual
F.	Femoral	Sma	Submandibular
I.	Inguinal	Sme	Submental
L.	Left		

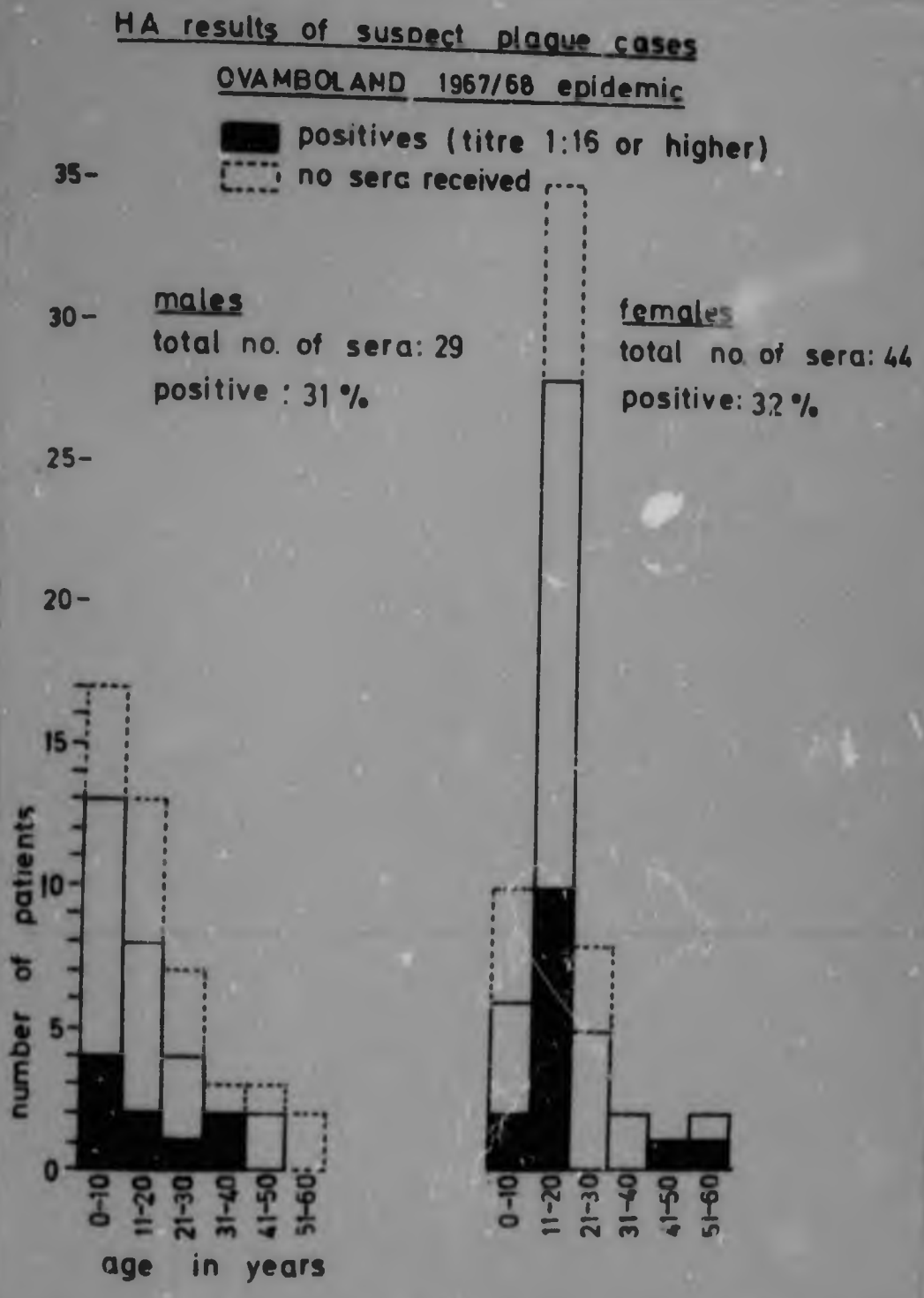

Fig.20 PHA results of 73 patients during the 1967/68 epidemic in Ovamboland.

5.6 Comments on Individual Cases and Discussion of Results

It has been our experience that it is sometimes difficult to obtain cooperation in the matter of submission of follow-up serum specimens to the laboratory from clinically suspect plague patients. A request for at least one repeat specimen is issued with each initial PHA report but the response has been poor.

A total of 179 suspected plague patients was notified during the 1966/67 and 1967/68 epidemics. Admission specimens of serum were received from 143 patients but follow-up sera from only 69 of these patients. Table VI shows the high percentage of positive PHA results which may be obtained when follow-up studies are carried out.

Table VI. Comparison of the percentage positive PHA results of suspected plague patients on admission and during convalescence.

	Total number of patients	Patients with positive PHA on admission	Patients with positive PHA on follow-up
Admission serum only studied	74	13 [= 18%]	no follow-up
Two or more sera studied	69	18 [= 26%]	42 [= 61%]

The maximum levels of antibody in the serologically tested patients during the two epidemics are shown in Fig.21. Serological follow-up was good during the 1966/67 epidemic but very poor in 1967/68 [see Tables III and V] and Fig.21 clearly reflects the difference in results, particularly with regard to the PHA titres.

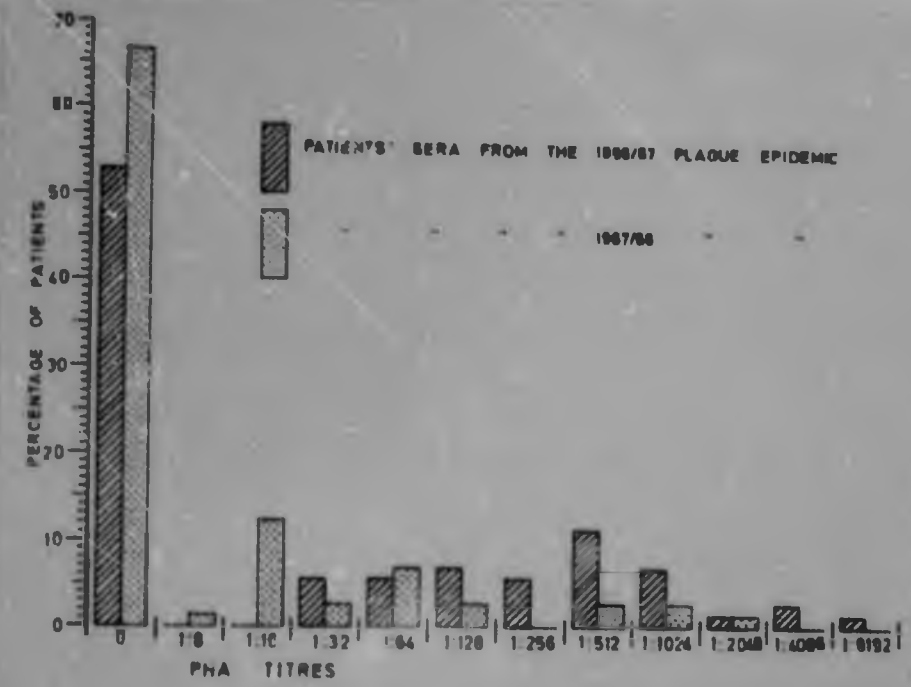

Fig.21 PHA titres of two Ovambo plague epidemics illustrating the greater percentage of positive sera and higher titres demonstrated during 1966/67 when serological follow-up was superior to that of the 1967/68 patients.

Meyer [1964] recommended serological testing of patients on the day of discharge from hospital. Consequently, since the PHA test was made available in this country we have recommended the minimum requirement of an admission serum and a discharge serum as a practical basis for reliable plague sero-diagnosis. Adherence to this recommendation would result in a considerably larger proportion of positive results, especially if the interval between tests measured at least several weeks.

Marshall et al. [1967a] also commented on the occurrence of a number of negatively reacting sera in the early phase of the disease in Vietnamese patients.

Several workers have made note of the apparent clinical mildness of plague in Ovamboland. Fourie [1932] drew attention to the low case mortality in this region at a time when specific chemotherapeutic agents were not yet available. He speculated on this anomaly and suggested the possibility of a 'mild type of infection or an enhanced resistance to the disease among the Ovambo'. Davis [1946] stated that 'no septicaemic or pneumonic cases have yet occurred' and that 'the mortality rate is low and has rarely exceeded 25 per cent'. Meyer (personal communication] also observed that the disease was relatively mild in Ovamboland.

The combined mortality rate of the 1966/67 and 1967/68 epidemics was 0,6 per cent of notified cases. There is good reason to believe that this mortality figure is artificially low due to the notification of plague on the flimsiest evidence, especially during the outbreak of 1967/68. Thus, Table V includes patients who were notified on no other basis than complaints of 'pain in the neck' [case numbers 50/67, 51/67 and 52/67], stiff neck [case number 54/67], 'pyrexia' [case number 64/67] and other vague symptoms. The dilution of the statistics with apparent 'non-cases' of this kind must result in unreliable data. Nevertheless, the fact remains that only a single fatality occurred as a result of the two outbreaks in which there were 59 patients with either bacteriological and/or serological evidence of plague in-

fection. Using this as a basis for estimating the mortality a figure of 1,7 per cent is obtained which is still uncommonly low.

Personal observations and enquiries revealed the remarkable absence of subjective symptoms of serious systemic infection. Patients with high temperatures, obvious buboes and occasional splenomegaly frequently arrived at a hospital after having walked several hours from their homes and complained of little else but headache and backache. There was no loss of mental alertness.

Although forms other than bubonic plague have not been reported previously in Ovamboland [Davis, 1946], the finding of soft, tender and enlarged spleens in some of the patients, as well as the isolation of Y. pestis from blood indicates the occurrence of septicaemic plague during the epidemics under discussion. Of interest in this respect is the history of an eleven year old boy who became ill during the 1967/68 epidemic. A specimen of serum was received with a provisional diagnosis of 'acute lymphadenitis and meningitis' and a request for a PHA test. The latter yielded a positive result to a titre of 1:64. On the supposition that this might be a case of plague meningitis a history was then requested from the attending medical officer, Dr D.K. Genis, who kindly supplied the following details.

The child was admitted to hospital with a painful left inguinal swelling of seven days duration. Examination revealed a pyrexia of 39,8°C, acute left inguinal lymphadenitis, mild pharyngitis, tachycardia, a systolic murmur and slight, but diffuse, abdominal

tenderness. The white cell count showed 24000 leukocytes/cmm blood with 69 per cent polymorphonuclear cells.

The patient was treated with parenteral penicillin which produced a favourable response at first but three days after admission the temperature rose again to 39,8°C and a typical picture of acute meningitis developed. Examination of the cerebrospinal fluid yielded the following results.

Polymorphonuclear leukocytes	:	innumerable
Lymphocytes	:	scanty
Erythrocytes	:	scanty
Protein	:	145 mg/100 ml
Sugar	:	7 mg/100 ml
Chloride	:	96 meq/L
Direct examination and culture	:	negative

Combination therapy with penicillin, sulphadiazine and chloramphenicol resulted in steady improvement.

The features of inguinal lymphadenitis, meningitis and the presence of PHA antibodies in a patient during an epidemic of plague strongly suggest this to be a case of plague meningitis.

A patient with very similar features has been described by Feeley and Kriz [1965]. Collins et al. [1967] reported a bacteriologically proven case of plague meningitis in a two-year old boy in New Mexico. This child was admitted with an axillary bubo, fever and meningismus. Plague was at first not suspected and the child was treated with penicillin and local heat.

Meningitis developed subsequently and Y. pestis was isolated from the cerebrospinal fluid.

The noteworthy similarity in all these cases was the wrong diagnosis on admission which resulted in inadequate treatment. Landsborough [1947] reviewed eight patients with plague meningitis and was of the opinion that this condition may represent a complication in the course of septicaemic spread from a primary bubonic focus. He also drew attention to the fact that meningitis tended to occur relatively late in the course of plague and that it is more frequent in treated patients.

The Ovambo patient discussed above is of interest not only as a probable example of relatively rare plague meningitis but also as an indication of the occurrence of septicaemic plague in Ovamboland.

Fiftyseven patients yielded positive PHA results during the two epidemics and these were analysed with a view to establishing the rapidity of sero-conversion. The results are presented in Table VII. While as many as 54 per cent of these patients already had antibodies on admission, almost 80 per cent were positive by the end of the second week in hospital and nearly all the patients by the end of the fourth week. These figures are in reality probably higher still as in 46 per cent of the patients the actual day of sero-conversion must have occurred some time before the second serum specimen was obtained. In trying to determine the significance of this rather rapid development of antibodies the conclusions drawn by Legters et al. [1970] are of great

Table VII. The appearance of demonstrable plague antibodies in relation to the number of days following admission to hospital of fifty-seven sero-positive Ovambo patients.

Number of days after admission	Number of sero-positive patients [accumulative]	Percentage of sero-positive patients [accumulative]
0	31	54 %
7	35	61 %
14	45	79 %
21	51	89 %
28	55	96 %
35	55	96 %
42	57	100 %

interest. These authors found PHA antibodies in most Vietnamese persons with clinically mild plague in their first or second serum samples but only at a much later stage in patients who were seriously ill. They based this difference on the belief [substantiated in one case] that mild plague occurred in those people who prior to their illness had been serologically stimulated by subinfective doses of Y. pestis via flea bites. This would then have the dual effect of mild plague resulting from subsequent exposure and the presence of antibodies early in the disease.

To a limited extent this explanation may apply also in Ovamboland, especially as a few of those patients who were sero-positive on admission to hospital claimed to have been ill for no more than one day. However, if prior immunization by natural infection played a major role in the mild character of Ovambo plague a relatively

high percentage of the healthy population in the plague affected area could reasonably be expected to have plague antibodies. This proved not to be the case. In marked contrast to other plague epidemic, and even quiescent endemic, situations in southern Africa the number of PHA positive healthy people was extremely low during the 1966/67 epidemic. Only 5 out of 337 people, or 1,4 per cent, were found to have demonstrable PHA antibodies. The survey was carried out right at the start of the epidemic and this could explain the low number of positives but, irrespective of the reason, this finding mitigates against prior immunization as a cause of the modified disease picture in this region. Artificial immunization has not been carried out in Ovamboland nor in the other regions discussed in this work.

