Transmission of *Nocardia farcinica* by two Ixodid species

by

B. M. Al-Janabi

B.V.Sc.(Baghdad)

Dr.Med.Vet.(Leipzig)

Thesis presented for the degree

of

Doctor of Philosophy of the University of Edinburgh

in the Faculty of Veterinary Medicine

APRIL 1976

Dedicated to the memory

of my mother, and

to the patience

and faith of

my wife

C O N T E N T S

C O N T E N T S (Cont'd)

C O N T E N T S (Cont'd)

C O N T E N T S (Cont'd)

ACKNOWLEDGEMENTS

I wish to express my gratitude to the Government of Iraq and to the Ministry of Higher Education and Scientific Research for the opportunity to complete my higher studies.

I am grateful to Professor Sir Alexander Robertson for the provision of laboratory facilities.

My special thanks are due to Dr. J. K. H. Wilde and Dr. D. Branagan for their supervision during this study, unfailing interest and invaluable kindness.

I am also grateful to Dr. G. Scott for his advice, help and interest.

I wish to thank Mr. A. C. Rowland for his assistance in post-mortem examinations; Mr. D. Danskin, Mr. W. G. MacLeod and Mr. A. Swales, for their technical assistance and also the staff of the photographic unit and animal house.

Finally, my thanks are due to Mrs. Margaret Allison for typing this thesis and to the librarians Mrs. L. Done and Mrs. H. London.

SUMMARY

The cultural, morphological and pathogenic characteristics of Nocardia farcinica were studied.

Guinea pigs were susceptible to Nocardia farcinica, showing typical lesions following injections with cultures or infected tick materials.

Rabbits readily acquired a generalized infection with Nocardia farcinica but only a small proportion of those so injected developed lesions.

Generalized Nocardia farcinica infections did not result from minor skin traumata (pin-pricks and scarification) made in rabbits' ears and guinea pigs' backs which had previously been painted with cultures of the organism, and neither did they result when ticks were fed on surfaces so treated.

The feeding performance and developmental periods of Amblyomma variegatum and Hyalomma anatolicum excavatum were also precisely studied.

Larvae and nymphs of Amblyomma variegatum acquired Nocardia farcinica infection from rabbits and, at periods of up to nine weeks, transmitted these infections to other rabbits when feeding as the subsequent instars. Trials proved that this period of retention of transmissible infection can extend up to 33 weeks.

Nymphs and adults of Amblyomma variegatum showed

their ability to retain transmissible infection under various environmental conditions.

Larvae and nymphs of Hyalomma anatolicum excavatum acquired Nocardia farcinica infection from rabbits and, at periods of up to eight weeks, transmitted the infection to other rabbits when feeding as the following instars. However, subsequent trials showed that Hyalomma anatolicum excavatum cannot retain naturally transmissible Nocardia farcinica infection for longer periods.

Trans-ovarial transmission was not achieved with either species.

These clear demonstrations of the trans-stadial transmission of the bovine farcy organism confirmed the existence of an epidemiological feature which has previously only been suspected.

CHAPTER I.

INTRODUCTION and REVIEW OF THE LITERATURE

1. General

Bovine farcy is a chronic infectious disease of cattle caused by an aerobic, acid-fast organism, Nocardia farcinica. The literature covering the disease and the manner in which it is transmitted is scanty.

The disease was first described in the Gironde and Charente departments of France in 1829 by Sorrilon, who called it Farcin du boeuf. It was described again by Maillet (1837) in the Province of Anjou, where the disease was known as Arboulets and was regarded as being of long duration and characterised by swellings and lesions confined to the limbs. Such swellings were also mentioned by Mousis (1837) and were - presumably - responsible for the disease being then regarded as a form of elephantiasis.

Observations on bovine farcy were made by Lafosse (1849) in cattle attending the clinic of the Toulousaine School. Later, Lafosse (1853) described cases seen in the Toulousaine region as being of a chronic nature and being similar to equine epizootic lymphangitis. This lymphatic involvement was commented on by Cruzel (1869), who described swelling and suppuration of the superficial

lymph glands and vessels. Some of these latter cases were said to display symptoms of pulmonary tuberculosis, but, in general, the disease was regarded by these early French writers as being of long duration and non-fatal.

In Guadeloupe, Couzin (1879) collected pus from lesions in autopsied cattle which had been showing symptoms of bovine farcy, and Nocard (1888) succeeded in isolating the organism from this material. Nocard & Leclainche (1903) commented that the disease was very widespread in France, but not in other European countries. Further, while the disease was of a severe character in Guadeloupe, it had assumed an endemic, less severe status in Mauritius. The disease was then described as existing in chronic form in Sumatra by Vryburg (1907) with lesions involving the skin, lymphatic system and lungs.

In India, Holmes (1908) described cases in which the early signs were subcutaneous nodules in the neck, shoulder and flank. This was followed by enlargement of the superficial lymph nodes (especially the prescapular and precrural). In all cases except one the disease followed a wound or an indolent sore in the region of the neck or hump. These sores are known locally as "Calcutta sores". The enlarged glands on section were found to have a thickened fibrous capsule. As described by Raymond (1910), characteristic features of an outbreak of a disease resembling bovine farcy among heavy draught

bullocks in Calcutta were the swelling and eventual abscess formation in lymph nodes and, sometimes, similar abscess formation in the internal organs. Schlegel (1913) described nodules and lesions in the lungs and pleura of a nine-month-old calf. In sections of the nodules he observed what he called branching streptothrix and small coccoid bodies both of which were acid-alcohol-fast. No growth could be obtained from isolates of these organisms in culture. The involvement of internal lymph nodes, visceral parenchyma and serosa was commented on by Bernard (1924; 1927).

The disease was described in Kenya by Daubney (1927), who offered the alternative names of bovine lymphangitis and tropical actinomycosis. Four cases of the disease were observed - all showing prolonged emaciation and debilitation before death. Hard, variously-sized subcutaneous nodules were accompanied by enlargement of the superficial lymph nodes. Udder involvement was accompanied by enlargement of the supramammary lymph nodes. In one case, an abscess occurred in the parotid region. In another, abscesses were seen at autopsy in the lungs and mediastinal lymph nodes. All of these animals were heavily infested with Amblyomma variegatum and, particularly so, in the vicinity of the lesions. Though Daubney associated the disease with this tick infestation, appropriate transmission experiments were not carried out.

In 1930, Pirani reported the disease in Eritrea, and, in 1935, Xiroudakis confirmed the persistence of bovine farcy in Guadeloupe, though he described it under the name "epizootic lymphangitis".

Sforza (1940) isolated acid-fast organisms from bovine farcy lesions and remarked that the disease occurs in a more severe form in the tropics (i.e. India and Africa) than in European countries. A similar comment was also made by Curasson (1942). The disease - with the familiar subcutaneous nodules and lymphangitis of the limbs - was then reported from Colombia by Luque (1946). A first recording from the Dakar region of Senegal was made in 1958 by Memery, Mornet & Camara, who described chronic inflammation and suppuration in the subcutis, the lymphatic system and the viscera.

A study of the disease in the Sudan was made by Awad & Karib (1958). They stated that the commonest form of the disease was a chronic purulo-granulomatous inflammation of the subcutis with appropriate lymphatic involvement. Lesions were most frequently encountered in the head, neck and precrural regions. The local name for the disease was given as "Giggeil el Baggar". The authors also described an internal form of the disease which had a superficial similarity to bovine tuberculosis and which sometimes could occur in animals already affected by the more common external form. The authors

emphasised that bovine farcy was a cause of considerable economic loss to the cattle trade in the Sudan.

Further studies of bovine farcy in the Sudan were carried out by Mostafa (1962; 1967a). In addition to the usual external form, he also described the internal form with characteristic farcy lesions in the lungs and in the bronchial, mediastinal and mesenteric lymph nodes.

In neighbouring Chad, the importance of losses in Zebu cattle through bovine farcy was remarked upon by Perpezat, Mariat, Destombes & Thome in 1963.

2. Cultural, morphological and pathogenic characteristics of *Nocardia farcinica*

The first recorded isolation of the organism responsible for bovine farcy was by Nocard (1888) from cattle in Guadeloupe. When stained with Gram's Weigert or Ziehl-Neelsen stains, the organism was seen in affected tissues as masses of branching Gram-positive and acid-fast threads. Nocard considered the organism to be a *Streptothrix* sp. and named it "bacillus du farcin". The name *Nocardia farcinica* was later bestowed by Trevisan (1889).

In an article published in 1903, Nocard & Leclainche described the organism as growing aerobically at temperatures between 30 and 40°C in various liquid and solid media. Guinea pigs were found to be very susceptible to

the organism, and would die between nine and 20 days following intravenous or intraperitoneal injections of N. farcinica culture. The lesions were similar to those of miliary tuberculosis. Guinea pigs which received subcutaneous injections of these cultures developed abscesses at the sites of injection. Rabbits, horses, dogs and cats were considered to be refractory.

In Sumatra, Vryburg (1907) stated the cause of bovine farcy to be a Gram-negative bacillus of variable size, and described similar pathogenic effects to those recorded by Nocard & Leclainche when cultures were injected into guinea pigs. Orchitis was noted following intraperitoneal injections.

Holmes (1908) reported a confused impression of the characteristics of the organism - culturally both aerobic and anaerobic, morphologically as rods, filaments and spores. He also mentioned the formation of single button-shaped colonies in incubated cultures, but apparently failed to continue the propagation of these cultures. The subcutaneous injection of pus from natural lesions produced hard localised abscesses in rabbits and guinea pigs. Cultures derived from these abscesses killed guinea pigs and rabbits in two to seven days (route of injection not stated).

A Gram-negative organism of variable size which grew slowly and aerobically in culture was also isolated by

Raymond (1910). A similar bacillus was recovered from guinea pigs after these had been injected with pus from natural lesions in cattle. Similar cultural and pathogenic properties were described by Sheather (1920) for a bacillus isolated from natural cases in Madras. Sheather considered that the cause of bovine lymphangitis might be due to Streptothrix (Nocard) together with other bacilli (Vryburg, Raymond and Sheather).

It should be noted at this juncture that the Gram-negative properties of the organism isolated in Sumatra and India and described by Vryburg (1907), Raymond (1910) and Sheather (1920) are at variance with accepted attributes of N. farcinica isolated by Nocard (1888) in Guadeloupe.

Bovine lymphangitis cases, as appeared in California, were described at some length by Traum (1919). Swellings and abscesses were mainly confined to the limbs and the adjacent lymph nodes, and the involvement of mesenteric lymph nodes was recorded in only one case. The abscesses were of variable size, and discharged through the skin with no tendency to heal or to respond to normal wound treatment. The disease was regarded as being somewhat similar to bovine tuberculosis in view of the absence of general disturbance.

When stained by Gram's and Ziehl-Neelsen's methods, smears prepared from pus and lesions showed a Gram-

positive, acid-fast organism which, in most instances, was pleomorphic and beaded, so that it appeared to be made up of two or more coccoid components. Some individual bacilli showed as fine straight rods; others were curved; and yet others were enlarged at one end. The organism grew readily at 37°C - and fairly well at 26°C - on plain and glycerine broth, on blood agar and Loeffler's blood serum. On glycerine broth, cultures developed a surface membrane which became wrinkled as the culture aged - the medium remaining clear except for a few flakes.