The late sero-conversion as described by Legters appeared to occur in a number of Ovambo patients and was definitely established in one case. The patient concerned was a fourteen-year old girl [case number 14/66, Table III] with bilateral inguinal buboes. Y. pestis was isolated on blood culture but serologically the patient remained negative until the twenty-second day of her stay in hospital when she was discharged and unfortunately lost to further study. Experimental work with other antigens, notably the pneumococcal polysaccharide, suggests that immune paralysis is readily induced on excessive antigen administration [Finland and Winkler,1934; Frisch et al.,1942] and this

type of response should be considered as a possible mechanism in the delayed or absent antibody production in the more severe cases of plague.

It could also be postulated that the absence of antibodies is an apparent one in that they may partake in the formation of immune complexes which would cause the antibodies to be no longer demonstrable by routine serological techniques. The occurrence of immune complex formation is highly likely in plague in view of some of the manifestations such as disseminated intravascular coagulation and bleeding reported to occur in severely ill patients. These clinical phenomena have been discussed in some detail by Finegold (1968] in relation to plague.

In the course of further attempts to find an explanation for the high proportion of mild plague among Ovambo patients a study was made in vitro of Y. pestis isolates obtained in this and other regions and the results are discussed in a later chapter. Suffice it here to state that no startling results were obtained.

The localization of primary buboes was accurately determined during the 1966/67 epidemic and the distribution was as follows.

Inguinal	:	73 %
Inguinal and femoral	:	7 %
Femoral	:	5 %
Inguinal and axillary	:	5 %
Axillary	:	7 %
Cervical	:	3 %

The cervical buboes in this series occurred in two male children aged four and six years [case numbers 36/66 and 34/66 respectively, Table III]. Conrad et al. [1968] discussed the significance of cervical buboes in two of their patients in a series of fiftyfive. Both were Vietnamese women and the authors referred to the association in Vietnam and Ecuador between the occurrence of cervical buboes and local habits of catching and killing lice and fleas with the teeth.

In Ovamboland the development of cervical buboes may have a different epidemiological basis. In this region they tend to be more common in children while the standard of personal hygiene in the population is comparatively high and pediculosis was not observed. The children's habit of hunting and eating small wild mammals and birds has been mentioned earlier. The two children under discussion attained high plague antibody titres of 1:1024 and 1:512 respectively. One of the boys [case number 36/66, Table III] had a pronounced right sided tonsillitis with membrane formation. The possible significance of this feature escaped our attention at the time and appropriate investigations were not done.

As early as 1927 Pirie and Murray stated that, especially in the African patient, the presence of cervical or axillary buboes is almost invariably associated with a history of having handled or eaten wild hares. It is likely that primary tonsillar plague with cervical lymphadenitis may develop in Ovambo children consequent to their handling of infected reservoir animals and infection via the finger-mouth route.

With regard to sex incidence of plague in Ovamboland there is an apparent overall female preponderance [Fig.19 and Fig.20]. This impression is however a false one created by the artificially uneven composition of the population which is permanently resident in the villages. The great majority of adult Ovambo males is absent during a large part of the year when they are working in the industrial centres of South West Africa. The adult population left behind therefore consists largely of women and this situation is clearly reflected by the plague notifications.

Among the old people there were only few cases of plague without a significant difference in sex incidence. But among the fortysix children in the 0-10 year age group who were notified as plague patients during the 1966/67 and 1967/68 epidemics the incidence was 59 per cent in the boys and 41 per cent in the girls. These percentages are identical to those established for 2755 male and female bubonic and septicaemic plague patients of all ages in Madagascar [Brygoo, 1968]. Brygoo ascribed this marked difference to the men engaging in outdoor activities to a greater extent than the women. In all probability the same reasoning is valid for the Ovambo children as the boys occupy themselves with herding cattle while the girls mostly remain close to their homes.

Caten and Kartman [1968a] showed a marked preponderance of males over females in eighty bubonic plague patients in the United States and also emphasized the

importance in this respect of the relatively closer contact of males than of females with the plague reservoir in enzootic regions.

6 PLAGUE IN THE CAPE PROVINCE: THE UITENHAGE DISTRICT

6.1 Description of the Area and its People

The Uitenhage district is in a low coastal region [altitude less than 100 meters] adjoining the Indian Ocean in the southeastern Cape Province and situated between latitudes 33° and 34° south, and longitudes 25° and 26° east. Its rainfall is evenly distributed throughout the year with an annual mean of less than 600 mm. The climate is mild and neither frost nor very high temperatures occur.

The natural vegetation consists mainly of Aloe, Euphorbia and Portulacaria (Cole, 1961].

The population mainly affected by plague in this region consists of the 'Cape Coloureds' who are an admixture of Caucasoid, South East Asian and African origins.

6.2 The Situation prior to 1965

The history of plague in this region dates back to the second Anglo-Boer war when murine plague affected most of the coastal centres including Port Elizabeth which is a port city in close proximity to Uitenhage.

Rats which had died of plague were first discovered in Uitenhage in April 1905 [Mitchell, 1905]. Subsequently plague established itself in the wild rodent population and today the Port Elizabeth-Uitenhage region is the only coastal plague focus in southern Africa, all other foci being situated inland and elevated well above sea level.

The first locally infected human cases in the Uitenhage district were reported in 1916 during an epidemic which involved the people of eight farms. There were 24 cases with a mortality of 54 per cent. The disease probably had its origin in wild rodents [Mitchell et al., 1927]. From 1916 onwards recrudescences occurred frequently and gradual expansion of the original focus took place [Davis, 1948].

The last outbreak of human plague was reported in 1959 when there were 10 cases [Hallett, 1967].

6.3 The Outbreak of 1967/68

After an interval of eight years one case of plague was notified in August 1967. The patient was a 29-year old Cape Coloured man who lived on a farm in the Uitenhage district [map reference 33.25.Dc]. He gave a four day history of illness and on examination was found to be pyrexial, mentally confused and having bilateral femoral buboes. He complained of severe generalized pains. The patient was treated with streptomycin, sulphadiazine and ampicillin and after ten days of intermittent pyrexia he recovered.

Y. pestis was isolated at the SAIMR, Port Elizabeth, from aspirated fluid of both buboes as well as from four Otomys unisulcatus found in the immediate vicinity of the patient's house.

The scene of the outbreak was not visited by the author who, however, requested sera from the patient, contacts and people living in the neighbourhood. In view of the then just published report by Marshall et al.

[1967b] on the finding in Vietnam of Y. pestis in the throats of people in plague affected areas a request was made for the performance of throatswab cultures. This was done at the SAIMR in Port Elizabeth on the 126 people who were bled for the serological tests but Y. pestis was not isolated.

The 126 serum samples received included those of the patient as well as four contacts. Fig.22 shows the incidence of antibodies in the sample population and Table VIII the titre distribution.

Table VIII. PHA titres in a human population during an epidemic in the Uitenhage district.

Number of sera	PHA titre	Percentage of total
67	0	53,2 %
24	1:4	19,0 %
17	1:8	13,5 %
11	1:16	8,7 %
6	1:32	4,8 %
1	1:128	0,8 %
Total 126		100,0 %

These results are of especial interest when compared with those obtained during a survey carried out in the same region one year prior to the 1967/68 outbreak and seven years after the previous outbreak; the findings are discussed in the next section.

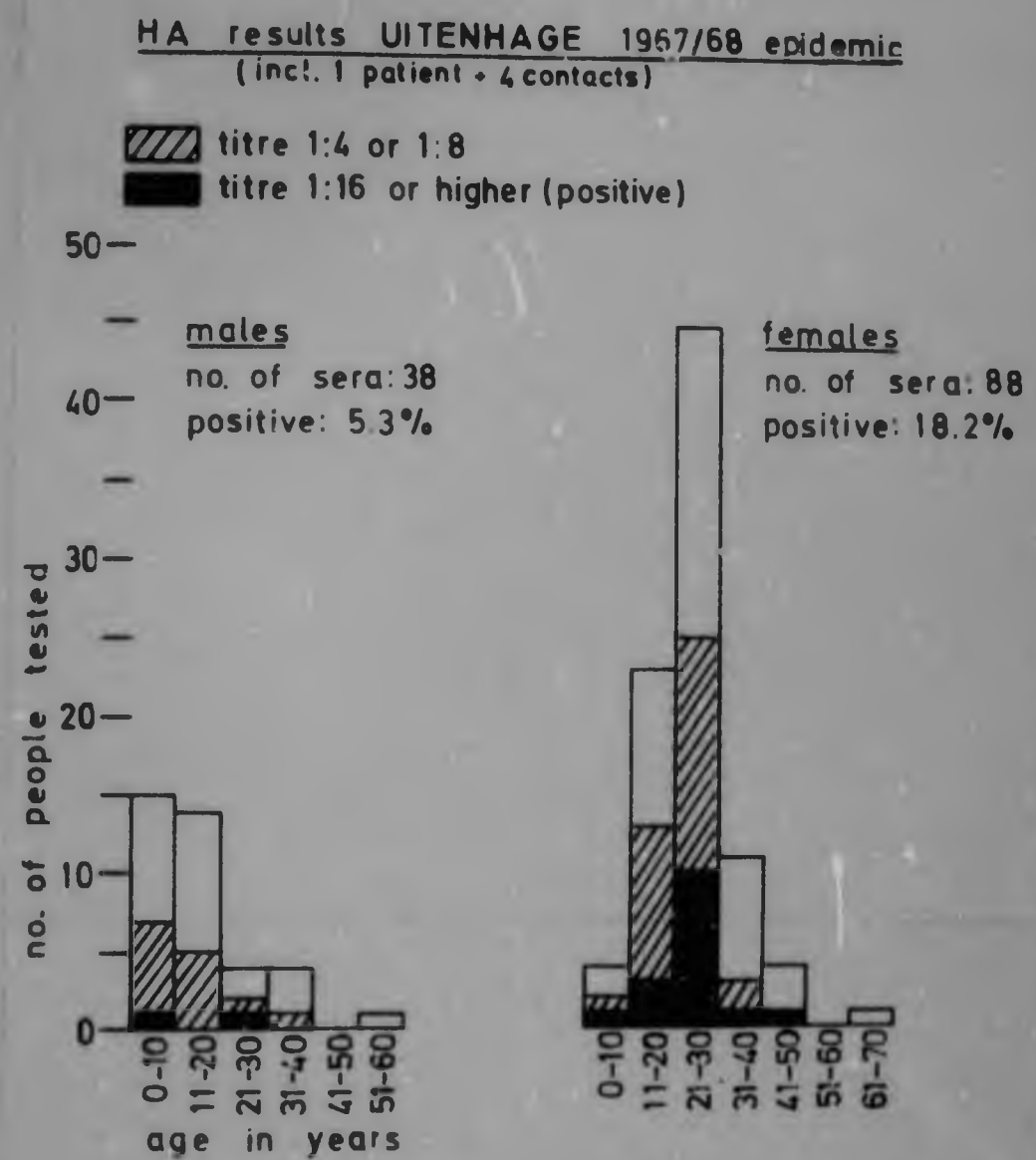

Fig.22 PHA results of 126 people resident in the plague affected area in the Uitenhage district during the 1967/68 outbreak.

6.4 Serological Surveys in the Uitenhage District and the Transkei during a Quiescent Period

Anticipating the introduction of the PHA test in 1967 blood was collected from 548 inhabitants of the Uitenhage district. The majority of these samples came from school children in the 11-20 year age group.

Of the sera tested 4,7 per cent [26 out of 548] contained PHA antibodies and 2,0 per cent had these to a titre of 1:16 or higher. Fig.23 shows the age and sex distribution of these results, and Table IX gives the titre distribution.

Table IX. PHA titres in a human population during an interepidemic period in the Uitenhage district.

Number of sera	PHA titre	Percentage of total
522	0	95,3 %
2	1:4	0,4 %
13	1:8	2,4 %
9	1:16	1,6 %
2	1:32	0,4 %
Total 548		100,0 %

The Uitenhage survey was immediately followed by one in the Transkei, a plague endemic area which in 1966 also enjoyed a state of quiescence. The last human plague cases had been reported there during the plague year 1961/62.

The Transkei is situated south of Natal and Lesotho and its southeastern border is formed by the Indian Ocean. The altitude does not exceed 1000 meters. The climate is mild and, although the main rainfall season

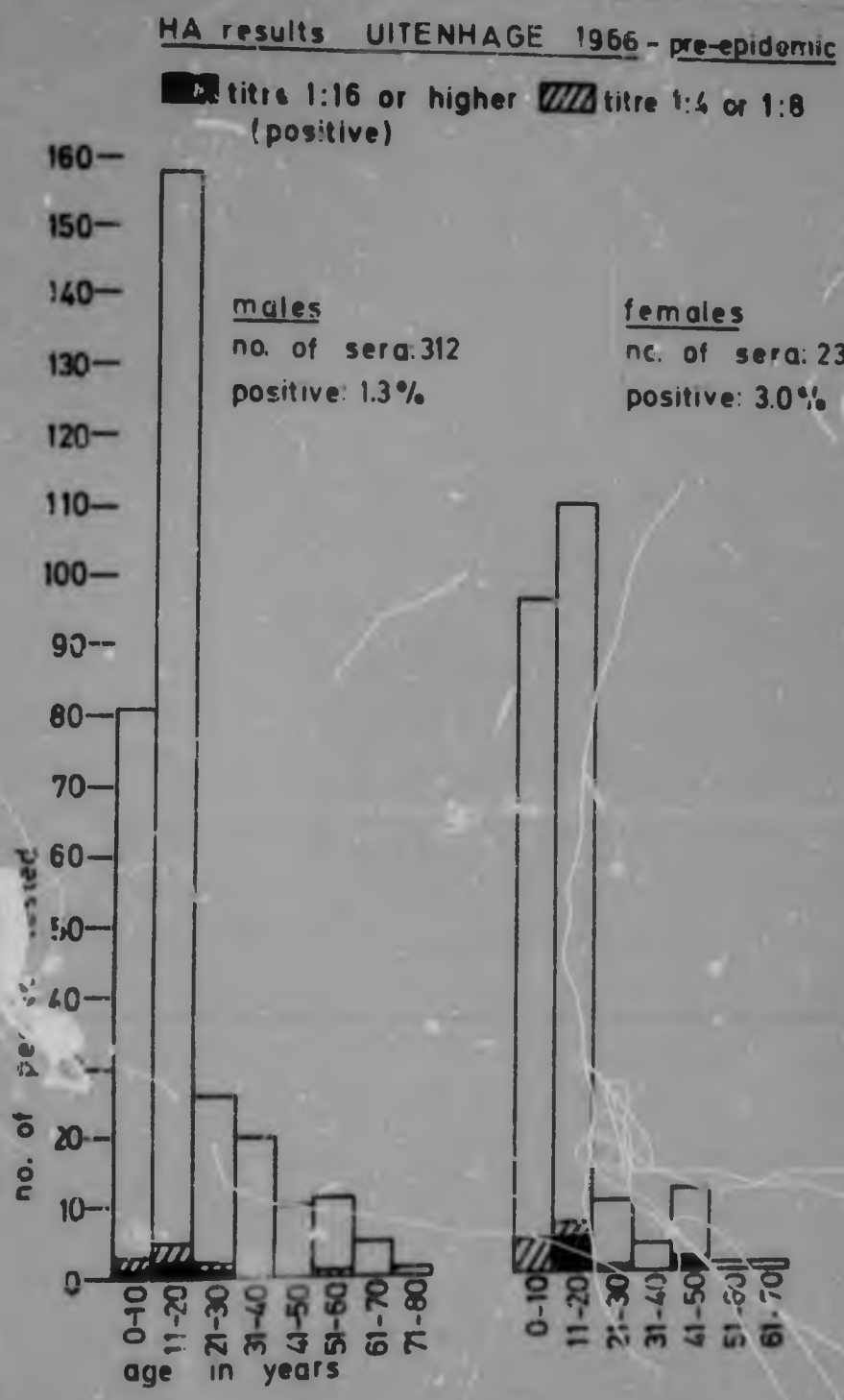

Fig.23 PHA results of 548 people resident in the quiescent plague endemic area of the Uitenhage district during 1966.

is in summer, some rain occurs also during the winter. Having a rich soil the region has excellent potential for extensive agriculture.

The inhabitants are Bantu speaking Africans of the Xhosa nation. The rural Xhosa exhibit a relatively low standard of personal hygiene and pediculosis is widespread. Epidemic louse borne typhus was therefore a major problem in the Transkei before the advent of DDT. It is possible that lice may play a role in the transmission of plague in the Transkei as it was found to do in Morocco by Blanc and Baltazard [1945]. At the SAIMR Y. pestis has been isolated on only one occasion [1950] from a specimen of Pediculus humanis capitis which had been removed from the body of a Bushman who had died of plague in Gobabis, South West Africa.

Laforce et al. [1971] implicated another human ectoparasite, Pulex irritans, in the transmission from man to man during a recent epidemic in Nepal.

During the Transkei survey in 1966 a total of 526 people was bled. Most of these also were school children in the 11-20 year age group. Of these sera 9,5 per cent [50 out of 526] had PHA antibodies and 5,1 per cent [27 out of 526] had PHA titres of 1:16 or greater. Fig.24 shows the results for each sex and age group while Table X gives the titre distribution.

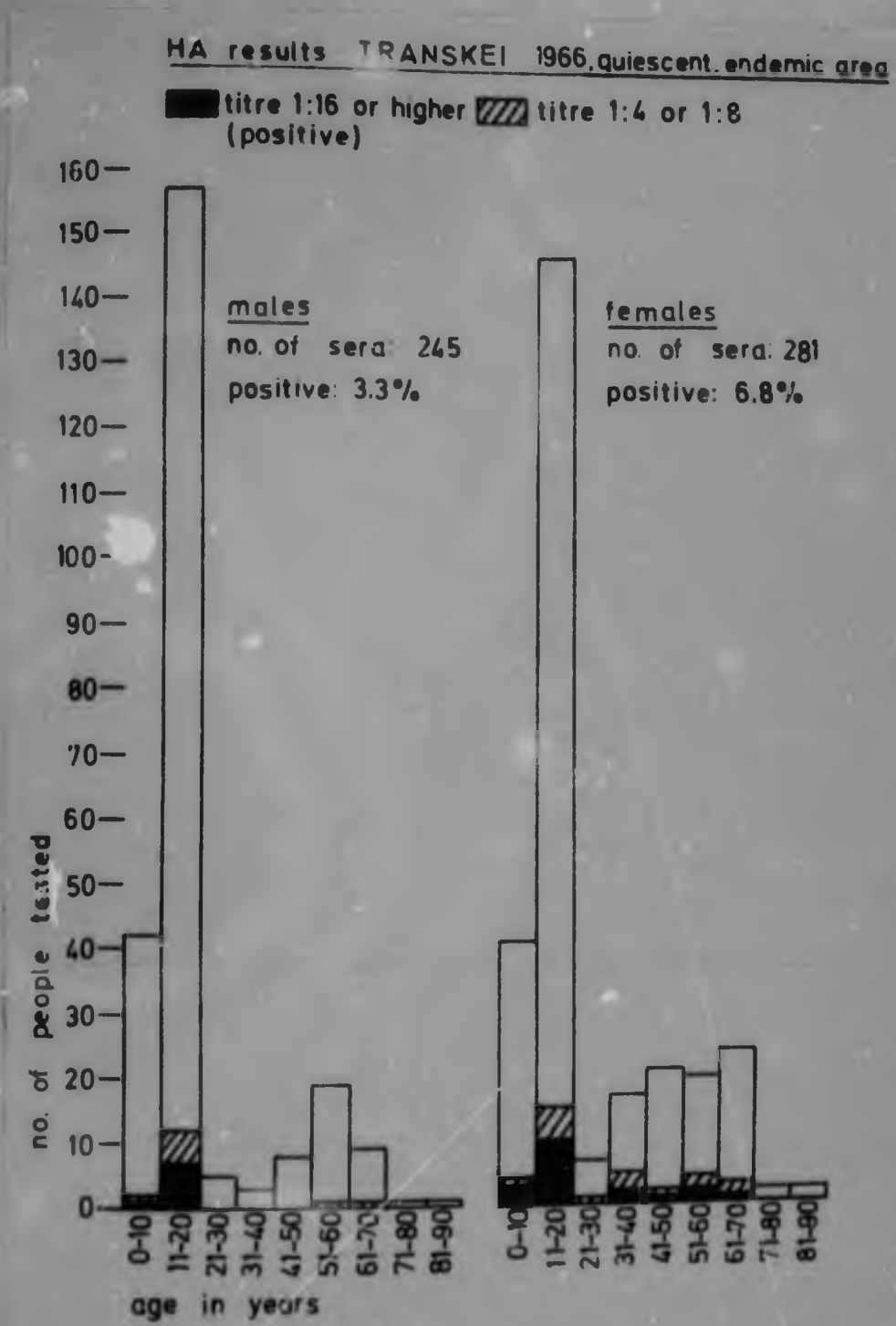

Fig.24 PHA results of 526 people resident in the quiescent plague endemic area of the Transkei during 1966.

Table X. PHA titres in a human population during a quiescent phase in the Transkei

Number of sera	PHA titre	Percentage of total
476	0	90,5 %
23	1:8	4,4 %
19	1:16	3,6 %
6	1:32	1,1 %
2	1:64	0,4 %
Total 526		100,0 %

A comparison was made between the results obtained during the three surveys and the salient features are summarized in Table XI.

Table XI. Plague antibody response in human populations of endemic regions during quiescent and active periods

Region	Percentage population with PHA antibodies [titre 1:4 or over]	Percentage population with PHA titres of 1:16 or over
Uitenhage 1966, 7 years post-epidemic	4,7 %	2,0 %
Uitenhage 1967/68, epidemic period	46,8 %	14,3 %
Transkei 1966, 5 years post-epidemic	9,5 %	6,1 %

The figures in Table XI show a tenfold increase in overall antibody incidence in Uitenhage during the epidemic while the percentage of sera with titres of 1:16 or higher underwent a sevenfold increase. These results leave little doubt that immunologically active, though apparently silent, plague infections were occurring

during the epidemic period. This is in keeping with results obtained by Legters et al. [1970] in Vietnam and by Payne et al. [1956] in Madagascar. The latter however drew attention to the possibility that sero-positive healthy people may be convalescents of pestis minor and evidence was obtained by us in Lesotho which reinforces this opinion.

While the occurrence of inapparent plague infections during epidemics is well recognized great care must nevertheless be exercised in the interpretation of positive serological results obtained during surveys on apparently healthy people. This is well illustrated by our findings during the Lesotho plague epidemic of 1967/68 when sixtyone members of the supposedly healthy survey population gave positive PHA results to titres of 1:16 or greater. Table XIII on page 108 shows that, of thirtythree people who were carefully questioned when found to be sero-positive, fourteen had recently suffered symptoms which were compatible with plague. Seven of these did not consult a medical practitioner and recovered spontaneously without conventional treatment.

During large-scale surveys it is virtually impossible to obtain a medical history from each serum donor. As demonstrated above, among the number of apparently silent infections revealed during a serological survey there may be hidden a proportion of overt, symptomatic plague patients. The occurrence of true silent infection was suggested by the high or changing titres found in six of the eighteen Lesotho people who emphatically

denied having been ill during the previous year [Table XIII, ref. no. L/204, L/224, L/462, L/537, L/659, L/669].

During the quiescent phase in the Uitenhage district and the Transkei in 1966 the people who were found to have PHA antibodies may have belonged to at least two groups. The one group is postulated to have consisted of those people who during this period were infected with low doses of Y. pestis or with relatively avirulent strains which stimulated the immune response without causing clinical illness, or which caused pestis minor for which medical treatment was not sought.

The other group may have consisted of people who were infected with Y. pestis which might or might not have resulted in clinical plague during a previous epidemic. It is of some interest that the percentage sero-positives in the Transkei was higher than that in the Uitenhage district in 1966. In the latter region seven years had elapsed since the previous epidemic while in the Transkei the interval was only five years.

We have found during a recent serological follow-up study in Lesotho that 53 per cent of sero-positive plague patients still showed the presence of PHA antibodies in titres ranging from 1:8 to 1:512 when three and a half to four years had elapsed since their illness [see chapter 8].

If it is postulated that the persistence of PHA antibodies for a number of years is a relatively common occurrence then the significant difference [p is less than 0,01] between the percentages of sero-positives in

the Uitenhage district and the Transkei is understandable when considering the difference in the number of years which had elapsed since the previous plague epidemics in the two regions.

7 PLAGUE IN THE CAPE PROVINCE: THE VRYBURG DISTRICT

A single case of human plague occurred in the plague year 1965/66 on the farm Alettasrus [map reference 26.24.Ad] which is situated at an altitude of approximately 1200 meters in the northeastern part of the Cape Province. The rainfall season is in summer and the area is within the twenty inch isohyet of mean annual rainfall.

The patient was a male Caucasian aged 39 years and self employed as a farmer. He became ill on the 9th October 1965, his main complaint being a unilateral painful inguinal swelling. A provisional diagnosis of strangulated hernia was made and three days after the onset of illness the patient was admitted to hospital for surgical intervention. The temperature on admission was 38,9°C.

At operation it was found that the swelling was glandular in nature. Gland tissue and sputum were then submitted to our laboratory with a provisional diagnosis of bubonic plague. Y. pestis was isolated from the gland but not from the sputum.

PHA antibodies were demonstrated in rising titre on four successive occasions, the highest titre being 1:512. The serological results were kindly supplied by Dr K.F. Meyer of San Francisco as the PHA tests were at the time not performed in South Africa.

Post-operatively the patient was given intensive chemotherapy commencing with chloramphenicol and strep-

tomycin. After six days this was changed to penicillin and streptomycin. Thirteen days post-operatively these were discontinued and the patient then received oral sulphadiazine for a further seven days.

The temperature returned to normal on the fifth post-operative day, recovery was quite uneventful and the patient was discharged three weeks after admission to hospital.

As soon as the diagnosis of plague was confirmed investigations were carried out on the patient's farm. Prior to the onset of illness the patient had been occupied with the transfer of grain from the threshing floor to storage tanks. No history could be obtained of unusual rodent mortality at the time. Investigations revealed the presence of one dead Tatera brantsii on the maize floor and another one was found dead in a burrow in the vicinity. Six live Tatera brantsii were dug up from burrows and one Elephantulis was caught in the field. Seven fleas of the genus Xenopsylla and five Echidnophaga gallinacea were removed from rodent burrows. Y. pestis was isolated from neither rodents nor fleas. The investigating team also found no signs of unusual rodent mortality.

This case of plague highlights the very real danger of making a wrong diagnosis especially when, as in this instance, there were no other cases and no associated or preceding epidemiological features which might otherwise have alerted local medical practitioners to the possibility of impending human plague.

The patient was not unique in having his inguinal bubo initially diagnosed as a strangulated hernia. For example, Reed et al. [1970] reported a case of bubonic plague in a 37-year old Indian man of New Mexico who was admitted to hospital with a provisional diagnosis of 'incarcerated hernia'. However, the occurrence of prior plague cases and a history of camping close to a prairie dog colony resulted in this instance in plague being considered as a diagnostic possibility soon after admission of the patient to hospital.

Caten and Kartman [1968b] published the case of a 21-year old American soldier who developed bubonic plague shortly after his return from Vietnam. The admission diagnosis in this patient too was 'strangulated hernia'.

The most recent description of such a case was given by Palmer et al. [1971] and concerned a 20-year old woman of New Mexico.

In recent years plague has frequently been misdiagnosed [Caten and Kartman, 1968b; Collins et al., 1967; Kartman et al., 1962; Kartman et al., 1966] with unfortunate consequences being a danger to the patient or the community or both. This was strikingly illustrated by the 1967/68 plague epidemic in Lesotho which went unrecognized for several months and developed into one of the worst outbreaks in modern times in southern Africa.

Paradoxically, the very successes in the prevention and control of plague are in a certain measure responsible for the periodic failures to prevent spread in a community and for the still high mortality. The present

comparative rarity of human plague has resulted in a generation of medical practitioners which is largely unaware of the still widespread and common occurrence of plague in wild animal reservoirs which constitute a constant potential danger to man.

8 PLAGUE IN THE ORANGE FREE STATE AND LESOTHO

8.1 Description of the Area

These two regions are discussed together as they adjoin each other and contain a contiguous plague focus.

The Orange Free State is one of the four provinces of the Republic of South Africa and its eastern region forms part of the 'Highveld Plateau' which has an elevation varying between 1200 and 1800 meters above sea level. Extensive maize and wheat cultivation and cattle and sheep farming are practised. Here too, the rainfall season is in summer and the plague enzootic foci are situated within the twentyfive inch isohyet of mean annual rainfall.