Cultures, pus and material prepared from nodules from natural cases were inoculated subcutaneously, intramuscularly and intraperitoneally into guinea pigs, and subcutaneously and intravenously into rabbits, calves and chickens. Similar materials were fed to mice. Some of the guinea pigs, rabbits and calves showed swellings at inoculation sites, and pus withdrawn from these lesions contained acid-fast organisms resembling those found in natural cases. In a few of the guinea pigs which had been inoculated intraperitoneally, small necrotic areas developed in the liver.

In his studies in Guadeloupe on the pathogenicity of the organism, Bernard (1924; 1927) described a condition of "generalised miliary pseudotuberculosis" followed by death in guinea pigs which had been injected intravenously with cultures. He also recorded lesions in the liver,

spleen, omentum, peritoneum and intestine of guinea pigs which had been injected intraperitoneally with similar cultures. Similar results were obtained in both cattle and sheep.

Cultures of a Kenyan isolate (Daubney, 1927) produced peritonitis and lesions in the liver, spleen and kidneys in guinea pigs seven to 14 days after intraperitoneal injection. Local abscess formation was observed in guinea pigs after subcutaneous injection with the same cultures. In one guinea pig, a suppurative orchitis developed three months after subcutaneous injection.

Two bulls were injected subcutaneously with these cultures and developed local, pus-discharging abscesses some five weeks later. Daubney was able to demonstrate an acid-fast organism indistinguishable from N. farcinica in material from both external and internal lesions in these injected animals and he concluded, first, that the lesions which he had observed were similar to these described by Nocard; and, second, that the disease in India is similar to that in Guadeloupe and Kenya.

An "actinomyces" was described by Bishop & Fenstermacher (1933) in lesions which were similar to those of bovine tuberculosis. This organism grew aerobically in various media, was partially acid-fast and was pathogenic in guinea pigs, rabbits, cattle and chickens.

Gray (1935) described a Gram-positive, acid-fast

organism isolated from cow's milk which formed long, branching and beaded filaments and which he considered to be related to Actinomyces farcinicus. This organism grew aerobically in ordinary media at temperatures of 23 to 40°C, and was pathogenic in guinea pigs and rabbits. In guinea pigs, death followed three weeks after the intravenous or intraperitoneal injection of cultures, and local abscesses were observed developing at the sites of subcutaneous injection.

Cases of bovine farcy in Colombia were at first thought to be tuberculosis by Luque (1946) since they gave positive reactions to the tuberculin test, and it was not until N. farcinica was isolated from lesions that the true identity of the pathogen was established.

The presence of tangled masses of branching, filamentous organisms was described by Awad & Karib (1958) in stained preparations from bovine farcy lesions in Sudan. These filaments varied in length and width. Filaments in older lesions had a tendency to break down into small rods resembling the tubercle bacillus, and these rods were occasionally swollen at one end. Gram-positive and acid-fast, the organism grew aerobically in liquid and solid media. Intraperitoneal injection of culture material into guinea pigs produced internal lesions and death in 11 to 34 days, whereas only local abscess formation at the site of inoculation resulted from subcutaneous

injection. The authors concluded, that the cultural, morphological and pathogenic characteristics of this organism conformed with those described by Nocard (1888).

Further, Awad (1960) isolated N. farcinica from the udders of two cows which had each shown a purulent mastitis, and also made similar isolations from the testes of three bulls. Again, El-Nasri (1961) isolated Actinomyces farcinicus from four cases of bovine farcy in which open abscesses were concentrated in the neck and shoulder regions.

In their paper on nocardiosis of rabbits and guinea pigs in Guadeloupe, Bonciu, Bonciu & Petrovici (1964) state that N. farcinica occasionally causes a fatal disease in laboratory colonies of these animals. This disease is characterised by the development of subcutaneous abscesses (usually large) with a creamy, purulent content. Histological examination showed a core of necrotic and calcareous material with many polymorphs surrounded by a zone of epithelioid cells, Langerhans' cells, mononuclear and polymorphonuclear cells. An outer fibroblastic reaction was enclosed within a capsule of connective and fibrous tissue. Visceral lesions were similar to these caused by tubercle bacilli. The disease could be reproduced experimentally by administering material isolated from naturally occurring cases.

Mostafa (1967b) examined over 500 smears of pus

during his mycological study on bovine farcy. The stained smears showed tangled masses of thin, closely-interwoven, branching filaments of variable length and width. The organisms were Gram-positive and partially acid-fast. They did not take stain in a uniform manner, but had a beaded appearance with darkly-stained Gram-positive granules scattered irregularly along the more lightly-stained filaments. With Ziehl-Neelsen stain, the filaments were red with deep violet granules. Further, stained preparations from both young and old cultures of N. farcinica showed Gram-positive - or partially acid-fast - refractive, branching filaments which were often broken up into short hyphae, pleomorphic rods and cocci.

The organism grew slowly and aerobically on various liquid and solid media at an optimum temperature of 37°C, with Lowenstein-Jensen medium giving the best results as regards yield and rapidity of growth. Creamy or pale yellow colonies could be observed at the sixth day of incubation, and "cauliflower" colonies were obtained in Lowenstein-Jensen and some other solid media if incubation was continued.

Mostafa (1967c) also studied the pathogenicity of N. farcinica by injecting rabbits, guinea pigs and calves with cultures or with pus collected from natural lesions. Rabbits injected subcutaneously either developed no

reaction or small swellings at the sites of inoculation. Of the rabbits injected intraperitoneally, only one developed internal lesions - i.e. fibrinous shreds in the peritoneal cavity which were composed of degenerate, partially acid-fast organisms. In guinea pigs, subcutaneous injection produced local swellings at the sites of inoculation, and from the pus of which N. farcinica could subsequently be isolated. Intraperitoneal injection of guinea pigs produced abscesses with involvement of the local lymph nodes, and also typical lesions - sometimes of a miliary character - in the internal organs. Smears from lesions - and from cultures derived from these lesions - showed typical N. farcinica filaments.

Subcutaneous inoculation of infective material into calves produced hard, slowly-developing nodules at the sites of inoculation. These nodules tended to coalesce and, later, discharge pus in which N. farcinica could be demonstrated. Intravenous inoculation in calves also produced local abscesses at the sites of inoculation, and there was abscess formation in the lungs and superficial and internal lymph nodes. Orchitis developed in two of these calves. Stained smears of pus from these abscesses revealed polymorphs, macrophages, cell debris and N. farcinica filaments.

Mostafa (1967b) concluded from this comprehensive study that the causal organism of bovine farcy in the

Sudan was N. farcinica and could not be distinguished from the organism described by Nocard (1888). Hence, he suggested that it was misleading to refer to this organism as Actinomyces farcinicus as had been done by Awad & Karib (1958) and El-Nasri (1961).

At this juncture, it is as well to mention that some confusion in the nomenclature and classification of the Actinomycetes existed until Waksman & Henrici (1943) proposed a system based on the presence or absence of mycelia and fragmentation. Thus, the Family Actinomycetaceae should include the true mycelia-producing forms which break down into bacillary or coccoid elements. Two genera belong to this family -

1. The Actinomyces - which include the anaerobic pathogens.
2. The Nocardia - which include the aerobic pathogens.

These proposals of Waksman & Henrici have been adopted in the 7th edition of Bergey's Manual of Bacteriology (1957), and the causal organism of bovine farcy can thus be classified as follows:-

Order:	Actinomycetales	Buchanan, 1917.
Family:	Actinomycetaceae	Buchanan, 1918.
Genus:	Nocardia	Trevisan, 1889.
Species:	farcinica	Trevisan, 1888.

3. The relationship between ticks and bovine farcy

Of the many authors who have commented on the frequent relationship between tick-infestation and bovine farcy, Neumann (1888) was probably the first to consider that ticks may be actual vectors of the disease, and mentioned their introduction to France and Guadeloupe during the importation of Zebu cattle from Senegal. The name given to these ticks by Neumann was merely "tique senegalensis" and, in 1913, Besson considered that bovine farcy was being transmitted by Hyalomma aegyptium. Again, Bernard (1924) stated that the organism of bovine farcy could possibly gain entrance to the host's body by means of tick bites, and pointed out that Martinique was free of both the disease and "tique senegalensis". He was of the opinion that major dissemination of the disease took place when cattle were driven through heavily tick-infested savannah.

In Kenya, the spread of the disease was associated by Daubney (1927) with infestations of A. variegatum, which he found to be heavy in most clinical cases - and particularly so over the sites of lesions. Daubney also suggested that previous authors might well have mis-identified ticks - e.g. was Besson's (1913) H. aegyptium a mistaken identification of an Amblyomma sp. and even possibly of A. variegatum?

However, in Guadeloupe, Xiroudakis (1935) again made

a definite association between bovine farcy and H. aegyptium, though he did not enlarge on the mechanisms of transmission. Again, Mostafa (1962) recorded the belief of Sudanese nomads as being that A. variegatum ("aleraiggit") was responsible for the occurrence of bovine farcy and, in later pathological studies, mentioned that the lesions occur most frequently in the softer parts of the body where infestations of this tick tend to be concentrated - e.g. perineum and groin. Further evidence of the disease being associated with different ticks in different countries was given by Kuseltan (1967), who suggested that "nocardiosis of the skin" in farm and wild animals in the republic of Tadzhick could be related to infestations of Hyalomma asiaticum. Nevertheless, no record could be found in the literature of any work which definitely established that bovine farcy could be disseminated in the manner of a truly tick-borne disease - i.e. that N. farcinica infection could be acquired by ticks while feeding on one host and then passed on to another host when feeding on the following instars.

4. The relationship between Amblyomma variegatum, Hyalomma anatolicum excavatum and other diseases

(i) Amblyomma variegatum

The first definite evidence of A. variegatum acting as a vector for a rickettsial species was given by Daubney

(1930), who successfully transmitted the causal organism of heartwater (Cowdria ruminantium) to susceptible animals with ticks which had previously fed on hosts suffering from the disease. Since then, Henning (1949) has considered that A. variegatum is a significant vector of heartwater in West Africa, and Hoogstraal (1956) makes an unequivocal association of the species with the disease in cattle, sheep and goats. Similarly, Neitz (1956) associated infestations of A. variegatum with heartwater in Kenya and in West and South Africa, and Edelsten (1975) observed that the distributions of the tick and the disease were coincidental in Somalia. Further, Reiss-Gutfreund (1956) infected A. variegatum larvae and nymphs by feeding them on rabbits which had been inoculated with fowl yolk sac material which had been seeded with Rickettsia prowazeki (the cause of typhus fever) isolated from man, domestic animals, lice and ticks. Trans-stadial transmission of the pathogen was then demonstrated by feeding the emerged nymphs and adults on susceptible rabbits. Trans-ovarial transmission was not demonstrated.