The independant Kingdom of Lesotho is situated approximately between latitudes 28°30' and 30°30' south, and longitudes 27°00' and 29°30' east. Topographically the country can be divided into two distinctly different regions i.e. the densely populated western lowlands which merge in the west with the eastern Orange Free State, and the sparsely populated eastern highlands. The division between the lowlands which are based on sandstone, [Cole, 1961], and the volcanic highlands is formed by the escarpment of the Maluti Mountains at an altitude of approximately 2000 meters. The designation 'lowlands' is a relative one as the elevation here is similar to that of the eastern Orange Free State while the highest point in the Maluti Mountains, Thabana Ntlenyana, is at 3481 meters.

Overgrazing and faulty agricultural practices in the past have resulted in very serious soil erosion, particularly in the lowlands of Lesotho. The introduction of modern soil conservation methods such as contour ploughing and forestry projects are showing success in reversing the process.

A striking feature in Lesotho is the absence of trees. The natural vegetation consists largely of grass while various species of Aloe are present in abundance and may frequently be found closely associated with the villages. Also of note is the absence of wild animals which are predators on small mammals. Domestic cats were never seen during repeated visits by the author to many of the villages.

These villages consist of solidly built square or round houses with thatched roofs. The quality of these dwellings is much superior to that found in Ovamboland which is probably in part a reflection of the widely differing climatic conditions [Fig.25]. Many of the villages are situated on rocky ledges and on hill tops. The absence of roads in the rural areas necessitates the use of four wheel drive transport and extensive detours are unavoidable when travelling from one village to the next. The local population makes use of the 'Basutu pony', a riding and transport animal which is sure-footed and exceptionally well adapted to the mountainous terrain.

The climate is temperate with a southern summer rainfall season. Heavy winter snowfalls are common in

Fig.25 A typical dwelling house in a village of the Lesotho foothills. Note its solid quality and the attractive decoration.

the foothills and the mountains, and winter temperatures frequently fall below freezing point. It is not without reason that Lesotho is referred to as the Switzerland of southern Africa.

The population consists mainly of the Sotho who even in the heat of summer may be seen enveloped in the national dress of colourful, but heavy, woollen blankets. The standard of personal hygiene of the Sotho is high.

8.2 The Situation prior to 1965

The first cases of human plague, sixtyeight in all, in the Orange Free State were reported during the years 1916-1918 and it was later established that these were probably secondary to wild rodent plague.

In Lesotho plague was reported for the first time in 1935 [Davis, 1953] and this was the result of a direct extension of the Orange Free State wild rodent plague reservoir across the border into the lowlands of what was then known as Basutuland. Several outbreaks have occurred since that time in southwestern Lesotho.

The last cases of human plague in Lesotho and the Orange Free State were notified in 1954 and 1956 respectively.

8.3 The Orange Free State Epidemic of 1967/68

During January and February, 1968, ten cases of suspected human plague were notified from the Steynsrus district [map reference 27.27.Dc]. The mortality was 50 per cent. Most of the early cases became ill and died without having sought medical attention. Consequently the information on most of the patients was obtained retrospectively and specimens were obtained from only four patients.

Splenic tissue from the exhumed body of one of the suspected plague patients gave a positive result for Y. pestis by means of the fluorescent staining technique. This finding was confirmed by Dr K.F. Meyer in San Francisco. Cultural and biological methods in this case failed, probably because of the very advanced state of decomposition and contamination of the material.

PHA tests could be carried out on three patients and the results left little doubt about the nature of the outbreak. Table XII shows the PHA findings as well as such data as could be obtained on the patients.

Table XII. Clinical and laboratory data on 10 cases of suspected human plague during the 1967/68 epidemic in the Orange Free State.

reference	sex	age in years	first PHA titre	second PHA titre	outcome	symptoms and other data
OFS/5	F	7			Died	Had a small left inguinal swelling and 'stomach upset'.
OFS/1	M	9			Died	Complained of unspecified glandular pain, headache.
OFS/W	M	70			Died	Had respiratory symptoms but was clinically taken to be a doubtful case of plague.
OFS/JH	M	20			Died	Son of OFS/SM; complained of chestpain, headache.
OFS/SM	F	40			Died	Complained of chestpain, headache. Post mortem splenic tissue yielded a positive plague fluorescent antibody test.
OFS/LM	M	70	0	1:512	Recovered	
OFS/JL	F	4	1:128	1:512	Recovered	Small left inguinal bubo and pyrexia.
OFS/IL	M	5	0	1:1024	Recovered	Small left inguinal bubo and pyrexia.
OFS/Si	M	15			Recovered	Occipital swelling and pyrexia.
OFS/4	F	11			Recovered	Post-auricular swelling.

Y. pestis was isolated from one dead Rattus rattus and from a pool of 24 fleas of the genus Xenopsylla which were collected in the plague affected area.

8.4 The Lesotho Epidemic of 1967/68

From the middle of February, 1968, a number of patients with symptoms of a pyrexial illness were admitted to the Mohaleshoek hospital [map reference 30.27.Ab] in south-western Lesotho. The majority of these people complained of feverishness and headache. Chestpain and coughing were fairly common symptoms and haemoptysis was experienced by some patients. The frequent occurrence of abdominal complaints resulted in a provisional diagnosis of typhoid being made in most cases. This was a reasonable assumption, particularly in view of the outbreak of this disease which was at the time being experienced in other parts of Lesotho. Abdominal symptoms are well known to occur in plague [Pollitzer,1954] and their presence was recently recorded by Palmer et al. [1971] in thirteen of a series of nineteen plague patients in the USA.

Though the presence of enlarged lymph glands was noted in some of the Lesotho patients the possible significance of these was at the time not appreciated.

The patients were treated with either oxytetracycline or chloramphenicol and recovered. Chloramphenicol has been shown to be highly effective in the treatment of plague [McCrumb et al., 1953] and the tetracyclines have recently been recommended as the antibiotics of choice by the World Health Organization [1970].

While the hospitalized patients were therfore being

adquately treated, the epidemic had not been recognized as one of plague and was able to pursue its n tural course.

On the 11th March, 1968, a 30-year old male inhabitant of the village of Kechane [map reference 29.27.Cd] was admitted to hospital. He complained of diarrhoea, abdominal pain and headache. Examination revealed the presence of an enlarged cervical lymph gland. Although the patient's temperature was then only 35,5°C it rose to 37,8°C within 24 hours.

On the same day a 60-year old male inhabitant of the village of Rampeli [map reference 30.27.Ba] presented himself at the hospital with very similar symptoms. A swab from this patient [anatomical origin unrecorded] was submitted two weeks later to the SAIMR laboratories in Bloemfontein. Scanty gram negative bacilli were observed but these failed to grow on culture. This result together with the clinical features of the two patients prompted the submission of blood samples to our laboratory with a request for plague antibody tests. Both patients yielded positive PHA results to titres of 1:1024 and 1:16 respectively.

By this time the Lesotho health authorities had been notified of an abnormal number of deaths which had occurred during the preceding months in several v llages and the accelerating admissions of patients with a pyrexial illness and lymphadenopathy to the hospitals in Mafeteng [map ref rence 29.27.Cc] and Mohaleshoek.

The serological results served to confirm the sus-

picion that a plague epidemic was in full swing.

Retrospective investigations showed that at least 15 villages were affected and that the population at risk numbered approximately 10000.

Control activities were commenced immediately and a South African plague team was invited to assist with control and to conduct epidemiological investigations. Besides the author the team included Dr D.H.S. Davis, State Ecologist; Miss T.P. Crowngold, Senior Medical Technologist, and Mr L.L. Hendricks, Chief Health Inspector.

During the course of the largely retrospective enquiries the picture of a classical plague epidemic was pieced together. Thus it emerged that as early as November, 1967, three people had died in the village of Kechane following what appeared to be some acute infective condition. As foul play was not suspected to be the cause of death the village chief was not required to notify these deaths immediately. Similar stories of sudden death were collected in other villages in one of which seven members of one family had died. The second hand histories always pointed to a respiratory infection or lymphadenopathy or both having preceded death.

In one village it was related how illness had broken out after a number of inhabitants had attended the funeral of relatives in another village.

When questions were asked concerning the rodent situation an old man bitterly complained about rats having attacked and injured his chickens some months earlier.

A school teacher volunteered the information that she had on three occasions found dead rodents near her house. It was at her house that inspection revealed the presence of gerbil burrows in the immediate vicinity of the entrance to the dwelling. Other people also spoke of dead rodents found in or near their dwellings.

During the course of these investigations several suspect plague patients were discovered in different villages and these were transported to hospital. At the Mount Tabor Mission [map reference 29.27.Cd] a 4-year old boy was presented by his mother with a history that he had been ill with a fever and a swelling in the right groin. The mother had not consulted a medical practitioner and the child's condition had improved spontaneously over the five days which had elapsed since the onset of illness. On examination there was a typical bubo in the right inguinal region. The surrounding skin was discoloured and showed evidence of superficial desquamation indicating the earlier presence of extensive cellulitis which is characteristically associated with plague buboes [Fig.26]. The child's PHA titre at that stage was 1:512.

Laboratory equipment was brought to the area in order to attempt the isolation of Y. pestis in the field. Due to unforeseen circumstances incubation facilities could not be procured while ambient temperature hovered mostly around freezing point. The urgent need for a suitable incubation temperature resulted in the impro-

Fig.26 Four year old plague patient in convalescent phase. This child recovered spontaneously and shows the typical features of a plague bubo.

visation of a 'biological incubator' in which the source of heat consisted of 40 white mice in a cage measuring 38,5 cm x 31,5 cm x 13,0 cm. The cage was wrapped in a folded horse blanket, leaving one side uncovered to permit entry of air. The wrapped cage was placed in a wooden box of which the front was open. Inoculated media were placed on top of the cage underneath the blanket and regular thermometer readings showed the temperature in this space to remain fairly constant at about 22°C. This very primitive contraption proved nevertheless highly effective and permitted the isolation and identification of Y. pestis from rodents and fleas.

During the month of May serum samples were obtained

from 6[illegible] people of whom 30 had been hospitalized as clinically suspect plague cases. The remaining 601 sera came from people residing in the villages where plague was believed to have occurred. The incidence of PHA antibodies is shown in Fig.27, grouped according to age and sex. The obvious lack of males in the young adult age groups is due to their absence from the villages while working, mostly in the South African mining industry. The results show no difference in sex incidence of antibodies nor does there appear to be any significant difference in age incidence.

Throatswabs were taken from 178 inhabitants of villages where pneumonic plague was suspected to have occurred as well as from the 30 above mentioned patients who by this time had been treated. The swabs were transported to the SAIMR in Johannesburg in Stuart's transport medium and processed within two weeks of their collection but Y. pestis was not isolated.

Y. pestis was cultured from fleas and rodents collected during June and July, 1968, in the plague affected areas 29.27,Cd and 30.27.Ab. The plague positive material consisted of:

2 dead Tatera brantsii

1 unidentified dead rodent

1 dead Praomys [Mastomys] natalensis

2 Dinopsyllus ellobius removed from above Praomys

24 pooled Xenopsylla
15 pooled Xenopsylla
54 pooled Xenopsylla
2 pooled Xenopsylla

} collected from rodent burrows

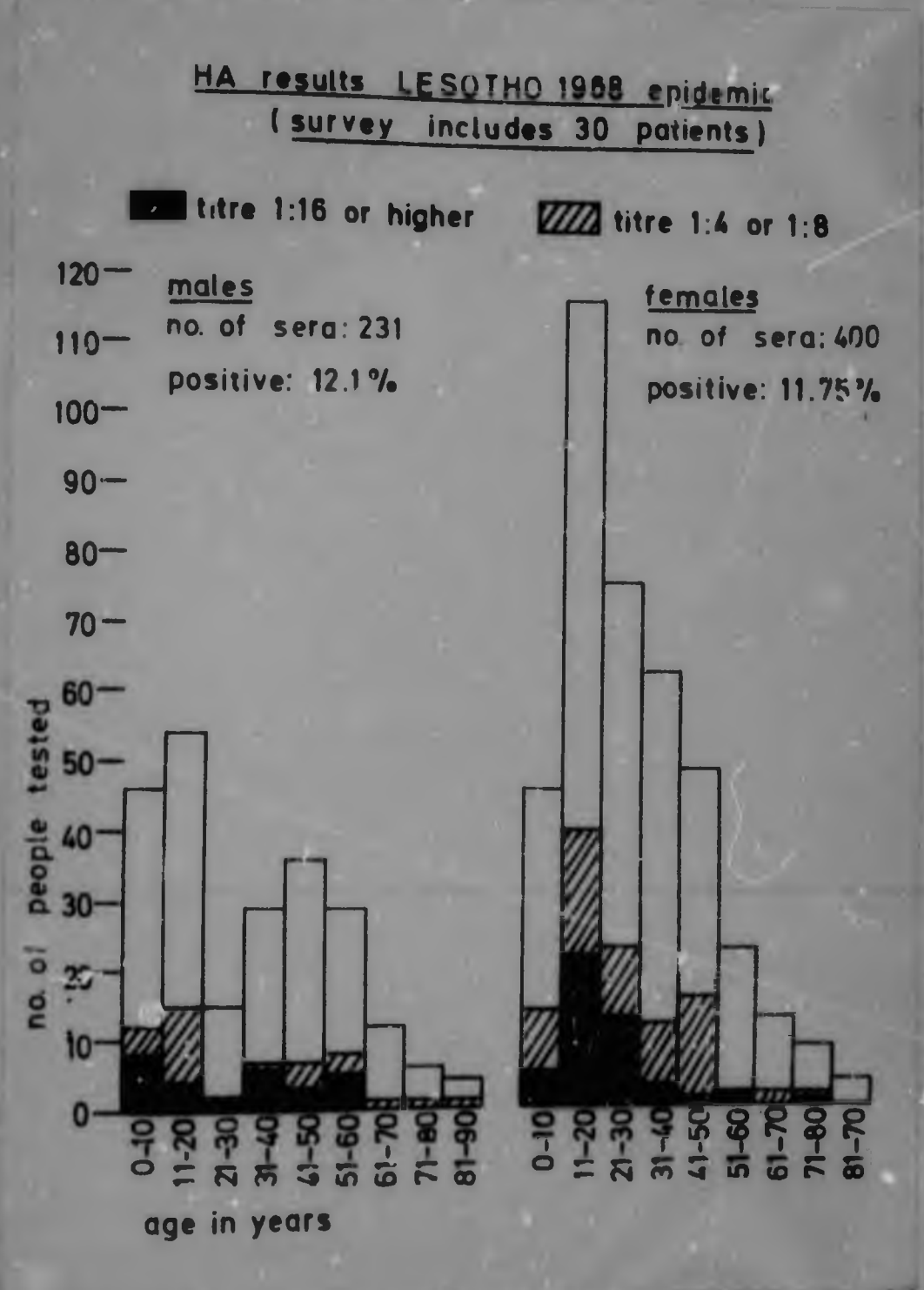

Fig.27 PHA results of 631 people, including 30 patients, who were inhabitants of the plague affected area in Lesotho during the 1967/68 epidemic.

Although the official notifications totalled 125 cases with 49 deaths these figures are based largely on retrospectively obtained data and the true incidence and mortality rate are unknown.

Two months after the initial survey a return visit was made in order to trace those 61 people who, although not suspected of having had plague, had PHA titres of 1:16 or greater. The purpose was to obtain retrospective histories and to repeat the PHA tests. The results of these investigations on 33 people who could be traced are presented in Table XIII. It may be seen that 14 of these people gave histories indicating that they had been ill and these may have been cases of pestis minor whereas the remaining 19 people include at least 6 who appear to have had true silent infections, as discussed in chapter 7.

In November 1971 an opportunity presented itself to re-test 19 people who were serologically positive on one or two occasions in 1968. This group included former plague patients as well as people who had no history of illness during the 1968 epidemic. Their serological data are also shown in Table XIII. The finding that as many as ten [53 per cent] still had PHA antibodies three and a half to four years after the epidemic was not entirely unexpected in view of similar observations by Payne et al. [1956] in Madagascar. The implications of this prolonged antibody persistence with regard to determining the reason for the occurrence of human PHA positives during plague quiescent periods was discussed in the previous chapter.

Table XIII. Lesotho plague epidemic 1967/68: PHA results and retrospective histories of seropositive people from whom follow-up sera were obtained.

Reference number	Sex	Age in years [1968]	PHA results in May 1968	PHA results in July 1968	PHA results in November 1971	Clinical data
L/1	M	4	1:128		1:8	Appeared to be a convalescent bubonic plague patient who first seen at Mount Tabor [see text, page 103] and is the child shown in Fig.26.
L/6	F	30	1:256		0	Suspected plague patient.
L/9	M	32	1:1024	1:4096	1:512	Index case. Admitted with pyrexia, diarrhoea and slight abdominal distention. Widal negative two weeks after admission.
L/185	M	8	1:512		1:4	No information.
L/204	M	47	1:256	1:64		No history of illness during previous year.
L/219	F	8	1:16	1:16		" " " " " " "
L/224	M	26	1:64	1:4	0	" " " " " " "
L/230	M	37	1:16	1:16		" " " " " " "
L/340	F	8	1:512		1:16	Suspected plague patient.
L/352	F	50	1:16	1:16		No history of illness during previous year.

Table XIII continued. For headings see page 108.

L/373	F	12	1:16	1:16		No history of illness during previous year.
L/380	F	17	1:64	1:64		" " " "
L/403	M	14	1:16	1:32		" " " "
L/43[illegible]	F	39	1:128	1:1024	1:64	Suspected plague patient.
L/439	F	80	1:16	1:32		No history of illness
L/447	M	7	1:32	1:64		Had a right axillary swelling in March 1968 for which the patient was treated with a 'liquid medicine' as an outpatient. Recovered after one week.
L/462	M	47	1:512	1:512	1:64	No history of illness during previous year.
L/481	F	24	1:64	1:64		Had a painful right axillary swelling in April 1968 and was treated as an outpatient with 'liquid medicine'.
L/485	F	14	1:16	1:16		Treated as an outpatient for a painful left pre-auricular swelling in April 1968.
L/515	F	11	1:64		0	No information.
L/537	M	35	1:128	1:64		No history of illness during previous year.
L/548	F	18	1:16	1:8		" " " " " " "
L/555	F	30	1:16	1:16		Treated as an outpatient for headache, generalized pains and a cough in April 1968.
L/613	F	8	1:64	1:16	0	Had a rash in April, diagnosed as chickenpox.
L/624	M	56	1:16	1:16		No history of illness during previous year.
L/626	F	39	1:64	1:32	0	Complained of headache, chest pain and slight abdominal pain in March 1968 but did not seek treatment and recovered spontaneously.

Table XIII continued. For headings see page 108.

L/632	F	19	1:32	1:8		Complained of a left axillary swelling, headache and backache in April 1968. Treated as an outpatient with unspecified tablets, medicine and ointment.
L/636	F	23	1:32	0		Headache and backache in January 1968. Did not seek treatment.
L/641	F	16	1:16	1:16		No history of illness during previous year.
L/646	F	28	1:32	0		Headache and facial rash in March 1968. Did not seek treatment.
L/659	F	26	1:16	0		No history of illness during previous year.
L/664	M	8	1:32	1:32		Had a headache, abdominal pain and rash in April 1968 and was treated as an outpatient.
L/669	F	13	1:32	1:4		No history of illness during the previous year.
L/686	F	12	1:512	1:256	1:64	Headache, painful eyes, vomiting and a cough in April 1968 but recovered spontaneously.
L/687	M	38	1:32	1:8		Headache, backache and chestpain in April 1968. Was treated as an outpatient.
L/708	M	10	1:2048		1:64	Suspected plague patient.
L/709	F	19	1:64	1:32	0	" " "
L/716	F	22	1:512	1:256		Had a headache, epigastric pain and fever in March 1968. Was treated by a witchdoctor and recovered.
L/723	F	12	1:64		0	No information.
L/753	M	10	1:256	1:64	1:64	Headache and chestpain in April 1968. Did not seek treatment.
L/761	M	54	1:32	1:32		No history of illness during the previous year.

Table XIII continued. For headings see page 108.

L/795	M	60	1:64		1:16	Headache and chestpain in March 1968. Did not seek treatment.
L/799	F	11	1:32	1:32		No history of illness during the previous year.
L/LM	M	60	1:16	1:128	0	Suspected plague patient.
L/LP	M	6	1:128		0	" " "

9 THE SPECIFICITY OF THE PLAGUE HAEMAGGLUTINATION TEST AND THE INTERPRETATION OF RESULTS OBTAINED IN MAN IN SOUTHERN AFRICA

The question of false positive reactions has been raised from time to time since the introduction of serological methods in the field of plague investigation. Particularly disturbing was the report by Baltazard et al. [1971] of high Y. pestis fraction 1 haemagglutinating titres [up to 1:320] which developed in previously negative rodents which had been fed Salmonella typhi-murium with antigenic patterns 1,4,12 and 4,5,12 and with Salmonella enteritidis. Feeding with Salmonella typhi-murium of antigenic patterns 4,12 and 1,4,5,12 did not result in the development of PHA antibodies. The authors concluded that 'the protein haemagglutination method, in wide and almost exclusive use at the present time [1969] in serological searches for rodent plague is not specific at the titres now accepted.'

Hudson et al. [1964] in their report on the serum antibody response in Microtus californicus considered titres of 1:32 and higher as positive. Dr K.F. Meyer [personal communication, 1967] recommended that a titre of 1:16 or higher be considered as significant in human sera, indicating active or recent infection, while lower titres should be regarded as evidence of exposure to the plague antigen in the more remote past.

In view of Baltazard's report, and considering the high incidence of salmonellosis in southern Africa, the

question arose as to the validity of the positive plague serological results obtained on human sera during our epidemiological investigations.

There is no doubt that cross-antigenicity exists between Y. pestis and not only the above mentioned bacteria but also E. coli, Shigella dysenteriae, Shigella sonnei and Salmonella paratyphi B [Barber and Eylan, 1971] and Y. pseudotuberculosis [Chen and Meyer, 1955; Currie et al. 1966; Goldenberg and Hudson, 1967]. In view of this close antigenic relationship serological cross reactions would therefore not be unexpected although cross reaction was not found to occur between plague and pseudotuberculosis in the complement fixation and passive haemagglutination tests employing the plague fraction 1 antigen [Chen and Meyer, 1955]. Currie et al. [1966] showed that haemagglutination did not occur between sheep erythrocytes sensitized with a plague antigen and a Salmonella typhi-murium antiserum but this observation does not necessarily invalidate Baltazard's findings which implicated only certain S. typhi-murium strains with defined antigenic patterns.

There are few serological reactions in bacteriology which can be regarded as absolutely specific and it was felt therefore that the important question is not whether the PHA test is entirely specific but rather to what extent do false positives occur in practice, in what titres may such reactions be found and how does their occurrence affect the usefulness of the PHA test in diagnostic and epidemiological work.

These questions prompted a priori a serological survey in the Eastern Transvaal lowveld region where plague is not known to have occurred since the introduction of the disease into this country at the turn of the century. On the other hand, Salmonella infections are highly prevalent.

The survey population consisted of 499 people of which 392 were staff, outpatients, inpatients and patients' relatives at the Shongwe Mission Hospital [map reference 25.31.Da]. Brief medical histories were obtained from most of these people but not from a group of 107 children attending a local school and who were included in the survey.

Serological tests carried out on the sera were the Y. pestis haemagglutination and haemagglutination inhibition tests as well as agglutination tests against antigens of S. typhi [somatic 9,12 and flagellar d], S. paratyphi A and B [flagellar a and b] and S. typhimurium [somatic 1,4,5,12 and flagellar 1,2,i]

The sera which reacted with Y. pestis fraction 1 were also tested against the somatic and flagellar antigens of Y. enterocolitica.

In eleven sera [2,2 per cent] antibodies against the antigen of plague were found to be present and this was confirmed by the PHAI test. The age and sex distribution of the survey population sample and the plague serological results are shown in Fig.28.

The titre distribution of the eleven reacting sera was as follows.

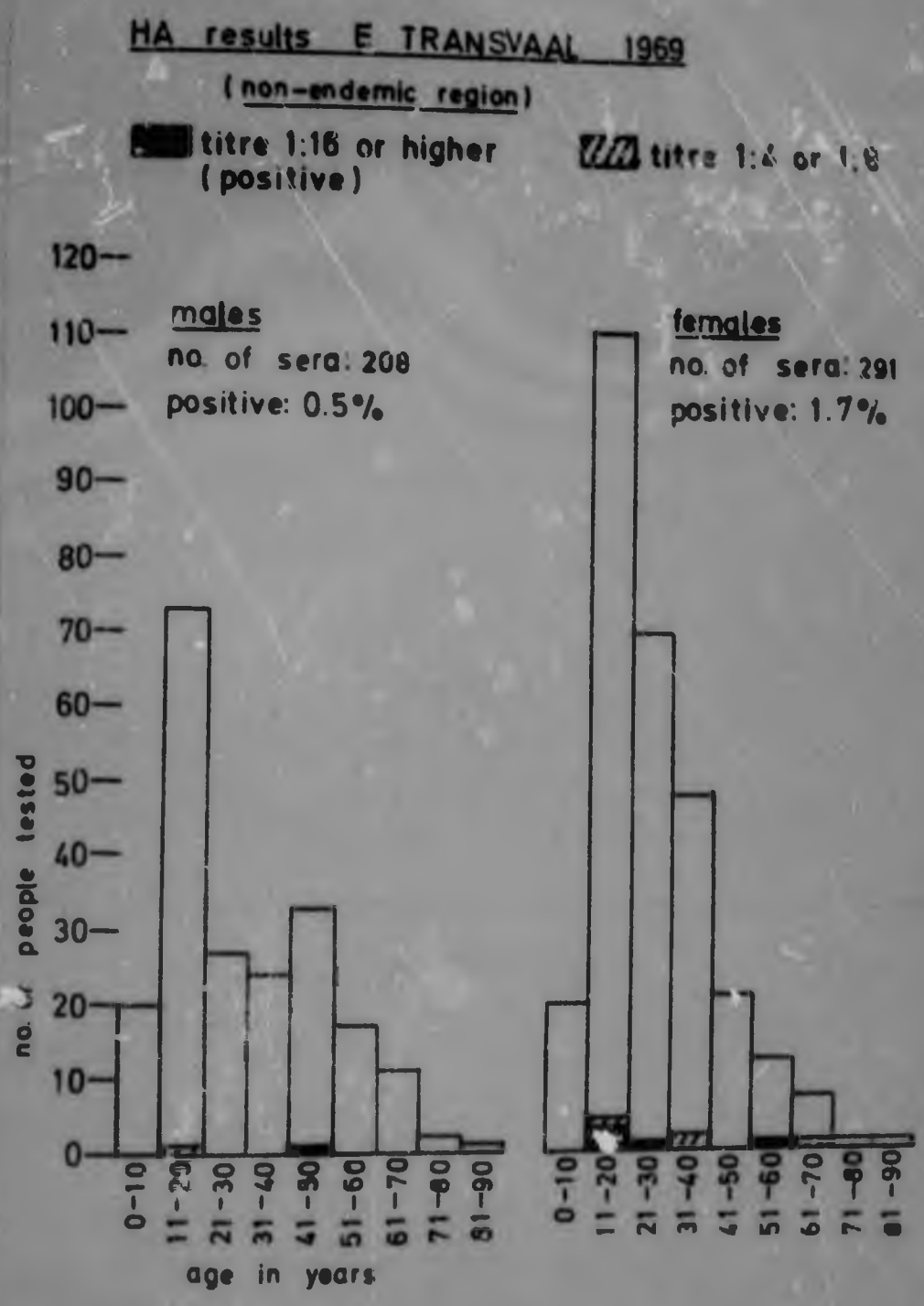

Fig.28. PHA results of 499 people inhabiting the Eastern Transvaal lowveld which is a plague-free region.

PHA titre	Number of sera
1:4	1
1:8	4
1:16	4
1:32	1
1:64	1

Ten of the plague reactors were permanent local residents and had not travelled beyond this area inasfar as they could remember.

Table XIV shows the relationship between agglutinations obtained against the Salmonella somatic antigens 1,4,5,12 and 9,12 and against the Y. pestis fraction 1 antigen.

Table XIV. Relationship between Salmonella and Y. pestis antibodies observed in 499 human sera from a plague-free region.

	Positive PHA sera	Negative PHA sera	Total
Positive Salmonella sera	5	62	67
Negative Salmonella sera	6	426	432
Total	11	488	499

The apparently higher proportion of PHA positive reactions among the Salmonella positive sera was confirmed by statistical analysis which showed the difference to be highly significant [$p \leq 0{,}01$]. Although these calculations are based on figures classed according to Salmonella somatic antigens, very similar results were obtained when the Salmonella typhi reacting sera only, or

when sera reacting against all the tested Salmonella antigens [including flagellar antigens] were used as a basis for calculations.