In Guadeloupe, Giroud, Capponi, Escude, Fauran & Morel (1966) were able to demonstrate the presence of Rickettsia conori - the causal organism of African tick-bite fever, or "fièvre boutonneuse" - from engorged larvae of A. variegatum. Again, Capponi, Floch, Chambon, Camicas, Carteron & Giroud (1969) isolated a rickettsial

species from A. variegatum larvae by injecting an emulsion of the infected ticks into male guinea pigs and gerbils. These animals developed an orchitis, and a similar strain of Rickettsia was isolated.

Jarvis (1918) appears to have been the first to associate infestations of A. variegatum with bacterial diseases - e.g. epizootic and ulcerative lymphangitis in horses, (caused by Histoplasma farciminosum and Corynebacterium pseudo-tuberculosis), and necrobacillosis in sheep, (caused by Fusiformis necrophorus). However, he based this association merely on the coincidence of the diseases and the supposed vector. Hoogstraal (1956) makes a definite association between bovine lymphangitis and A. variegatum, and considered that a similar association probably exists between the tick and epizootic lymphangitis of horses. In reporting a form of lymphadenitis in sheep and goats in Somalia, Pegram (1973) suggested that the bacteria incriminated in this disease (e.g. Rickettsia sp., Corynebacterium pyogenes, Streptococcus and Staphylococcus sp.) could well be transmitted by A. variegatum.

In 1956, Plowright described a relationship between bovine streptothricosis (Dermatophilus congolense) and A. variegatum and showed experimentally that the disease could be limited by effective tick control. Further studies on this relationship were carried out by Macadam

(1962a) who was able to transmit Dermatophilus congolense infection to rabbits by means of A. variegatum which had previously fed on affected cattle. On bovine ears, which had been swabbed with cultures of D. congolense, persistent and typical lesions were set up at the sites of tick-bites. Macadam (1962b) also reported that, during the course of the studies on the transmission of bovine streptothricosis by A. variegatum, three out of four rabbits died of a septicaemia caused by Pasteurella multocida - thus giving circumstantial evidence of tick transmission of this pathogen. This circumstantial evidence was later supported by the successful transmission of P. multocida from cattle to rabbits by means of A. variegatum - as shown by the recovery and biochemical identification of P. multocida from the heart blood, liver, lungs, spleen and kidneys of these rabbits.

(ii) Hyalomma anatolicum excavatum

In comparison with A. variegatum, there is very little information on the disease relations of H. a. excavatum.

Neitz (1956) stated that adult H. a. excavatum obtained from Iran were shown to transmit Rickettsia bovis. This organism - the cause of bovine rickettsiosis - was transmitted by an undetermined species of Hyalomma which had also been obtained from Iran (Donatien & Lestoquard,

1936). In 1937, these latter authors reported that another batch of an undetermined Hyalomma species from Iran was allowed to feed on an ox in which Eperythrozoon wenyoni, Rickettsia bovis and Theileria dispar were all subsequently demonstrated. The transmission of E. wenyoni must, however, remain in doubt since the parasitaemia could as well have indicated a recrudescence of a previous infection as one that was freshly established.

According to Hoogstraal (1956), H. a. excavatum is commonly found infected with the rickettsiae of Q fever (Coxiella burnetti) in Northern Africa, southern Europe and Uzbekistan. He also emphasised the importance of that tick as vector of cattle theileriosis (Theileria annulata).

It is noted that Hyalomma spp. are the commonest Ixodid ticks infesting domestic livestock in Near and Middle East countries. Collections made in Baghdad (Al-Janabi, 1969) in summer (July to September 1967) and in the following winter (December 1967 to February 1968) from cattle, sheep, goats and camels showed that Hyalomma spp. formed the bulk of tick infestations in both seasons. Further, H. a. excavatum was the second commonest species occurring on cattle in both seasons - and possibly also on sheep and goats, though the preponderance was less clear. Hence, H. a. excavatum has an important potential vector status for any pathogen it is capable of transmitting.

5. The ticks, their identification, distribution, host relationship, biology and ecology

(i) Amblyomma variegatum (Fabricius, 1794)

The A. variegatum ticks used in this work were drawn from the laboratory colony which has been maintained at the Centre for Tropical Veterinary Medicine since 1969. This colony was founded from original stock collected by Dr. D. Branagan from cattle in the Kedong Valley, Kenya. Identification was checked against the descriptions by Robinson (1926) and Hoogstraal (1956), and against type specimens kindly supplied by Miss J. B. Walker.

According to Hoogstraal (1956), A. variegatum is distributed throughout the Ethiopian Faunal Region apart from the northern Sudan, most of Southwest Africa and Mozambique and the whole of the Republic of South Africa. It is also found in the highlands of Southwest Arabia and in Madagascar, and has become established in the Cape Verde Islands and the West Indies by means of importations.

Within this general range, the precise distribution of A. variegatum is clearly limited by water relations. In East Africa, for example, heavy infestations are not commonly encountered in areas with less than 635 mm (25 inches) rainfall (Yeoman & Walker, 1967; Walker, 1974). Wilson (1953) drew attention to the association between A. variegatum and Rhipicephalus appendiculatus Neumann, 1901, in which the distribution of the more drought-

resistant former species contains - and overlaps by a considerable margin - the more confined distribution of the latter. From this has emerged the sound general rule that a resident population of R. appendiculatus is a firm indication of a co-existent resident population of A. variegatum. On the other hand, the reverse is true only if the A. variegatum infestations are consistently very heavy.

The wide host range of A. variegatum has been admirably collated and summarised by Hoogstraal (1956), Yeoman & Walker (1967) and Walker (1974). As Hoogstraal says, A. variegatum is often the commonest species in cattle in these areas where it occurs, but infestations are not as heavy on other domestic animals. Large ungulates are the most important non-domesticated hosts. Nymphs are said to infest small to medium-sized mammals more often than adults, and larvae attack birds and small mammals such as hares and goats. In this respect, it is worth noting the comment by Walker (1974) when referring to the listings by Lewis (1932; 1939) and Theiler (1959) of several birds and mammals as hosts for immature A. variegatum - "the morphological differences between the larvae and nymphae of this species and those of Amblyomma gemma and Amblyomma lepidum have not yet been analysed properly and it is somewhat doubtful whether these identifications are specific".

Even without the influence of specific pathogens, the commonly heavy infestations of A. variegatum can be a substantial hazard to animal health. Tick "worry" by this species is often a debilitating factor in young calves, and local abscess formation is common at the attachment sites of adults - usually involving Staphylococcus spp. Massive larval infestations can be a serious nuisance factor in humans.

In human disease, A. variegatum is known to transmit Rickettsia conori, the causal agent of tick typhus (Heisch, McPhee & Rickman, 1957) and Coxiella burnetti, the causal agent of Q fever (Blanc, Bruneau & Chabaud, 1950). The disease relations with animals have been discussed elsewhere.

A. variegatum is strictly a three-host tick, and the life-cycle periods given by Walker and Lewis (as quoted by Hoogstraal, 1956) compared with Branagan's unpublished data are as follows in Table 1.

With respect to the figures in Table 1, it is noted that Walker and Branagan both fed larvae and nymphs on rabbits, and adults on rams, whereas Lewis (1932) used hares, chickens and sheep as hosts. The pre-feeding periods are arbitrary. As with other Ixodidae, the influence of temperature on the duration of developmental periods is dominant - e.g. Branagan (1970) gives the ranges of larva-nymph and nymph-adult premoulting periods

TABLE 1.

Summaries of life-cycle periods of A. variegatum as quoted by Hoogstraal (1956) compared with Branagan's unpublished data

Life-cycle period	Duration - days		
	Summary 1	Summary 2	Summary 3
Pre-oviposition	12	18 (at 24-27°C)	9-13 (at 25°C)
Oviposition to hatching	53	86	
Larval pre-feeding period	7	7	
Larva feeds	5	7 (5-8)	6-14
Premoulting period	14	22 (at 25-27°C)	16-26 (at 25°C)
Nymphal pre-feeding period	7	7	
Nymph feeds	5	7 (6-13)	5-12
Premoulting period	19	24 (at 24-27°C)	18-25 (at 25°C)
Adult pre-feeding period	7	7	
Adult female feeds	12	10 (14-22)	14-31

Note: Summaries 1 and 2 are these of Walker and Lewis respectively as quoted by Hoogstraal (1956). Summary 3 is Branagan's unpublished data.

at 18°C, as 46 to 67 days and 102 to 120 days. It seems unlikely, therefore, that A. variegatum would be able to complete more than one life-cycle per year except in the very hottest sectors of its distribution, and one suspects that impressions of two or three generations being completed in one year in Kenya and Uganda (e.g. Wilson, 1950; 1953) would have arisen from extended conditions suitable for hatching rather from any acceleration of developmental processes.

The survival of A. variegatum is - as with other Ixodidae - governed by water relations. Lewis (1939) records the longevity of A. variegatum under laboratory conditions as 732 days and, in quoting this, Hoogstraal (1956) queries the precision of the statement - i.e. did it refer to unfed adults? Branagan (unpublished data) gives the longevity of A. variegatum nymphs and adults at 18°C and 87 per cent relative humidity as - respectively - nine to 12 and 20 to 26 months. Certainly, A. variegatum is not a "dry country" tick, and its longevity will thus be strongly influenced by both general and microclimatic humidity. That this is so is illustrated by the Kenyan distribution of the species as given by Walker (1974) and which shows its virtual confinement to the more humid southwestern quarter of the territory.

Yeoman & Walker (1967) record that the favourite sites of attachment of A. variegatum adults were found to

be ears, neck, head, genitalia, tail, thorax and abdomen.

Hoogstraal (1956) also mentioned that the most common sites of attachment of A. variegatum nymphs and adults were on the udder, scrotum, flanks, dewlap and brisket. However, Wilson (1948) found that larvae fed only on the ears and heads of their hosts.

(ii) Hyalomma anatolicum excavatum (Koch, 1844)

The Hyalomma sp. used in this work was obtained from the Razi Institute, Teheran, as Hyalomma anatolicum and these original samples of adult instars were used to establish a colony of the species at the C.T.V.M. The precise identity of this species was originally questioned by Mrs. Susan Hood (a technician in the Protozoology section at the C.T.V.M.), who considered that the adult stages in the colony conformed to the features described for the sub-species H. anatolicum excavatum by Hoogstraal & Kaiser (1959). The writer was of a similar opinion, but samples were supplied to Dr. J. A. Campbell, Department of Zoology, University of Edinburgh, for a definite opinion. Dr. Campbell's opinion was that the samples did indeed conform to the description of H. a. excavatum, but then raised the question of the validity of this subspecies. His opinion (personal communication) was that H. a. excavatum as supplied from the C.T.V.M. colony conforms to the tick described by Delpy (1949) and

Feldman-Muhsam (1954) as H. excavatum and is sufficiently distinctive to stand on its own as a separate species. In this paper the tick will be referred to as Hyalomma anatolicum excavatum.

According to Hoogstraal (1956), H. a. excavatum is distributed throughout Northern Africa and ranges through the Near East, Southern Russia to India, and is particularly common in Egypt, Palestine, Yemen, Syria, Turkey, Iran and Iraq. It is also found in parts of southern Europe - Spain, France and Portugal. It also occurs in the Sudan, Kenya, Ethiopia and Eritrea.