The PHA titres obtained in the two groups are shown in Table XV.

Table XV. PHA titres obtained in sera with and without antibodies against Salmonella somatic antigenic patterns 1,4,5,12 and 9,12.

PHA titres	Number of sera in Salmonella positive group	Number of sera in Salmonella negative group
1:4	0	1
1:8	1	3
1:16	2	2
1:32	1	0
1:64	1	0
Total	5	6

The mean PHA titre in the Salmonella positive group of sera was $1:2^{4,4}$ and in the Salmonella negative group it was $1:2^{3,2}$. This series is too small to permit definite conclusions to be drawn from this marked difference in mean titres but it indicates the need for future investigation.

The Eastern Transvaal results appeared to be consistent with Baltazard's experimental observations on rodents fed with Salmonellae. They were communicated to Dr. K.F. Meyer and thence to Col. J.D. Marshall,Jr. of the Walter Reed Institute for Medical Research. At that time there was no clear-cut explanation for these apparent anomalies but the concensus of opinion was that caution should be exercised when interpreting the significance of a few

positive PHA results, especially when the titres are low as they were in this instance.

An opportunity to investigate further the question of a possible Y. pestis-Salmonella cross immunogenicity in man presented itself in May 1971 when an epidemic of typhoid occurred in Lesotho. The affected area was one where plague has not been known to occur. Serum specimens were kindly supplied at our request by the medical officers attending the patients.

Fortysix patients were included in the study and Salmonella typhi was isolated from blood and stool or urine of 26 patients. A pre-treatment serum was received from each patient and this was followed in most cases by one or two further serum samples at 5-day intervals. Widal tests were done on the sera of 41 patients and all but three individuals showed positive results with high and/or rising titres.

The PHA tests yielded negative results in each case. Although the sera were absorbed prior to the haemaglutination test with fresh sheep erythrocytes from the same source as those used in the test, heterologous agglutinations nevertheless occurred in 12 patients. It is possible that heterologous antibodies might have been eliminated if tanned erythrocytes had been employed for the absorption procedure.

Heterologous antibodies against tanned sheep erythrocytes became demonstrable in some patients only in the second or third serum sample and in others they increased slightly in titre during the course of their illness.

It should be emphasized at this point that our absorption procedure since the PHA test was introduced in this country has been quantitatively standardized. Thus 0,15 ml of packed red cells are added to 0,5 ml of serum and the mixture is shaken and allowed to stand at room temperature for 20 minutes. Erythrocytes from one sheep only have been used. During 1967 attempts were made to remove persistant heterologous antibodies by repeating the absorption process up to three times. This proved to be unsuccessful and repeated absorption was eventually abandoned. It was also noted that in the absence of any absorption procedure almost all our sera yielded heterologous agglutinations, sometimes to high titres.

This excessive reaction is probably a peculiarity of the particular sheep employed by us as a source of cells. Species specific isophile sheep red cell antigens are known to occur. It was however mentioned in chapter 3 that our sheep was selected after a period of frustrating attempts at obtaining reproducable results and the relatively minor nuisance of a small proportion of heterologous reaction [after absorption] was accepted as the price to pay for a reasonably reliable PHA procedure. It was therefore not only on general scientific principle that rigid controls have been carried out in our work but also in recognition of the occurrence of a number of heterologous agglutinations even after absorption.

The variation found in 1967 when different sheep

were tried for the sensitization process was probably also associated with genetically different sheep erythrocytes as both phenomena are dependant on cell membrane function. Recent work by Bell et al. [1972] and Leddy et al. [1972] again draws attention to this genetic difference and its importance in relation to immune haemolysis. Marked differences were found by these authors in the susceptibility to lysis by erythrocytes from three genetically different types of sheep and importance was attached in this respect to the conformation of the red cell membrane.

During the Lesotho typhoid epidemic the heterologous antibodies which were still present in the absorbed sera of 12 patients ranged in titre from 1:4 to 1:64. Of interest was the finding that these antibodies appeared to be absent in the early stages of typhoid in 4 patients and became demonstrable only in the second or third serum specimens while in another 2 patients a slight rise in titre [1 dilution] occurred.

These observations serve to underline that the execution of the PHA test without strict controls may not only give rise to erroneous 'positive' reactions but also to apparent sero-conversions and even to apparently rising PHA titres.

The serological results of the Lesotho typhoid patients show that Salmonella typhi can almost certainly be excluded as a cause of false positive PHA results.

At the time of writing Rust et al. [1972] reported

reported their finding of a minor protein contaminant in all fraction 1 preparations which in high concentrations can give rise to non-specific PHA reactions in sera from people who have had no contact with Y. pestis. These authors then presented a method by which the consequences of the protein contaminant can be minimized.

As the Y. pestis fraction 1 antigen prepared at the SAIMR yielded a gel precipitation pattern similar to that of an American fraction 1 preparation it can be assumed that the SAIMR antigen also contains this protein contaminant and this factor may be responsible for at least some of our unexpected findings.

With the PHA test hitherto in use such false positives would not be detected by the PHAI test which is subject to the same disadvantage.

Rust and his coworkers did not discuss the nature of the antibody with which the protein contaminant reacts. In the light of our Eastern Transvaal findings and of Baltazard's results in experimental animals it is tempting to speculate whether this antibody is associated with certain Salmonella infections or if it arises independently in situations in which such Salmonella infections also tend to be common.

Typhoid, mentioned as a diagnostic red herring in chapter 8, not uncommonly causes confusion in plague work. This aspect was also discussed by Legters et al. [1969] who did not believe that S. typhi infections which occurred during a plague outbreak in Vietnam were

responsible for positive PHA results in people who had no plague symptoms and had not been vaccinated against plague.

Our Lesotho results are highly reassuring in this respect and current studies on a combined typhoid/plague outbreak in Lesotho confirm the previous findings. The work on the current outbreak is still in progress but results to date show that, whereas clinically diagnosed plague patients show the development of high titre antibodies against Y. pestis fraction 1, the clinically and bacteriologically confirmed typhoid patients have S. typhi antibodies in high titres but their sera are uniformly negative in respect of plague antibodies. The situation described by Marshall et al. [1967c] in which typhoid and plague occurred side by side in the same patient has not been observed in southern Africa and must, fortunately, be a relatively rare phenomenon.

With regard to false positive PHA reactions the work of Rust and his coworkers goes a long way to rendering the PHA method more specific. It also confirms the suspected occurrence of false positive results in the past. Time alone will tell whether the new technique will eliminate all non-specific reactions but absolute specificity, though to be strived for, is not a prerequisite for an epidemiologically and dignostically useful procedure. At present, in the awareness that false positive reactions have occurred, we are still cautious and adopt certain criteria for the interpretation of plague serological results according to the circumstances.

The occurrence of false positive PHA reactions was also suspected during the Lesotho plague epidemic of 1967/68. On referring back to Table XIII on page 108 it will be seen that there were 18 asymptomatic people with positive PHA results. Six of these are believed to represent true silent infections [see page 88] but the remaining 12 had titres of 1:64 or less and which remained virtually unchanged when the test was repeated two months later. It is possible that a high antibody peak occurred during the interval and was missed. It is however likely that at least some of these reactions may represent false positives.

In the light of experience with the PHA technique [as described by Chen and Meyer) in southern Africa we regard a titre of 1:128 as highly suggestive of active plague regardless of the immediate epidemiological circumstances. The isolated finding of a titre below 1:128 should [in southern Africa, though not necessarily elsewhere] be treated with reserve and, although such a finding requires further investigation, it should not be the signal for the institution of emergency measures of control. It is our well considered opinion that an unsubstantiated cry of 'Plague !' can do much harm to the affected population. When such a population, as is so frequently the case in Africa, is labouring under poor socio-economic conditions, the publicity which is inevitably associated with plague control operations results in a decrease in revenue [from e.g. tourism and exports] which it can ill afford.

The usefulness of the PHA test as an epidemiological tool is without question. Table XVI presents a summary of all our plague survey results on human sera in southern Africa.

Table XVI. Human PHA results of surveys conducted in southern Africa during the period 1966-1971

Region	Number of sera studied	Number of sera with PHA antibodies	Number of sera with PHA titre 1:16 or higher
Ovamboland [epidemic]	480	63 [13,1%]	60 [12,5%]
Lesotho [epidemic]	631	165 [26,1%]	75 [11,9%]
Uitenhage [epidemic]	126	59 [46,8%]	18 [14,3%]
Uitenhage [inactive]	548	26 [4,7%]	11 [2,0%]
Transkei [inactive]	526	50 [9,5%]	23 [6,1%]
Eastern Transvaal [plague free]	499	11 [2,2%]	6 [1,2%]
Orange Free State *	500	22 [4,4]	5 [1,0%]
Total	3310		

* Results of a recent [1971] survey includes sera from hyper-endemic and completely plague free parts of this region.

The striking difference shown between results obtained during active and quiescent periods has been discussed in chapter 6. The overall plague antibody incidence in the Eastern Transvaal is the lowest of all the regions. The very high incidence found during the Uitenhage epidemic will be discussed in chapter 10 as the results of the virulence studies on the Uitenhage Y. pestis isolates may have a bearing on the antibody incidence.

10 CHARACTERIZATION OF SOUTHERN AFRICAN YERSINIA PESTIS ISOLATES

An increasing amount of work is currently in progress in several research centres to gain a better understanding of factors determining the virulence and pathogenicity of the plague bacillus. Such work includes the biochemical characterization as well as the study of certain virulence factors and the role they play in the pathogenesis of the disease.

10.1 Biochemical Reactions of 66 Southern African Isolates of Y. pestis

Sixtysix strains of Y. pestis isolated since 1949 were still viable and were passaged through white mice prior to conducting biochemical tests. These tests were carried out in the media recommended by Baltazard et al. [1956] which were incubated at 27°C for 21 days. Also tested were 4 known reference strains namely EV76[Paris], EV51f, Tjiwidej and MP6. The EV strains were obtained through the courtesy of Dr K.F. Meyer of the George Williams Hooper Foundation in San Francisco and the MP6 strain by courtesy of Dr A.F. Hallett of the Medical Ecology Centre, Johannesburg.

Inspection of the media was carried out daily where applicable. Table XVII presents the results of these studies.

In 1927 Pirie and Murray announced the unexpected finding of 4 glycerol fermenting isolates obtained from 1 human source and 3 rodents of the northwestern

Table XVII. Biochemical reactions of 66 Y. pestis isolates obtained during the period 1949-1972 and of 4 reference strains. Inspection of the media was carried out daily for 21 days [where applicable] and the figures indicate the days on which the relevant reactions were observed.

Reference number	Lactose	o-nitrophenyl-β-D-galactopyranoside	Glucose	Mannitol	Sucrose	Maltose	Rhamnose	Melibiose	Urea	Salicin	Glycerol	Nitrate reduction	Source	Map reference
V338/49	-	+1	+1	+1	-	1	+6	-	-	+3	-	+3	X.brasiliensis	
V201/50	+17	+1	+1	+1	-	+1	-	-	-	+3	-	+3	X.pirei	31.26.Dd
V19/50	+9	+1	+1	+1	-	+1	-	-	-	+4	-		X.phyllomae	24.28.Ac
V13/50	+9	+1	+1	+1	-	+1	-	-	-	+3	-	+3	X.philoxera	27.26.Bb
V44/50	+16	+1	+1	+1	-	+1	+10	-	-	+3	-	+3	Fleas	24.28.Ac
V203/50	-	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Chiastopsylla, Dinopsyllus and Xenopsylla sp.	30.26.Db
V199/50	+9	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Chiastopsylla, Dinopsyllus and Xenopsylla sp.	30.26.Dd
V205/50	+12	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Chiastopsylla, Dinopsyllus sp.	

Table XVII continued.

Ref. no.	Lact.	ONPG	Gluc.	Mann.	Sucr.	Malt.	Rham.	Meli.	Urea	Sali.	Glyc.	Nitr.	Orig.	Map ref.
H10/51	+6	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Human autopsy	32.27.Dd
F473/52	+15	+1	+1	+1	-	+1	-	-	-	+5	-	+3	Xenopsylla sp.	29.27.Ac
4464/15/9/53	+14	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Bubo fluid	Unknown
F607/53	+14	+1	+1	+1	-	+1	-	-	-	+3	-	+3	X.eridos	31.21.Db
V59/54	+12	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Human	31.20.Dd
F652/54	+17	+1	+1	+1	-	+1	-	-	-	+3	-	+3	X.philoxera and Dinopsyllus sp.	27.27.Ad
F668/54	+8	+1	+1	+1	-	+1	-	-	-	+3	-	+3	X.philoxera and Dinopsyllus sp.	27.27.Bc
F724/54	+17	+1	+1	+1	-	+1	-	-	-	+3	-	+3	X.philoxera and Dinopsyllus sp.	27.27.Ad
H2/54	+8	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Bubo fiuid	30.22.Dc
F20/55	+12	+1	+1	+1	-	+1	-	-	-	+3	-	+3	X.brasiliensis, L.segnis	27.27.Cd
F28/55	+14	+1	+1	+1	-	+1	-	-	-	+3	-	+3	X.brasiliensis, Ctenocephalides	27.27.Da
F535/56	+8	+1	+1	+1	-	+1	+10	-	-	+3	-	+3	Fleas and mites	27.27.Bc
H2/56	+8	+1	+1	+1	-	+1	+8	-	-	+3	-	+3	Bubo fluid	27.27.Ca
PE5448/57	+8	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Human	Unknown
F394/61	+9	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Xenopsylla sp., Ctenocephalides Echidnophaga	31.27.Cd

Table XVII continued.