The host relationship of this tick was summarised by Hoogstraal (1956). He considered that it is principally a parasite of large and medium-sized domestic animals but would also infest dogs and human beings. Hares appear to be an important wild host.

Larvae and nymphs frequently attack calves, but they were also found to parasitize rodents, lizards and gerbils in the desert. Delpy (1949) considered birds to be an important host for nymphs.

Serdyukova (1946a) considered H. a. excavatum as a three-host tick but Lototsky & Pokrovsky (1946) described it as a two-host tick. Serdyukova (1946a) observed that these ticks feed only during the warm periods of the year (July to August). He studied the effect of host size on the life-cycle of this tick. When larvae were fed on

rabbits (an unusual host for this tick), some of them had detached as engorged larva while others moulted on the animal. Some of the resulting nymphs wandered on the host without feeding but others attached, engorged and dropped off as replete nymphs. On the other hand, when he applied larvae on calf ears, they all engorged and detached as replete larvae. No engorged or moulting larvae or larval exuvia were observed on these hosts. Tick collections made in the calf shed showed freshly engorged and moulting larvae and unfed nymphs. Therefore he concluded that H. a. excavatum develops as a three-host tick when feeding on usual hosts but could adapt this behaviour to a two-host cycle when feeding on unusual or unsuitable hosts.

Feldman-Muhsam (1948) observed that some larvae may remain on the host throughout the nymphal stage. Delpy (1946) thought that, if they do so, they first detach and wander to another place, moult and then reattach. The life-cycle of this tick is summarised in Table 2.

Parthenogenesis of H. a. excavatum was observed by Pervomaisky (1949), i.e. a few F_1 females of the tick were reared from batches of eggs laid by some females in the absence of males. Feeding behaviour of this tick has been studied by Pavlovsky, Pervomaisky & Chagin (1954). They observed that the feeding of a large number of ticks on a small restricted area of the host skin resulted in

TABLE 2.

Summaries of life-cycle periods of H. a. excavatum as quoted by Hoogstraal (1956)

Life-cycle period	Duration - days	
	Summary 1	Summary 2
Pre-oviposition	7 - 9	-
Oviposition	15 - 19	-
Eggs hatch	23 - 26	-
Larva feeds	2 - 4	4 - 6 (30°C and 75% r.h.)
Larva moults to nymph	6 - 12	6 (30°C) 4 - 5 (38°C) 30 - 50 (17°C)
Nymph feeds	4½ - 6	9 - 12
Nymph moults to adult	12 - 20	12 - 20 11 - 24 (35°C)

Note: Summary 1 and Summary 2 are these of Serdyukova and Feldman-Muhsam respectively as quoted by Hoogstraal (1956).

poor engorgement or even death of the ticks. This fact is generally applicable to most ixodid ticks, since similar observations on the feeding behaviour of R. appendiculatus were made by Branagan (1974). Branagan described some factors affecting the feeding mechanism and engorgement of ticks. Some of these factors were due to the tick, e.g. prolonged storage of ticks resulted in reduction in vigour, and low environmental temperature could prolong engorgement. Other factors were originated in the host, such as the responses by rabbits in which resistance has been induced by successive infestations. Pomeranzev, Matikashvily & Lototsky (1940) described H. a. excavatum to be a species adapted for survival in desert, semi-desert and steppe. It does not prefer forested areas. These findings agree with Hoogstraal (1956) in Anatolia. Hoogstraal (1956) recorded that the favourite sites of attachment of H. a. excavatum were found to be the scrotum, perineum and in the inguinal and axillary areas. Nymphs generally feed on the neck.

CHAPTER II.

MATERIALS AND METHODS

1. The pathogen

Origin

The original cultures of *Nocardia farcinica* were obtained from the Medical School, University of Glasgow, through the kindness of Dr. John Gordon. These original cultures were obtained in Lowenstein-Jensen (LJ) medium.

Dr. Gordon was at this time engaged in studies on the criteria by which *Nocardia* and associated organisms are classified and, in turn, had obtained his original samples of *N. farcinica* as No. 4524 of the National Collection of Type Cultures, Public Health Laboratory Service Board, 24 Park Crescent, London W.1 (NCTC 4524).

A working stock was first built up by subculturing the original samples from Glasgow into LJ medium. These subcultures were incubated at 37°C for seven days before storing at 4°C. Throughout this work, the stock of *N. farcinica* was thus stored in LJ medium at 4°C.

Maintenance in cultures

The LJ medium was supplied ready for use in bijou vials by Messrs Oxoid, London.

Various solid and liquid media were used for the different requirements of the work. Blood agar was used

in the preliminary studies on cultural characteristics, and also in cases where contamination with another organism was suspected. It was prepared each month in the laboratory.

Tryptose phosphate broth (TPB) was prepared in batches as and when required. In universal vials, it was used for the isolation of the pathogen from blood samples and from tick material. It was also used for the preparation of inocula and N. farcinica extract. On occasions, glycerine was added to TPB to enhance growth, and is referred to as TPB + glycerine in the text.

Prepared in the laboratory, nutrient agar was used for studies on morphological and cultural characteristics. Sabouraud's agar and broth were similarly prepared, and were used for the same purposes.

The methods by which these media were prepared are given in Appendix II.

Staining of smears and histological sections

Smears from cultures and lesions were air-dried, fixed with heat and then stained by Gram's and Ziehl-Neelsen's methods. Tissues for histological examination were fixed in buffered formol saline for about 48 hours and were then prepared in an automatic tissue processor (Histokinette, by Messrs Elliott, Liverpool). The resulting sections were then stained with haematoxylin-eosin and by Gram's.

These staining methods were broadly used according to Cruickshank (1969) and Drury & Wallington (1967). The exact procedures used throughout this work are, however, described fully in Appendix I. The treatments carried out by the automatic tissue processor are also given in Appendix I.

Preparation of inocula

Original infections were conveyed to rabbits and guinea pigs by means of inocula prepared from TPB cultures. These cultures had been incubated for seven days at 37°C after being seeded from stock cultures in LJ medium. They were injected as the crude culture - 1 ml intravenously in the marginal ear vein of rabbits, 1 ml subcutaneously for rabbits and guinea pigs, and 0·1 ml intradermally for rabbits and guinea pigs.

Inocula from tick material were prepared, first, by cleansing whole ticks in 70 per cent ethyl alcohol for 30 minutes; second, by grinding the cleansed ticks in a mortar and pestle with 20 ml of TPB to which 0·1 ml of a penicillin-streptomycin solution had been added; third, by straining the whole through four layers of surgical gauze (BPC standard) and incubating for one hour at 37°C; and, fourth, by centrifuging the whole at 3,000 rpm for 15 minutes and discarding the supernatant fluid. Inocula for two animals were then prepared from the sediment by adding 2 ml of TPB as a vehicle for injection,

and then dividing the whole into two lots of 1 ml each.

Other possible modes of infection

Ten day cultured organisms of N. farcinica were applied to rabbits' ears and guinea pigs' backs after cleaning and sterilizing. Multiple scarification was then made using a sterile disposable lancet - care being taken not to draw blood. Observations were made daily and blood cultures made at intervals through the disappearance of the scabs until the 36th day.

Isolating the pathogen

The presence of N. farcinica in the circulation was tested by withdrawing 1 ml of whole blood, seeding this into 6 ml of TPB, and incubating at 37°C. This incubation of the original isolation was continued for 21 days unless a positive result was obtained sooner with subcultures made on the 7th and 14th days. Each of the subcultures (also in TPB) was incubated at 37°C for a maximum period of eight days. These TPB cultures were examined at intervals for typical surface growth and by means of stained smears. Blood samples were drawn from the marginal ear veins of rabbits with 23 swg x 1 inch needles, and from the hearts of guinea pigs with 18 swg x 1½ inch needles - both with full sterile precautions. Samples were collected at intervals of four, seven, 10, 14, 18, 22, 25, 30, and 36 days following

the application of infected ticks or infection.

The presence of N. farcinica in lesions and pus was tested by seeding suitable samples into TPB and incubating for seven days at 37°C. These cultures were then examined at intervals for typical surface growth and by means of stained smears.

The presence of N. farcinica in ticks was tested by preparing inocula - as described above - for injection into guinea pigs.

Preparation of "nocardin"

Extracts of N. farcinica ("nocardin") were prepared in two ways. In the first method, heavy growths of the organism were scraped off the slopes of LJ medium which had been kept in stock for about one month. These scrapings were seeded into 6 ml of TPB and incubated for 24 hours at 37°C. The resulting culture was then centrifuged for 15 minutes at 3,000 rpm, and the supernatant fluid passed through a Swinnex filter (GSWP 025 00, GS 0·22 u). The filtrate was then used for intradermal injection into guinea pigs and rabbits at 0·1 ml doses using a 26 swg x $\frac{5}{8}$ inch needle.

In the second method, extracts of N. farcinica were prepared from the blood of infected rabbits. Blood was drawn (1 ml) and seeded into 6 ml of TPB and incubated as above. From these cultures, a platinum loop of culture

was then seeded into 6 ml of TPB, incubated for seven days at 37°C, filtered, centrifuged and used as described above.

Collection and checking of sera

Samples of whole blood (3 ml) were collected from infected and control rabbits and guinea pigs, incubated for one hour at 37°C and then transferred to 4°C for two to three hours. These samples were then centrifuged at 2,000 rpm for five to 10 minutes. The sera were then removed and mixed with 6 ml TPB to which had been added a loopful of culture growth from LJ medium. The whole was then incubated at 37°C, and checked at days three, five and seven for the degree of inhibition of growth of N. farcinica which could be attributed to the presence of antibodies in the sera.

2. The ticks

Origin

The ticks were drawn from the colonies of Amblyomma variegatum (Fabricius, 1794) and Hyalomma anatolicum excavatum (Koch, 1844) at the C.T.V.M. The colony of A. variegatum was originally obtained in East Africa in 1967, and had been maintained at the laboratories of the East African Veterinary Research Organization (EAVRO) at Muguga by the methods of Bailey (1960) until 1969 when it

was transferred to the C.T.V.M. Since then, it has been maintained by slightly modified methods (see below). The colony of H. a. excavatum was founded from stock supplied by the Razi Institute, Iran and which had originally been collected in the vicinity of that institute. The colony has since been maintained at the C.T.V.M. by methods similar to those for A. variegatum.

The sources, identification and confirmations of identity of both A. variegatum and H. a. excavatum have been described in the Review of the Literature.

Tick feeding

The methods used at the C.T.V.M. for feeding ticks on rabbits are more or less similar to those described by Bailey (1960) for the feeding of R. appendiculatus. One modification to the method is the use of a "sleeve" instead of an ear-bag. These sleeves are affixed to the rabbits' ears by zinc oxide adhesive plaster in the same way as ear-bags, and are closed at the distal ends by elastic bands, both closed ends being then secured to the top of the leather collar described by Bailey. This use of sleeves greatly eases the daily examination of infestations (see Fig. 1).

Immature stages of both tick species feed readily on rabbits' ears. Both A. variegatum and H. a. excavatum are, however, much larger ticks than R. appendiculatus,

and hence the size of infestations was controlled as described later together with feeding performances.

Adults of both tick species tended to produce substantial host reactions on rabbits' ears - local oedema and distinct malaise being quite frequent. When these reactions were severe, the infestation was terminated, for, apart from humanitarian aspects, the feeding performance of the attached ticks could not be considered as being normal.

As a check on the normality of feeding performance, ticks were weighed on an Oertling substitution balance (Model R.20) immediately after completing engorgement. Larvae and nymphs were weighed in randomly selected samples of 100 and 10 respectively, and females individually.

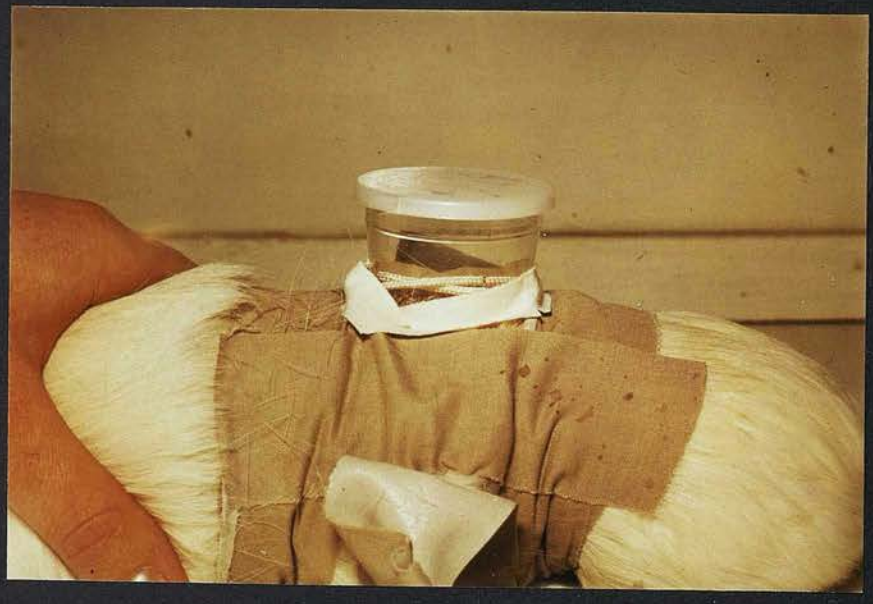
Ticks were applied to guinea pigs within a contrivance made from a wide-necked, screw-topped plastic bottle cut off at the "shoulders". This was held in place over a clipped area of a guinea pig's back by adhesive tape across the "shoulder" part of the bottle, and the ticks placed within the bottle neck and retained there by the screw-cap which had a gauze-covered hole in it (see Fig. 2).

Incubation and storage of ticks

Ticks were contained in a variety of flat-bottomed glass vials stoppered with gauze-covered cotton wool.

FIG. 1. Tick feeding on a rabbit's ear showing partially engorged one female and two male A. variegatum.

FIG. 2. Tick feeding on a guinea pig.



Egg batches were divided into two or three equal parts at the completion of oviposition, and each of these parts was placed in a 50 x 10 mm vial. This size was convenient for placing directly within an ear-sleeve when the larvae had emerged and were ready to feed. Engorged larvae were placed in batches of 50 to 100 in 50 x 10 mm vials, or in batches of 100 to 200 in 50 x 20 mm vials. This, again, was convenient for direct placement within ear-sleeves. Engorged nymphs were placed in batches of up to 100 in 75 x 25 mm vials. The emerged adults were counted out of these 75 x 25 mm vials into one or two 50 x 10 mm vials for application to the ear of a rabbit. Engorged females of both species were contained singly in 50 x 35 mm vials which were "floored" with filter paper and which had a strip of filter paper lining the lower 2 cm of the inside wall of the vial (see Figs. 3, 4 and 5).

These vials were all kept in large screw-topped jars of the type used for confectionary display, and had inside dimensions of 10 x 10 cm by 12 cm high. Within these closed jars, the vials were kept in an atmosphere in which relative humidity (r.h.) was sustained at a constant 85 to 87 per cent by means of about 2 cms depth of a saturated solution of potassium chloride - the vials being raised clear of this solution by standing them on a platform of vinyl flooring material resting on three

FIG. 3. Engorged female A. variegatum.

Scale division = 1 mm.

FIG. 4. A. variegatum during oviposition.

FIG. 5. A. variegatum during eclosion.

FIG. 6. Incubation jar containing A. variegatum in labelled tubes over saturated KCL solution to maintain relative humidity at 85 - 87%.


27/3
3371

horizontal 75 x 25 mm vials (see Fig. 6). This method of maintaining constant relative humidity was recommended by Buxton & Mellanby (1934), and has two advantages in that, first, it does not give off toxic vapours as does, for instance, various dilutions of sulphuric acid; and, second, it can be seen at a glance if the saturated solution is in the required condition (a hydrometer is necessary to gauge the correct concentrations of glycerol when these are used to control humidity).

All developmental stages were incubated at a constant 25°C until hatching or moulting had been completed. Thereafter, the emerged instars were stored at 18°C. Hence, relative humidities within the closed containers were 85 per cent during development, and 87 per cent during storage.

Ticks were handled with a No. 6 sable hair water colour brush, and miniature spatulae were used for dividing and moving egg batches.

Examination of ticks for the presence of infection

All larvae, nymphs and adults were washed thoroughly for at least 30 minutes in 70 per cent ethyl alcohol before being prepared in a variety of ways for examination for the presence of N. farcinica.

Engorged larvae were squashed on slides and the material spread immediately to avoid the opacity which

would result if the blood, haemolymph, etc. were allowed to congeal. These spread preparations were then fixed by heat, stained by Gram and Ziehl-Neelsen methods and examined for the presence of N. farcinica.

Engorged larvae, unfed and engorged nymphs, and unfed and engorged adults were ground up singly in a pestle and mortar, and the material cultured in TPB and on blood agar at 37°C for seven days. These cultures were checked for evidence of N. farcinica as shown by typical growth characteristics and in stained smears of culture material. Further, individual adults were secured dorsum-uppermost in paraffin wax, covered with sterile phosphate-buffered saline (PBS), and the tick's scutum removed by means of a scalpel (No. 11 Swann Morton blade). Intestine and salivary glands were then removed by means of watchmakers' forceps for separate culture in TPB. Again, material for culture was drawn from engorged females by syringe and needle.

Eggs were examined for the presence of N. farcinica by grinding in a pestle and mortar with 20 ml TPB to which 0·1 ml penicillin solution had been added. The resulting fluid was then seeded into various media, and checked as before by examining for typical growth and by means of stained smears. Additionally, small portions of egg batches were crushed on occasions between two slides and then stained by Gram, Ziehl-Neelsen and

haematoxylin-eosin.

3. Experimental animals

All rabbits and guinea pigs used in this work were supplied by the Edinburgh University's Centre for Laboratory Animals, Easter Bush, Roslin, Midlothian.

The rabbits were usually Porton or Porton-Chinchilla crosses, and occasionally New Zealand White. Of either sex, all rabbits were at least four months old (younger rabbits tend to become unduly upset by the trappings of tick-feeding) and had not been used previously as hosts for ticks or for any experimental purpose.

Guinea pigs were of either sex, were at least 250 g in weight and were up to nine months old when used.

The rabbits were kept singly in cages measuring 22 x 12 x 18 ins and fitted with slatted floors. These cages were set over drop trays filled with peat moss. Guinea pigs were kept in pairs in polypropylene cages measuring 22 x 7 x 14 ins with standard stainless steel wire lid. Both rabbits and guinea pigs were fed on compressed pellet diets (SG.11, by Messrs Oxoid) supplemented with hay. Ascorbic acid was added to the drinking water in the guinea pig cages. The room temperature in both cases was maintained at 18°C.

Rabbits were chosen as principal hosts in transmission experiments in view of their outstanding merits for

tick feeding. Since, however, they were reputed to be somewhat unsatisfactory subjects for N. farcinica, immunosuppressants were used in the initial experiments with a view, first, to raising the levels of pathogen in the blood meal of ticks and, second, to giving emphasis to the pathogen's presence in rabbits' blood followed attempted transmission by ticks.

In some experiments, guinea pigs were used for routine checking for the presence of the pathogen since this species is known to be particularly susceptible to N. farcinica infection.

The marginal ear veins of rabbits were used for the withdrawal of blood samples and also as a route for intravenous inoculation. With two rabbits, heavy tick infestations prevented the collection of blood from marginal ear veins with the requisite degree of sterility. In these cases, the samples were withdrawn by heart puncture.

Since blood samples were drawn from guinea pigs by heart puncture, collections were made less frequently than with rabbits, i.e. on days seven, 14, 21 and 28.

4. Miscellaneous

Weighing

All weighings of ticks were carried out with a substitution balance (Oertling R.20, by Oertling Ltd.,

Orpington) which has a sensitivity of 0·1 mg with an accuracy of ±0·05 mg. Adult ticks were weighed singly; nymphs in groups of 10 or 20; and larvae in groups of 100. These weighings were carried out immediately after the ticks had completed engorgement as a check on the normality of feeding performance - i.e. substandard engorged weights indicated an inadequate feeding performance and thus a reduced intake of blood.

Incubators and refrigerators

Three incubators were used throughout this work. One was used for cultures of N. farcinica being incubated at 37°C, and a second was used for the incubation of all developing stages of ticks at 25°C. Both of these incubators were water-jacketed models by LTE, of Oldham.

Emerged ticks were stored at 18°C in a cooled incubator by Gallenkamp (IH-270). Stock cultures of N. farcinica were stored at 4°C in a normal household refrigerator.

These temperatures were checked at periodic intervals by means of independent mercury thermometers.

Maintenance of humidity

All ticks were kept under conditions of constant humidity sustained within closed culture jars by means of saturated salt solutions as described by Buxton & Mellanby (1934). The level of humidity for all developing and

emerged instars was 85 to 87 per cent relative humidity (r.h.) sustained by saturated solutions of potassium chloride.

In the work concerning the effect of varying levels of humidity, saturated solutions of potassium carbonate were used to sustain a level of 44 per cent relative humidity, and of potassium acetate a level of 18 to 20 per cent relative humidity.

Drugs and chemicals

Two immunosuppressant drugs were used in this work. Cyclophosphamide (Endoxana, by Ward Blenkinsop) was supplied in vials containing 1 g cyclophosphamide B.P. and 0·45 g sodium chloride B.P. Calculated at 72·5 mg per kilo body weight, the correct dose for each animal was dissolved in 2 or 3 ml of sterile distilled water and administered to rabbits by intraperitoneal injection. The other immunosuppressant drug was betamethasone (Betsolan injection, by Glaxo). It was supplied in 50 ml bottles as an aqueous solution, each ml containing 2 mg of betamethasone. It was administered by intraperitoneal injection, at the rate of 1·6 mg per kg body weight.

Antibiotics added to TPB in which whole ticks were being ground were drawn from a stock solution of penicillin and streptomycin at concentrations of 50,000 IU and 50,000 µg respectively per ml. To each 20 ml of TPB,

0.1 ml of this stock solution was added, thus giving a concentration of 250 IU of penicillin and 250 µg of streptomycin per ml of the whole.