Ref. no.	Lact.	ONPG	Gluc.	Mann.	Sucr.	Malt.	Rham.	Meli.	Urea	Sali.	Glyc.	Nitr.	Orig.	Map ref.
H1[illegible]2	+13	+1	+1	+1	-	+1	+7	-	-	+4	-	+3	Human	Ovambo
H13/62	+9	+1	+1	+1	-	+1	+8	-	-	+3	-	+3	"	"
H14/62	+14	+1	+1	+1	-	+1	+8	-	-	+3	-	+3	"	"
H3/63	+10	+1	+1	+1	-	+1	+8	-	-	+3	-	+3	"	"
D8/64	+13	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Myotomys	31.21.Db
F219/64	+14	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Chiast.coraxis	31.21.Db
H/5/65	+13	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Bubo fluid	26.24.Ad
F321/66	+14	+1	+1	+1	-	+1	+8	-	-	+3	-	+3	X.philoxera	17.15.Cb
F361/66	-	+1	+1	+1	-	+1	+10	-	-	+3	-	+3	X.philoxera	17.15.Cd
H4/66	+8	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Human	Ovambo
H13 /66	+6	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	"
H14/66	+9	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	"
H19/66	+11	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	"
H20/66	+9	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	"
H21/66	+9	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	"
H105/66	+13	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	"
H113/66	+11	+1	+1	+1	-	+1	+10	-	-	+3	-	+3	"	"
H125/66	+8	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	"
H140/66	+10	+1	+1	+1	-	+1	+10	-	-	+3	-	+3	"	"
H141/66	+14	+1	+1	+1	-	+1	+8	-	-	+3	-	+3	"	"

Table XVII continued.

Ref. no.	Lact.	ONPG	Gluc.	Mann.	Sucr.	Malt.	Rham.	Meli.	Urea	Sali.	Glyc.	Nitr.	Orig.	Map ref.
H142/66	+15	+1	+1	+1	-	+1	+11	-	-	+3	-	+3	Human	Ovambo
H143/66	+9	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	"
D54/67	+11	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Myotomys	33.25.Da
D56/67	-	+1	+1	+1	-	+1	+5	-	-	+5	-	+3	Myotomys	33.25.Da
H4/67	+16	+1	+1	+1	-	+1	-	-	-	+3		+3	Bubo fluid	33.25.Da
H32/67	+13	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Human	Ovambo
F44/68	+13	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Xenopsylla sp.	27.27.Bc
F308/68	+8	+1	+1	+1	-	+1	-	-		+3	-	+3	D.ellobius	30.27.Ab
F329/68	+7	+1	+1	+1	-	+1	-	-		+3	-	+3	Xenopsylla sp.	30.27.Ab
F340/68	+8	+1	+1	+1	-	+1	+12	-		+3	-	+3	Xenopsylla sp.	30.27.Ab
F351/68	+14	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Xenopsylla sp.	29.27.Cd
F357/68	+8	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Xenopsylla sp.	29.27.Cd
D4/68	+16	+1	+1	+1	-	+1	+9	-	-	+3	-	+3	R.rattus	28.27.Ab
D9/68	-	+1	+1	+1	-	+1	-	-	-	+4	-	+3	R.rattus	27.27.Dc
D16/68	+10	+1	+1	+1	-	+1	+7	+18	-	+4	-	+3	Tatera brantsii	30.27.Ab
D17/68	+8	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Rodent	29.27.Cd
D20/68	+7	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Mastomys	30.27.Ab
H3/68	+9	+1	+1	+1	-	+1	+15	+7	-	+3	-	+3	Human	Ovambo
H6/68	+12	+1	+1	+1	-	+1	-	-	-	+3	+21	+3	"	"

Table XVII continued.

Ref. no.	Lact.	ONPG	Gluc.	Mann.	Sucr.	Malt.	Rham.	Meli.	Urea	Sali.	Glyc.	Nitr.	Orig.	Map ref.
H14/68	+9	+1	+1	+1	-	+1	-	-	-	+3	-	+3	Human	Ovambo
H19/68	+10	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	"
H8/71	+17	+1	+1	+1	-	+1	-	-	-	+3	-	+3	"	26.24.Dc
H1/72	+7	+1	+1	+1	-	+1	+16	-	-	+3	-	+3	"	Ovambo
Tjiwidej	+15	+1	+1	+1	-	+1	-	-	-	+3	-	+3		
EV76g	-	+1	+1	+1	-	+1	-	-	-	+3	-	+3		
EV51f	-	+1	+1	+1	-	+1	+19	-	-	+3	-	+3		
MP6	+8	+1	+1	+1	-	+1	-	-	-	+3	-	+3		

Cape Province.

Bezsonova [quoted by Pollitzer, 1954] in 1928 first drew attention to the distinct geographic distribution of glycerol positive and glycerol negative strains and this idea eventually culminated in the concept by Devignat [1951] of three plague races which could be distinguished from each other on the basis of glycerol fermentation and nitrate reduction. Accordingly, the southern African isolates generally belong to Y. pestis var orientalis which reduces nitrate but does not ferment glycerol.

The finding of 4 glycerol positive strains has therefore puzzled South African plague workers ever since they were reported and it is especially tantalizing that these strains are no longer available in our collection for further study.

Chen [1949] reviewed the question of stability of the glycerol fermenting property and came to the conclusion that this would probably prove to be a stable characteristic.

Table XVII shows that 65 of our isolates were glycerol negative after 21 days of incubation while a single isolate fermented this sugar on the 21st day. The latter observation was confirmed by repeat testing. All our isolates reduced nitrate and these findings support the validity of classifying southern African Y. pestis within the oceanic race.

The prompt acidification of rhamnose is used as a property to differentiate Y. pseudotuberculosis from

Y. pestis. A fairly high proportion of our isolates [20/66 or 30 %] were found to be late rhamnose fermenters, the earliest reaction being noted on the fifth day of incubation. Rhamnose fermentation was much commoner among the Ovamboland isolates [46 %] than among those from other regions [21 %].

Englesberg [1957] showed that Y. pestis does normally not utilize rhamnose but that all are potentially capable of doing so by giving rise to rhamnose utilizing mutants. He showed this mutation to be of a very low frequency, namely $2,6 \times 10^{-11}$. Furthermore, the appearance of mutants did not occur until the sixth day, appeared at a constant rate from the seventh until the twelfth day and thereafter decreased. This type of mutation appears to be responsible for the late rhamnose fermentation in our isolates.

Utilization of melibiose is also used as a differentiating characteristic when comparing Y. pseudotuberculosis with the plague bacillus. Brubaker [1972], however, reported that wild type Y. pestis can yield melibiose meiotrophs. Two of our isolates appear to be of that variety in that they were capable of fermenting melibiose as a late phenomenon. Both isolates also fermented rhamnose but were otherwise unremarkable. Melibiose positive isolates were recently reported by Richaud and Rasjenison [1971] in Madagascar but their reactions became positive after 60 hours.

According to Pollitzer [1954] and Baltazard [1956] late acidification of lactose may occur occasionally.

In this respect the southern African isolates differ considerably in that late lactose fermentation was the rule rather than the exception. Only 3 out of 66 of our isolates and the two EV strains failed to ferment lactose by the 21st day of incubation when the experiment was terminated.

In view of this unexpected finding the ONPG [ortho-nitrophenyl-beta-D-galactopyranoside] test was subsequently carried out on all the strains. The ONPG test, a test for beta-galactosidase, rapidly demonstrates the property of potential lactose fermentation and gave positive results in each case, including the 4 reference strains, after 24 hours incubation. This suggests that even the 5 lactose negative plague cultures would eventually have become positive if incubation had been prolonged beyond 21 days.

Salicin fermentation is reported as being variable by most authors. All our isolates were salicin negative after 2 days but the majority became positive on the 3rd day and the remainder on the 4th and 5th day of incubation.

On the basis of these observations on isolates obtained over a period of 24 years it was possible to draw up a reference table of biochemical reactions which can be expected from southern African Y. pestis after short incubation [for diagnostic purposes] and after 21 days of incubation. Table XVIII presents these features.

Table XVIII. Reference table of biochemical reactions to be expected from southern African Y. pestis isolates after short and long periods of incubation.

Name of test or substrate	Result after 48 hours of incubation	Result after 21 days of incubation
Glucose	+	
Mannitol	+	
Sucrose	-	-
Maltose	+	
Lactose	-	+[a]
ONPG	+	
Salicin	-	+[b]
Glycerol	-	-[c]
Nitrate reduction	+[d]	
Urea	-	-
Rhamnose	-	v
Melibiose	-	-[a]

a : with a few exceptions
b : acidification occurs within 3-5 days
c : rare, very late exceptions may occur
d : after 72 hours
v : variable

10.2 Virulence Factors of Southern African Y. pestis

Consequent to the publication of an article by Surgalla et al. [1970] describing simple in vitro methods for the detection of certain plague virulence factors some of these techniques were applied to our own isolates.

It was hoped that the virulence tests might throw some light on the Ovamboland enigma of mild plague.

In this section the example of Brubaker [1972] is followed in adopting the recommendations by Demerec et al. [1966] who proposed a uniform nomenclature in bacterial genetics.

10.2.1 Pesticinogeny and Fibrinolytic Activity

The attenuated strains EV76 [Paris] and EV51f were used as pesticin negative [pst^-] and pesticin positive [pst^+] controls respectively.

Y. pseudotuberculosis type I, kindly supplied by Prof. H.H. Mollaret of the Pasteur Institute in Paris, was used as the indicator strain known to be sensitive to pesticin I.

Two of our plague isolates, namely H6/68 and H14/68 were found to be pst^- and also devoid of demonstrable fibrinolytic activity. The two characteristics are genetically linked, together with a third comprising coagulase production [Brubaker et al., 1965]. The latter activity was not tested in our isolates.

Surgalla and Beesley [1971] showed that Y. pestis which is pst^- but vwa^+ [produces V and W antigens], pgm^+ [able to pigment] and fra^+ [produces fraction 1 antigen] is fully infectious for mice and guinea pigs but that the lethal effects are considerably reduced.

Brubaker [1969] demonstrated that all pst^-, pgm^+ strains of Y. pestis tested [with the exception of the Dodson strain, a human isolate from Arizona, USA] proved to be sensitive to pesticin I while bacterial cells which were pst^-, pgm^- were resistant to this substance.

Our two pst^- isolates were both of human Ovambo origin and H14/68 is an example of a pst^-, pgm^+ strain. Using this as the indicator strain with EV51f as a producer strain, it was found to be sensitive to pesticin I. On the other hand, H6/68 which is pst^-, pgm^- [the loss of pigmentation is thought to be due to mutation under storage conditions;

see section 10.2.3] was shown to be resistant to pesticin I. These findings therefore correspond with those noted by Brubaker.

Beesley and Surgalla [1970] considered pesticin production to be a highly stable characteristic and indicate its absence in wild strains of Y. pestis to be relatively rare. Thus only 3 pst⁻ strains were identified among 1100 Vietnamese isolates, 2 such strains were found in the USA [1 from fleas isolated in 1940 and 1 from a child, the Dodson strain, in 1967] and 3 pst⁻ strains were found among 370 isolates by Russian workers. The latter isolates were obtained in 1923, 1945 and 1960. These observations make the finding of 2 pst⁻ strains among 66 southern African isolates all the more interesting, especially as they both originated in Ovamboland, during the same epidemic and are both of human origin.

Unfortunately no clinical information is available on one of the patients concerned who was not notified as a case of plague [isolate H6/68]. Isolate H14/68 was obtained from case number 11/68 [see Table V] who was a 19 year old young woman, admitted to hospital with a left inguinal bubo and a temperature of 37,8°C. Her PHA titre on admission was 1:16 and 29 days later it was 1:64.

10.2.2 The Virulence Factor

Burrows and Bacon [1956] described the V and W antigens which they believed to play a role in virulence, an opinion which was subsequently confirmed. It has been shown that these antigens are required for Y. pestis infectivity and generally strains which do not produce these antigens [vwa⁻] are noninfectious for mice or guinea pigs in doses

below 10^3 or 10^4 bacterial cells by any of the usual experimental routes [Surgalla and Beesley, 1971]. It was established that virulent Y. pestis has a nutritional requirement for calcium when grown at 37°C but not at 26°C, and Higuchi and Smith [1961] established that the mutation rate of calcium dependence at 37°C by virulent cells to calcium independence, or avirulence, was extremely high, namely 10^{-4}.

Burrows and Bacon had associated loss of virulence with loss of V and W antigens and thus the three properties of virulence, production of V and W antigens, and calcium requirement at 37°C were shown to be related. The latter property was utilized by Higuchi and Smith [1961] to develop a differential plating medium, poor in calcium and containing magnesium oxalate, for the detection of calcium dependent Y. pestis.

The finding of an avirulent, calcium independent strain of Y. pestis which however produced V and W antigens led Brubaker [1972] to suggest that it is the expression of calcium dependence per se which is correlated with pathogenicity.

Using the EV51f and Tjiwidej strains as positive and negative controls respectively, 6 of the southern African isolates were found to be calcium independent at 37°C and presumed to be lacking V and W antigens [vwa$^-$] and avirulent. The six isolates were the following.

V44/50, isolated from fleas in 1950;

H10/51, of human origin in 1951;

F607/53, isolated from fleas in 1953;

H113/66, of Ovambo human origin in 1966;

H4/67, of Uitenhage human origin in 1967;

D54/67, isolated from Uitenhage Myotomys in 1967.

The cultures used for these tests had been stored at room temperature in semisolid agar and been subcultured at irregular intervals varying from 6 months to several years. It is important to note however that all 66 isolates were subcultured at the same time and great care was exercised to transplant a representative bacterial population in each case.

The finding of only 6 out of 66 [9,1 per cent] vwa$^-$ strains in a culture collection dating back to 1949 led us to believe that these are not necessarily the products of mutation but may be true vwa$^-$ wild strains, especially in the case of the 3 more recent isolates. If this were so, then strains H4/67 and D54/67 would furnish an explanation of some of the findings in the Uitenhage plague outbreak of 1966/67. H4/67 was isolated from the bubo of the single notified and bacteriologically confirmed plague patient and D54/67 originated in the bonemarrow of a Myotomys found dead near this patient's house. A third isolate , also from a Myotomys, was found to be calcium dependent. The serological survey on human sera at the time showed that 46,8 per cent of the healthy population had PHA antibodies [see chapter 6]. In addition to the single confirmed plague case there had been a few deaths, believed to have been due to plague but unconfirmed as exhumation was not considered. These observations indicate a high attack rate with a rather low case incidence. If it is

postulated that vwa$^-$ strains were prevalent together with vwa$^+$ strains then the above observations become less perplexing in that a large part of the population may have been naturally immunized by avirulent Y. pestis which is able to infect in sufficiently large doses. The patient, from whom the vwa$^-$ strain was obtained, may either be an example of an atypical, severe reaction to this strain or may have received a very high infective dose. Meyer [1970] and the present author [quoted by Meyer, 1970] have shown that avirulence with respect to Y. pestis is a relative concept by demonstrating lethal reactions in non-human primates to the administration of the attenuated EV51f strain. Meyer also referred to racial differences which have been observed in reactions to live, attenuated vaccine strains.

The possibility that the PHA positives found during the Uitenhage epidemic are in fact false positives can almost certainly be excluded on the basis of the previous year's serological results obtained in the same population.

10.2.3 The Pigment Factor

This factor, like pesticin and capsular material, is not implicated in infectivity but is concerned with the lethality of the plague bacillus.

Brubaker [1969], by making use of the earlier demonstrated pesticin I sensitivity of pst$^-$, pgm$^+$ Y. pestis and the pesticin I resistance of pst$^-$, pgm$^-$ strains, developed a method for calculating the mutation rate of pigmentation to non-pigmentation and arrived at the high frequency of 10^{-5} mutations per bacterium per generation.

The Congo red agar medium for the detection of pgm$^+$ Y. pestis was developed by Surgalla and Beesley [1969] who also suggested that Y. pestis may have a common binding site for Congo red and for haematin which was previously used for the demonstration of pigmentation.

In the studies on the southern African isolates the pgm$^+$ MP6 and Tjiwidej strains, and the pgm$^-$ EV76 [Paris], EV51f and Y. pseudotuberculosis were used.

In this instance duplicate sets of stock cultures [of both wild and reference strains], one set of which had been stored at room temperature and the other at 4-10^{o}C, were used for the pigmentation tests. All refrigerated plague cultures were shown to be capable of absorbing the Congo red dye but a large proportion of the isolates stored at room temperature, including the Tjiwidej strain, had lost this property. The refrigerated Tjiwidej culture also showed the presence of some non-pigmented colonies. To confirm a suspicion that the non-pigmentation was due to mutation during storage, single pigmented colonies of 6 isolates were subcultured on Congo red agar and, as expected, produced only pigmented offspring. These clones were then stored in semi-solid agar at room temperature and subcultured on Congo red agar 3 months later. All 6 showed the presence of non-pigmented colonies to a greater or lesser extent. These findings indicate that retrospective pigment studies may lead misleading results and the only conclusion to be drawn from the studies on the southern African isolates is that of 32 refrigerated cultures 29 were capable of pigmentation but no quantitative opinion

can be given. The remaining three isolates [H6/68 which was also pst$^-$, H3/68 and D9/68] were completely non-pigmented but this may be the result of storage.

10.2.4 Fraction 1, the Envelope Antigen

Burrows [1960] stated that strains of Y. pestis which are competent to produce fraction 1 are symbolized as F1$^+$ and those that are incompetent to produce this material under any conditions are F1$^-$. These strains are now designated as fra$^+$ and fra$^-$ respectively. In addition Burrows described a rare intermediate type termed F1* which in virulence studies behaves as fra$^-$ [virulent to mice, avirulent to guinea pigs] but behaves like fra$^+$ in immunogenicity studies. An example of such a F1* Y. pestis is the Bryans strain which caused fatal plague in a 4-year old girl [Winter et al., 1960].

The work of Janssen and Surgalla [1969] has shown that the envelope antigen [or capsular material] is not required for immunization or infectivity but plays a role in determining the lethality of Y. pestis. This was further substantiated by Donovan et al. [1961] who established that the infectious and lethal dose of fra$^+$ Y. pestis MP6 to be one cell for guinea pigs while a lethal dose of fra$^-$ Y. pestis M23 required close to 10 000 cells in the guinea pig.

Mutation from fra$^+$ to fra$^-$ also occurs at a high rate but the reverse has not been reported.

Little and Brubaker [1972] drew attention to the irreversibility of the mutational loss of the genes responsible for pst$^+$, pgm$^+$, vwa$^+$ and fra$^+$ of which only the loss of

pst$^+$ occurs at a very low rate.

It was intended to test our isolates for the presence of fraction 1. An attempt was therefore made to produce specific Y. pestis fraction 1 antiserum by applying the methods described by Surgalla et al. [1970] to the use of SAIMR plague vaccine instead of the Cutter vaccine which was used by these authors. The gel precipitation tests with the antiserum thus obtained in rabbits yielded unsatisfactory results and it was clear that the use of this material would give rise to unreliable data about the fra$^+$ status of the plague isolates.

However, in 1963 Dr. K.F. Meyer quantitatively tested 7 Ovambo plague isolates for fraction 1 and found this to be present in concentrations typical for virulent strains.

11 SUMMARY AND CONCLUSIONS

Following a review of the history of plague in general and of southern Africa in particular an account is given of the ecological conditions which favoured a recrudescence of plague during the years 1965-1968. No cases of plague were reported during the year 1969. During this cycle a sharp increase in incidence was noted as compared with previous recrudescences and this was compared with some other regions in the world where the same phenomenon was experienced. Our increase is believed to have been an absolute one in contrast to Vietnam where Reiley and Kates [1970] were of the opinion that the increased incidence was at least in part due to improved reporting procedures.

Although outstanding work has been done on the epidemiology and ecology of southern African plague since the introduction of this disease at the turn of the century, little has been published about the clinical aspects of plague in man. An attempt was therefore made to fill this gap to a certain extent by describing the disease as observed in this part of the world.

The epidemics of 1966/67 and 1967/68 in Ovamboland are described and it is shown that, in spite of the generally mild plague peculiar to that region, the septicaemic form nevertheless occurred and a probable case of plague meningitis and one of pharyngeal plague are discussed. It is also demonstrated that in spite of the notification of suspected plague on very slender evidence the mortality rate is very low. Comparison of the two outbreaks, the first of

which was investigated in person, demonstrated the need for improved serological follow-up of suspect cases as shown by the much superior results obtained during 1966/67. It cannot be overemphasized that single serum specimens are virtually useless for plague diagnosis and may in fact do harm by inducing a false sense of security on receipt of a negative PHA report.

The Y. pestis isolates obtained from this region showed a rather high proportion of rhamnose meiotrophs in comparison with isolates from other regions.

Two Ovambo isolates were pesticin negative and these were the only such isolates detected in Y. pestis obtained over a 24-year period from various parts of southern Africa. This finding may have a causal relationship to the mildness of Ovambo plague . One of these pst$^-$ strains, which was pigmented on Congo red agar, was itself sensitive to pesticin I.

Another peculiarity that was noted in Ovamboland was the very low incidence of asymptomatic but serologically positive people.

The relationship of cervical and axillary buboes seen in Ovambo children to their habits of catching, killing and eating of small mammals and birds is discussed.

A plague outbreak in the Uitenhage district is reported and a peculiarity in this instance was the occurrence of a very low case incidence associated with an extremely high [46,8 per cent] proportion of healthy, sero-positive people living in the affected area. The possibility of these being examples of false positive PHA results can

probably be excluded on the basis of the findings obtained during a serological survey in the same area in the previous year when the incidence of PHA antibodies in the population was only 4,7 per cent.

A hypothesis is presented that a proportion of PHA positive sera found during quiescent periods may be 'left-overs' from a previous epidemic and not necessarily due to current antigenic stimulation by Y. pestis. This opinion is based on the finding of plague antibodies to high titres in 53 per cent of Lesotho people up to four years following active plague infection. This hypothesis is further supported by the finding of a higher percentage of PHA positives found in a region with a shorter interval since the previous epidemic than in a region with a longer interval.

Two out of the three Y. pestis isolates from the Uitenhage outbreak which were tested were found to be vwa$^-$. Among 66 isolates obtained over 24 years there were six such observations. The high frequency of mutation to vwa$^-$ is discussed. This did not seem to be common in our stock cultures but the probability of this being the cause of our vwa$^-$ findings cannot be ignored. Nevertheless, the observation of two vwa$^-$ strains of Y. pestis in related sources [patient and rodent near patient's house] led us to wonder if this might have resulted in the observed high attack rate and low case incidence.

A single case of bubonic plague initially diagnosed as 'strangulated hernia' is discussed and compared with similar cases observed elsewhere in the world. There were no obvious epidemiological features indicating the pre- or co-existence of an epizootic.

The circumstances of a plague epidemic with bubonic and probable pneumonic components in Lesotho, and the delay in diagnosis which resulted from the well known confusing factor of co-existing typhoid, are presented. All the features of a classical, severe and unchecked plague epidemic were observed.

Attempts at demonstrating the presence of Y. pestis in the throats of plague patients and their contacts in the Uitenhage and Lesotho epidemics were unsuccessful.

The question of the occurrence of false positive PHA results in man was investigated and it was found that this does in fact occur. However, it is not believed that false positives significantly affect the results of large scale serological surveys, particularly if these are comparative surveys.

We have established that Salmonella typhi did not appear to be a cause or co-existent factor in the occurrence of false positive PHA results but a correlation was demonstrated between the occurrence of non-specific PHA reactions and infection with Salmonellae other than S. typhi. From both the diagnostic and the epidemiological points of view it is concluded that careful evaluation of PHA results in relation to the circumstances surrounding such findings will minimize the drawing of wrong conclusions.

It is to be expected that the new method described by Rust et al. [1972] will reduce or even eliminate the occurrence of false positive reactions.

It was demonstrated that the well known principle of proper controls being essential in serological work in

general is even more the case in plague serology in that the absence of such controls may not only result in 'false positives' but also in an erroneous demonstration of seroconversions and rising titres. The antibodies responsible for this type of error are heterologous ones against sheep erythrocytes and not cross reacting antibodies.

The biochemical patterns of 66 Y. pestis isolates obtained over 24 years were established. These were not remarkable except for the very high incidence of late lactose fermentation. This finding was confirmed by the ONPG test which was uniformly positive after 24 hours. It is therefore recommended that the ONPG test is included in the routine battery of biochemical tests in plague diagnosis.

All the isolates were shown to belong to Y. pestis var orientalis on the basis of nitrate reduction and failure to ferment glycerol promptly.

The conclusion was reached that plague is by no means a disease of the past in southern Africa and, in spite of effective preventive measures being available and practiced, a sharp increase in incidence was noted not only in Africa, but also in parts of Asia and the Americas. The tendency by some local authorities to toy with the idea of reducing or even suspending plague vigilance on the basis of prolonged absence of the disease is to be deplored as it is the seemingly unrewarding vigilance which is largely responsible for plague being a relatively uncommon disease. The relaxation of such vigilance must sooner or later result in disaster.

Plague is firmly established in southern Africa and there have been signs that expansion of at least one plague focus is taking place. It is beyond our present ability to halt this expansion, not to mention eradication of wild rodent reservoirs, but the new methods developed in all fields of plague work over the past two decades are making it increasingly easy to effect early detection of plague resurgence and to minimize its consequences.

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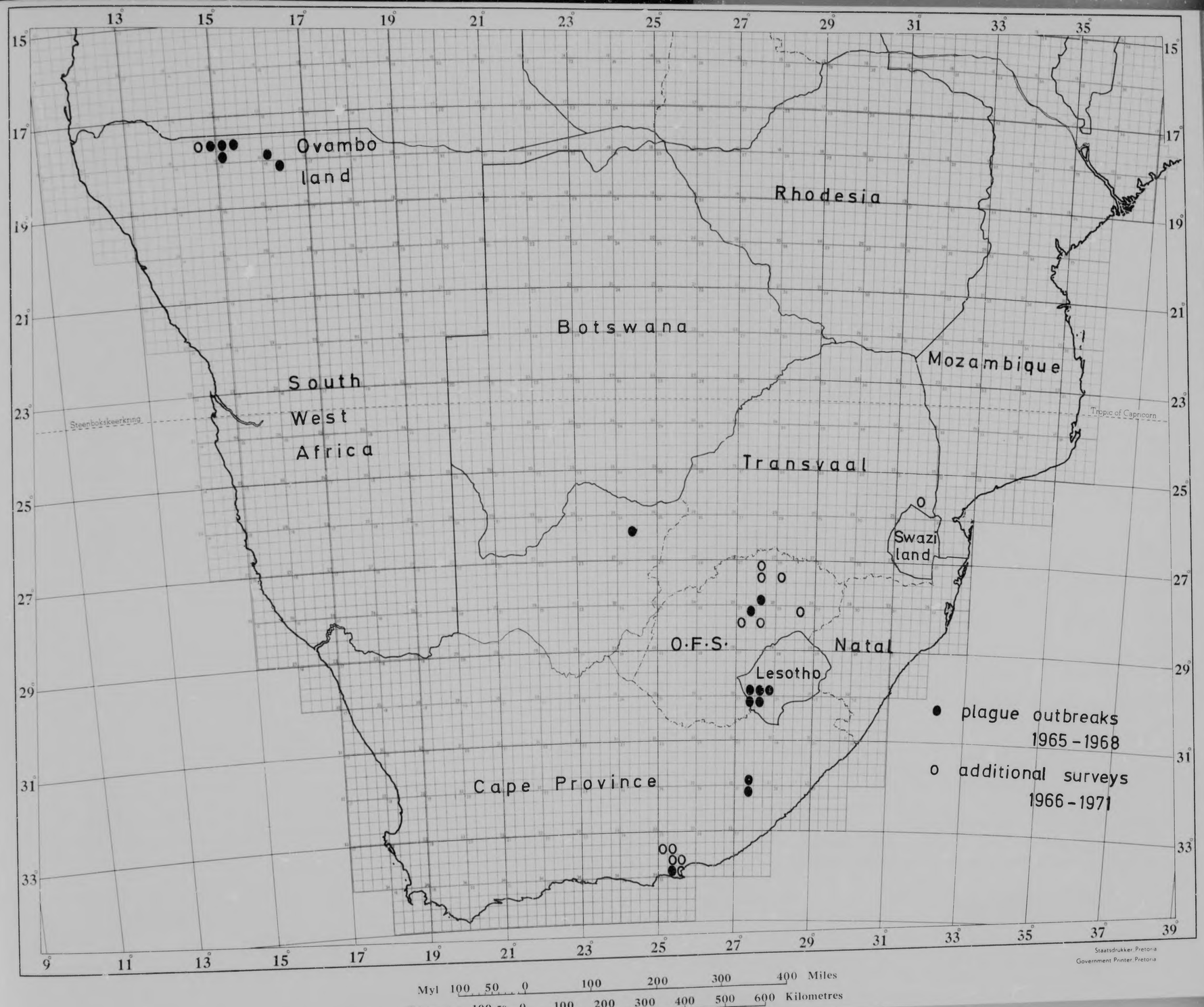
Ovambo land
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plague outbreaks 1965 - 1968
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Staatsdrukker Pretoria
Government Printer Pretoria