Pentobarbitone sodium B. vet. C. was used for the euthanasia of rabbits and guinea pigs. An antiseptic cream (Savlon, by ICI) was used for the treatment of sores caused by tick infestations and - occasionally - by the chafing of head collars. Ethyl alcohol at 70 per cent was used to cleanse ticks before grinding, and to clean skin surfaces before inoculations or blood withdrawals.

CHAPTER III.

PRELIMINARY EXPERIMENTS AND OBSERVATIONS

1. Demonstration of the characteristics of the organism

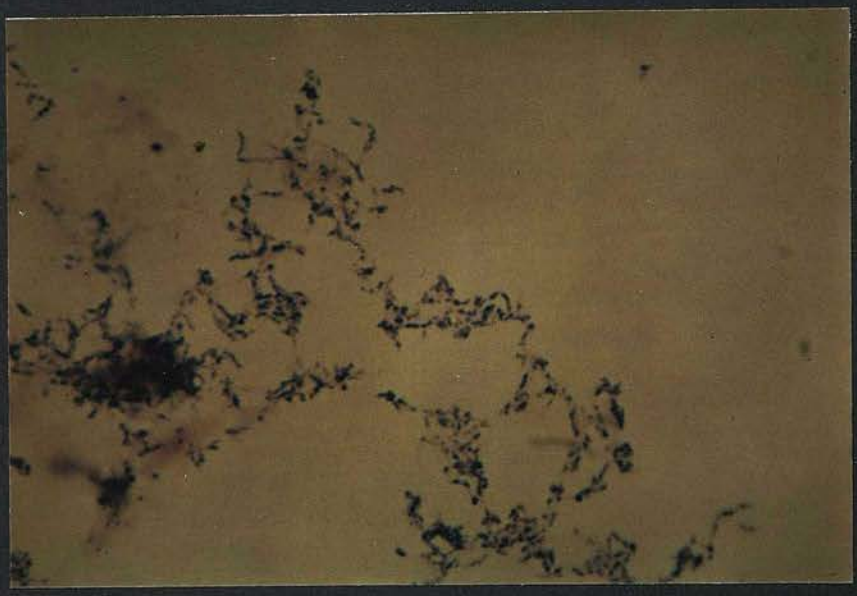
(i) Morphological Characteristics

As seen in stained smears prepared from young or old cultures grown in liquid or solid media, the morphological characters of N. farcinica consist of tangled masses of closely-interwoven, thin, branching filaments which vary in length and width. They are Gram-positive and acid-alcohol-fast. Gram's stain is not taken uniformly - this giving a beaded appearance with darkly stained Gram-positive granules scattered irregularly along the lightly-stained filaments (see Fig. 7). The organism takes modified Ziehl-Neelsen stain in a more even manner showing red-stained filaments with a few deep violet granules (see Fig. 8). In some of the stained preparations, the filaments were found to be broken into short hyphae, rods, bacillary forms and cocci (see Figs. 9 and 10). This phenomenon is clearer in preparations made from old cultures.

The morphology of the organism was also studied in stained smears prepared from blood cultures drawn from infected guinea pigs and rabbits, and also from pus, tissue materials, tick-infected materials, tick salivary

FIG. 7. *N. farcinica* showing beaded appearance. Gram's stain.

FIG. 8. *N. farcinica* showing branching filaments. Ziehl-Neelsen's stain.



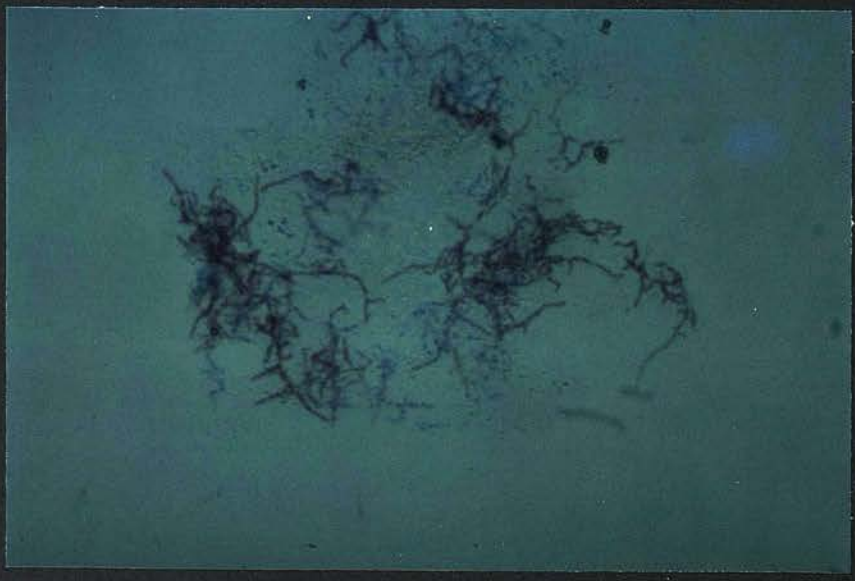


FIG. 9. Broken filaments of N. farcinica showing bacillary and coccoid forms. Beading clearly shown. Gram's stain.

FIG. 10. Old culture of N. farcinica bacillary, coccoid and chained forms. Gram's stain.

glands and tick faeces which were cultured in TPB. Positive preparations revealed the presence of large numbers of tangled masses of the branching filamentous organism, which were again Gram-positive and acid-alcohol-fast. The beaded appearance was also clear and the filaments were arranged singly or in long chains (see Figs. 11, 12, 13 and 14). They were also seen in the broken forms mentioned above.

In some stained preparations made from various cultures or from positive blood cultures, budding was very clear, i.e. there was an enlargement at one end of the hypha while the other end was still attached to the mother filament (see Figs. 15 and 16).

The organism was never positively identified in stained tissue sections, even though positive cultures were prepared from the lesions through which these sections were taken. This was thought to be due to a small number of organisms being present in those lesions.

(ii) Cultural Characteristics

The organism showed optimum aerobic growth at 37°C. No growth was observed in cultures kept at room temperature but they remained viable for the purposes of subculturing for a considerable time (see below).

Cultivation of the organism was tried in different solid and liquid media. Very satisfactory results were

FIG. 11. *N. farcinica* isolated from *A. variegatum* faeces showing bacillary and coccoid forms. Gram's stain.

FIG. 12. *N. farcinica* from cultured *A. variegatum* salivary glands showing long chains and bacillary forms. Gram's stain.

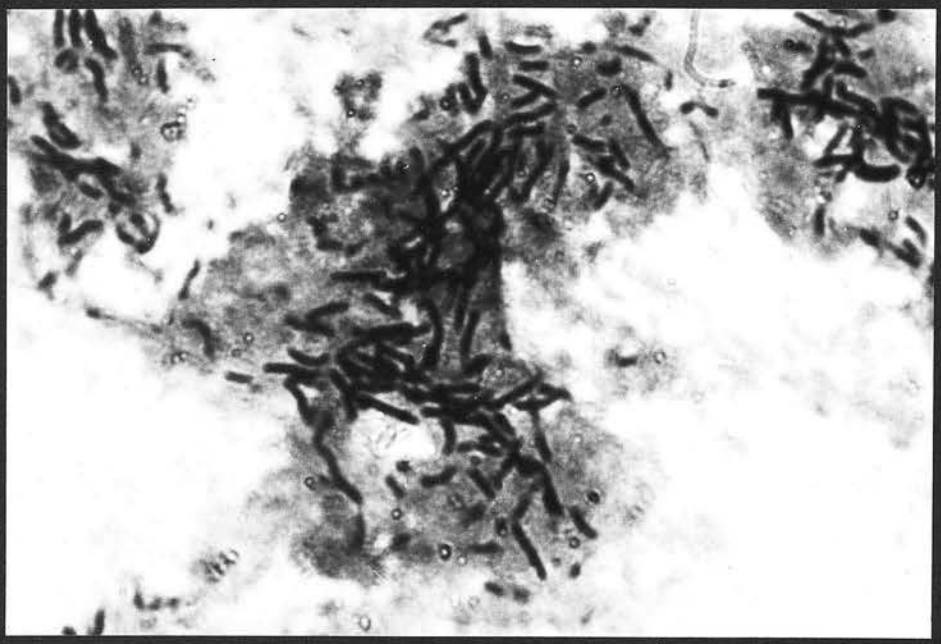


FIG. 13. *N. farcinica* from cultured ground-up *A. variegatum* nymphs showing long chains and broken filaments. Gram's stain.

FIG. 14. *N. farcinica* from cultured infected rabbit blood showing clumped bacillary and coccoid forms. Gram's stain.

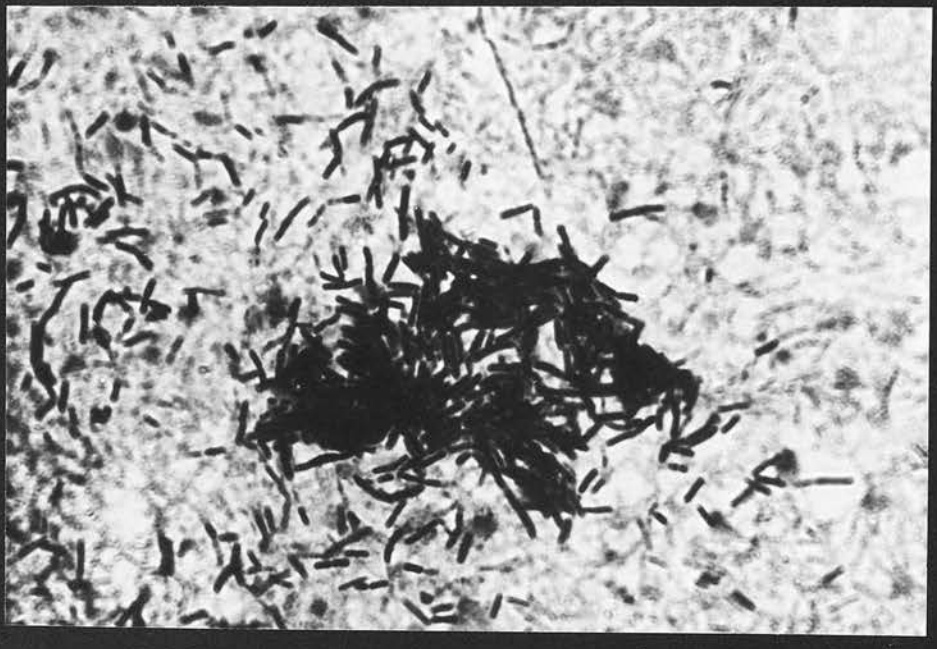
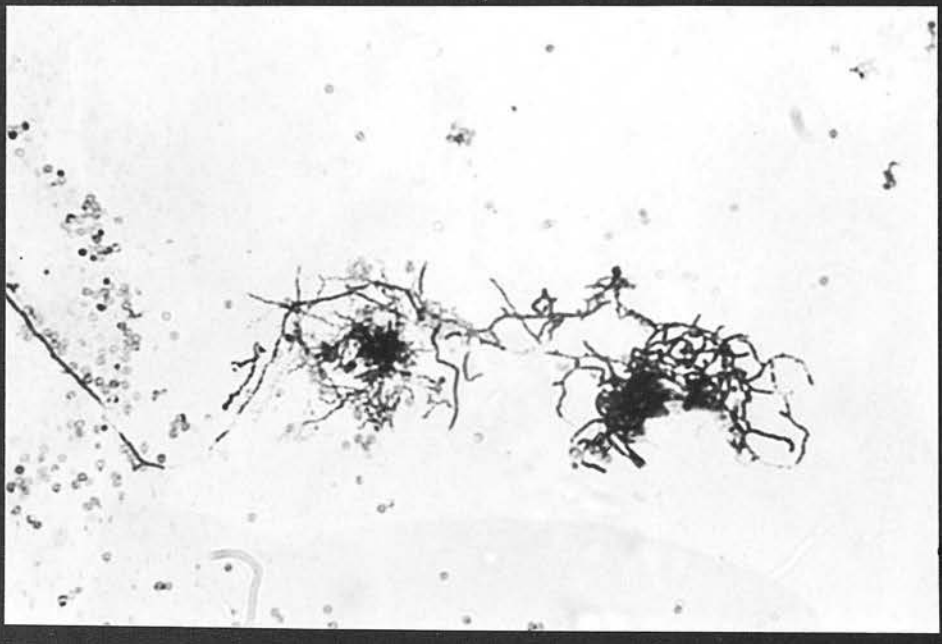


FIG. 15. N. farcinica from cultured infected rabbit blood showing branching filaments with budding.
Gram's stain.

FIG. 16. N. farcinica from cultured infected A. variegatum material showing branching filaments with budding.
Gram's stain.



obtained with tryptose phosphate broth (TPB). The growth was rapid and took place after three to four days of incubation at 37°C. The colonies were small, pellicle-like and yellowish-white in colour. Growth started at the bottom of the universal vials which contained this medium (see Fig. 17). It was observed that the colonies started to grow on the surface of the medium at day 7. At first, these surface colonies were flaky, yellowish-white and coalesced later to form one, thick, wrinkled layer. This kind of growth is termed "surface growth" (see Fig. 18). When pus material or scrapings from the lesions were cultured in this medium, heavy inoculations were usually made since the number of the organisms in such material could be very small. Subcultures into this medium were made from the inoculated blood cultures, at days 7, 14 and 21. Clearly discernible growth was obtained in these subcultures by the end of the second or third day of incubation. Growth was slightly enhanced in this medium by the addition of 1·2 per cent glycerine. Good growth was also obtained in cultures in Sabouraud's and nutrient broths. By the fourth or fifth day of incubation, small yellowish-white colonies were seen at the bottom of the universals. Typical surface growth was also observed when these cultures incubated beyond nine days.

The organism has also been cultivated on Sabouraud's,

FIG. 17. Sixteen-day culture of N. farcinica in TPB showing "bottom growth".

FIG. 18. Twelve-day culture of N. farcinica in nutrient broth showing "surface growth".

nutrient and blood agars. In all these media, clearly discernible growth was observed by the fourth or fifth day of incubation. Colonies were small and yellow to yellowish-brown. Typical cauliflower colonies were seen when these cultures were incubated for 10 days or more, and became larger, opaque and raised from the medium surface with irregular edges (see Figs. 19, 20 and 21).

Blood agar was used always in cases of suspected contamination, but it was usually difficult to isolate N. farcinica in these instances since growth was greatly inhibited by contaminants. The latter grew faster and appeared to starve the slower growing N. farcinica colonies of nourishment.

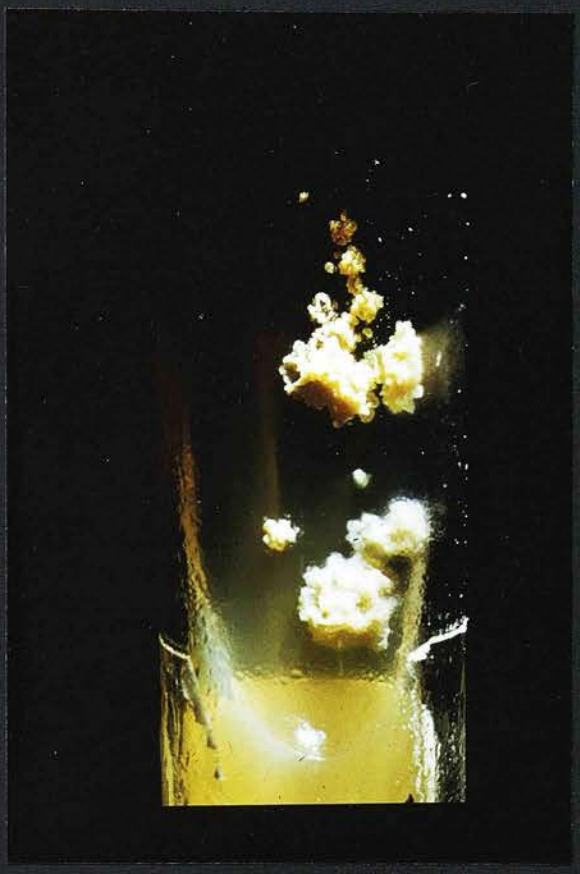
Another medium used was Lowenstein-Jensen's medium. This was found to be superior to all other media as regards yield and rapidity of growth. Usually, the colonies could be seen at the third day of incubation as small, pale yellow nodules. After eight or more days of incubation, these colonies adopted a pronounced "button-shape" which was raised from the medium surface and varied in colour from greyish-white to pale yellow (see Fig. 22).

(iii) Viability and Resistance

As shown by positive results from subcultures, N. farcinica cultures remained viable for two and a half

FIG. 19. Nine-day culture of N. farcinica in Sabouraud's agar showing typical cauliflower colonies.

FIG. 20. Twenty-two-day culture of N. farcinica in nutrient agar showing typical cauliflower colonies.



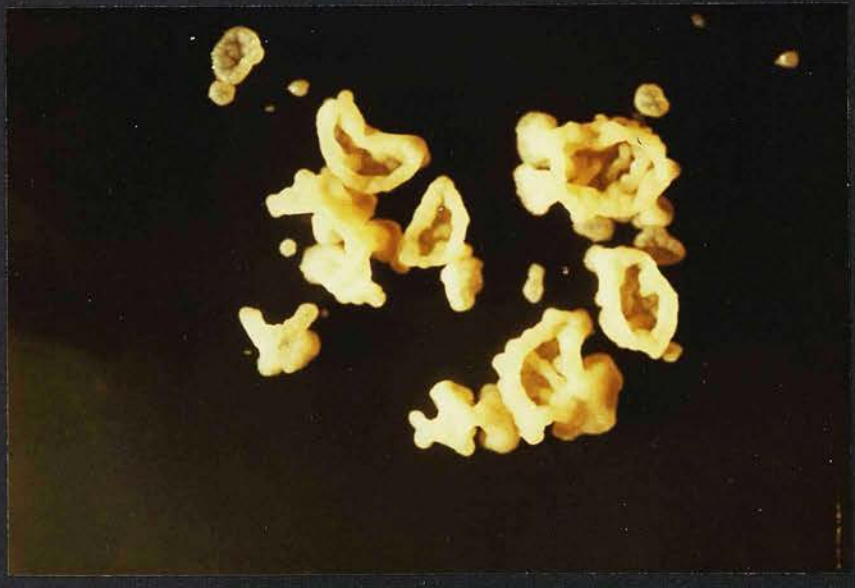
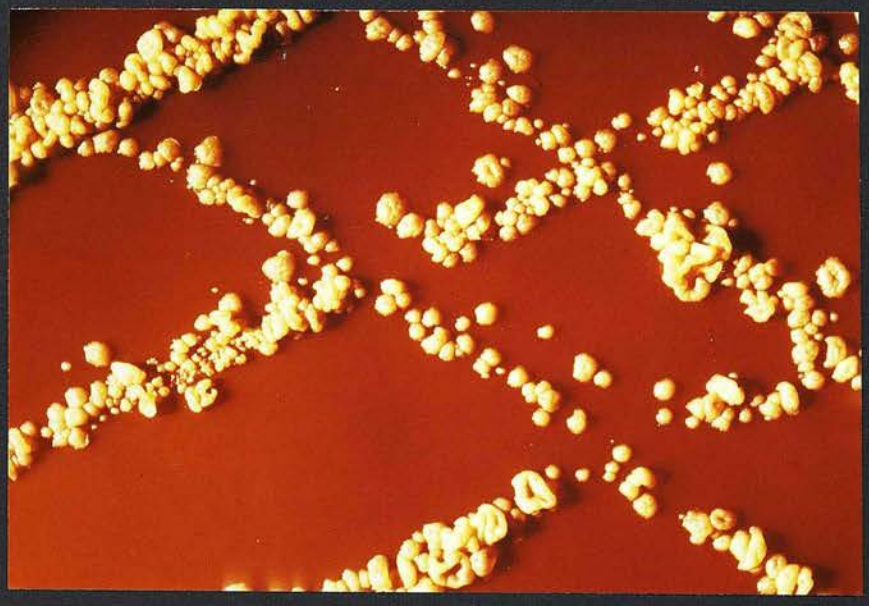


FIG. 21. Twenty-day culture of N. farcinica on blood agar showing typical cauliflower colonies.

FIG. 22. Culture of N. farcinica on Lowenstein-Jensen's medium.

Right - 9 days, showing "button-shaped" colonies.

Left - 145 days, "button-shaped" colonies still visible.



years at 4°C on Lowenstein-Jensen's medium and for approximately eight months at room temperature on both Lowenstein-Jensen's and Sabouraud's media. No growth was apparent in either medium, either at room temperature or 4°C.

Cultures on Lowenstein-Jensen's medium and in TPB remained viable for one to two months at 37°C. When subcultures were made from these into various liquid and solid media, discernible growth was seen on the third day and reached a maximum degree of growth at 10 to 12 days. When cultures of N. farcinica on Sabouraud's agar were kept at room temperature for longer than eight months, the cauliflower-type colonies usually became dry and the tips of the growth turned dark brown. When relative humidity was increased to 85 per cent it was noted that growth on nutrient agar was faster and the number of colonies increased in comparison with cultures kept at lower relative humidities (i.e. 15 per cent and 44 per cent) at the same incubating temperature of 37°C.

Cultures of N. farcinica were readily destroyed by 15 minutes immersion in a 70 per cent or absolute alcohol. Cultures did not survive heating at 70°C for 20 minutes.

(iv) The induction of *Nocardia farcinica* infections in rabbits and guinea pigs

Rabbits

Initially, 1 ml of a 15-day *N. farcinica* culture in TPB was inoculated subcutaneously into one rabbit and 0·2 ml of the same culture into a second rabbit intradermally. No evidence of swelling at the site of inoculation or other reaction was observed in the first rabbit. It was killed after 369 days and no *post-mortem* lesions were observed.

The second rabbit developed an erythematous lesion 2 mm in diameter at the site of inoculation by the third day. This had disappeared by the fifth day. This rabbit died 26 days later. No significant lesions were found at *post-mortem* examination. The cause of death was severe dehydration not associated with the *N. farcinica* infection.

Guinea pigs

As a comparison with the rabbits, two guinea pigs were inoculated in the same manner. The guinea pig receiving the subcutaneous inoculation developed a very hard swelling at the site of inoculation (see Fig. 23). This persisted from the third to the seventh day, when it measured 15 mm in diameter. Thereafter, it diminished in size and finally disappeared around the 16th day.

This animal was killed 197 days later. Post-mortem examination revealed a hard small abscess with caseous material at the site of inoculation. Cultures made from this material in TPB demonstrated the presence of N. farcinica in stained smears.

The second guinea pig developed a pustular lesion at the site of injection on the third day. This lesion erupted on the fifth day, discharging a creamy-coloured pus. Cultures of this material in TPB revealed the presence of N. farcinica in stained smears on the fifth day.

(v) Production of generalized Nocardia farcinica infection in rabbits with or without the use of immunosuppressants

An experiment was set up to study the effect of the organism when injected into the general circulation.

Three rabbits were injected intravenously with 1 ml of 10-day N. farcinica culture in TPB. The first rabbit was treated with cyclophosphamide, commencing on the day of injecting the organism and continuing for four successive days. The second rabbit was treated with Betsolan injectable solution - treatment starting on the day of injecting the organism and being continued for 18 days. The third rabbit was not treated with any immunosuppressants and considered as an infected non-treated control.

Results showed that positive blood cultures were obtainable from the first (cyclophosphamide) and second (Betsolan) rabbits on days 7, 10, 14, 18 and 22, while positive blood cultures could be obtained from the third (non-treated) rabbit on days 10, 14, 18, 22 and 25 only.

Serum from these rabbits was also checked for the presence of antibodies by its degree of inhibition of N. farcinica growth.

Serum from a fourth normal rabbit "non-infected, non-treated", was checked for the same purpose and considered as control. The growth of the organism was checked on the third, fifth and seventh days of incubation at 37°C.

From these results it could be concluded that the bacteraemia in the animals treated with immunosuppressants started earlier than in the non-treated rabbit.

In the serum tests it was very clear that serum from the first (cyclophosphamide) and third (untreated) rabbit caused a remarkable inhibition of growth of N. farcinica and a lesser degree of inhibition was caused by the serum of the second (Betsolan) rabbit. On the other hand, no inhibition at all was caused by the serum from the fourth normal non-infected, non-treated rabbit.

The first rabbit died eight months later. Autopsy revealed the presence of profuse, creamy-white pus in the pleural cavity and a dissemination of the same material in

parts of the lung tissue. This material was of lymphocytic origin and cultures in TPB established the presence of N. farcinica

The second and third rabbits were killed 293 days later and no post-mortem lesions were observed.

2. The feeding performance and developmental periods of Amblyomma variegatum

All developing stages were incubated at 25°C and 85 per cent relative humidity, and all emerged stages were stored at 18°C and 87 per cent relative humidity. Transfer from incubatory to storage conditions was done as soon as hatching or moulting was seen to have been completed. Larvae and nymphs were kept in storage for two to three weeks and adults for at least a month after emergence in order to allow the ticks to "harden" before applying to a host.

The numbers of ticks applied varied considerably according to circumstances, but infestations of 2,000 larvae, or 400 nymphs, or 20 adults on each rabbit ear were regarded as maximal. Exceeding these figures gave inferior results, causing undue damage to the rabbits' ears and thus possibly interfering with the continuation of the feeding process.

With only two exceptions, adult infestations were always made up with equal sex ratios or with a greater

number of males than females. Possibly because of the long engorgement period, replete detachment did not seem to be delayed by a reduced proportion of males.

Larvae almost always attached within 24 hours of application - a few taking as long as 48 hours. The great majority of nymphs attached within the first 48 hours, but adults usually wandered on the ear surface for three to seven days before attaching. Some females were still unattached 18 days after application. It is suggested that this delay in attachment might be due either to the unsuitability of rabbits as hosts for adults, or to applying the adults before they had "hardened" sufficiently.

The great majority of larvae and nymphs attached on the external surface of the ear, with but a few on the inner surface. Adults, on the other hand, attached either to the tip of the ear or at the side of the marginal ear vein (see Fig. 1).

The feeding performances of all three instars is summarised in Table 3, and the distributions of their engorgements is illustrated by histograms in Figs. 24, 25 and 26.

It should be noted that females which did not achieve a minimum degree of repletion have been excluded from the data given in Table 3. This was judged by whether or not repletion was sufficient to allow oviposition, so that all

females which did not lay eggs were regarded as not having completed a normal engorgement. The lowest engorged weight of a female which subsequently laid viable eggs was 0·3985 g (weight of egg batch 0·0830 g).

A check was kept on the duration of developmental periods by recording the commencement of hatching and moulting in each batch of larvae and nymphs; the commencement of oviposition by each female; and the commencement of hatching in each egg batch. Pressure of other work did not allow the progress of these developmental processes to be monitored day by day, and hence a summary of these minimum developmental periods at 25°C is given in Table 4.

Both feeding performance and the duration of developmental periods were used as a check on the normality of the ticks.

The mean weight of egg batches laid by 44 A. variegatum females was 0·9020 g, or 44·53 per cent of their engorged weight.

3. The feeding performance and developmental periods of Hyalomma anatolicum excavatum

The H. a. excavatum colony was maintained in the laboratory in the same way as A. variegatum concerning incubation and storage of developing and emerged stages. Hardening of the emerged stages before applying to a host

was assured by keeping the larvae and nymphs for two to three weeks and the adults for at least four weeks in storage. The number of H. a. excavatum applied was less than that of A. variegatum, i.e. 400 larvae, or 100 nymphs, or 28 adults on each rabbit were the maximum. Larvae always attached within 24 hours of application. The great majority of nymphs attached within the first 24 hours of application - a few taking as long as 48 hours. Adults attached within one to three days of application. The great majority of larvae and nymphs attached on the external surface of the ear with but a few on the inner surface. Adults, on the other hand, attached either to the tip of the ear or at the sides of the marginal ear vein.

H. a. excavatum was seen to behave as either a two or three-host tick, i.e. when larvae were applied, engorged larvae, flat and engorged nymphs were recovered. The feeding performance of these larvae is summarised in Table 5 and the distribution of engorgement is illustrated by histogram in Figs. 27 and 28.

The feeding performance of nymphs when applied as flat nymphs and that of adults is summarised in Tables 6 and 7, and its distribution of engorgement is illustrated by histogram in Figs. 29 and 30.

It should be noted that females which did not achieve a minimum degree of repletion have been excluded from the

data given in Table 7. This was judged by whether or not repletion was sufficient to allow of oviposition, so that all females which did not lay eggs were regarded as not having completed a normal engorgement. The lowest engorged weight of a female which subsequently laid viable eggs was 0·1786 g (weight of egg batch 0·0725 g).

A check was also kept on the duration of developmental periods by recording the commencement of oviposition, hatching and moulting.

A summary of minimum developmental periods at a constant 25°C and 86 per cent relative humidity is given in Table 8. Both feeding performance and the duration of developmental periods were used as a check on the normality of the ticks.

The mean of egg batches laid by 30 H. a. excavatum females was 0·3130 g, or 45·73 per cent of their engorged weight.

4. Experiments on the mechanical transmission of the parasite

It was realised that feeding of non-infected ticks on an animal which might be harbouring the parasite in its skin might be expected to carry the infection into the blood stream. In order to examine this possibility the following experiments were carried out:-

(i) Application of Nocardia farcinica culture on rabbit's skin

The right ear of one rabbit was washed and cleaned with soap and warm water, then swabbed with a culture of N. farcinica in TPB. This ear was then pricked with pins (see Materials and Methods). Swabbing with culture and pricking was repeated three days later. Blood samples were collected from the other ear at various intervals as described previously. None of the blood samples so obtained established the growth of N. farcinica when seeded into culture media and incubated.

A slight congestion of the ear veins and some inflammation at the sites of the pricks persisted from the fifth till the ninth day following the first application of culture.

The negative results from cultures of blood were taken as indicating that generalized infection of N. farcinica did not arise from such minor traumata as pin pricks.

When the rabbit was killed and autopsied 92 days later no gross evidence of any pathological lesions was discerned at autopsy.

(ii) Scarification and application of Nocardia farcinica culture on rabbit's and guinea pig's skin

One rabbit and one guinea pig were used in this

experiment.

The right ear of the rabbit was washed and cleaned with soap and warm water, then swabbed with a culture of N. farcinica in TPB. The swabbed area was then scarified with lancet. After scarification the area was swabbed again with the same culture. There was a slight rise in body temperature (0·1 to 0·4°C) on the second and third day following the application of culture. Scabs formed at the site of scarification and persisted from the fourth day till the 18th day, after which the scabs sloughed and complete healing of the wound took place. Cultures in TPB of scab materials did not establish any growth of N. farcinica but growth of contaminants such as Staphylococcus spp. was obtained. Blood samples were also collected from this rabbit, but none of these established the growth of N. farcinica in TPB cultures.

When the rabbit was killed and autopsied 150 days later, no gross evidence of any pathological lesion was discerned.

The guinea pig was shaved on the back over an area of 1½ inches. After cleaning and washing as before, the shaved area was swabbed with a culture of N. farcinica in TPB. Scarification of the shaved area was then made with lancet and the whole area was swabbed again with the same culture. There was a slight elevation of body temperature (0.1 to 0·5°C) on the second and third day following

scarification.

Scabs formed on the seventh day and persisted until the 17th day. Cultures of the scab material in TPB established no growth of N. farcinica but revealed the presence of Staphylococcus spp.

When this guinea pig was killed and autopsied 197 days later no gross evidence of any pathological lesions was discerned.

(iii) Tick feeding on skin swabbed with Nocardia farcinica culture

The right ear of a rabbit was washed, cleaned and swabbed with a culture of N. farcinica in TPB. Three female and six male adult A. variegatum were applied to each ear. After most of the ticks had attached, the right ear was swabbed again with the same culture. All females from both ears completed their engorgement 18 to 25 days after application. Blood samples from this rabbit were negative for N. farcinica.

All detached ticks were first cleansed externally by washing for 30 minutes in 70 per cent alcohol; then washed in sterile normal saline and ground up in a pestle and mortar. When seeded into TPB and incubated, this macerated material produced no growth of N. farcinica.

When this rabbit was killed and autopsied 135 days later no gross evidence of any pathological lesions was

discerned.

Two guinea pigs were shaved on the back over an area of 1½ inches. Culture of N. farcinica was then painted on the shaved area after cleaning and washing as before. One hundred larvae of H. a. excavatum were fed on the first guinea pig, while on the second guinea pig 10 nymphs and three female and three male adults of the same species were fed (see Materials and Methods).

Only 10 larvae and three nymphs completed engorgement in six to 10 days and six to nine days respectively. All adults did not attach.

Blood samples from both guinea pigs were negative for N. farcinica.

Cultures in TPB were made from the detached ticks, as mentioned above, but none of them produced any growth of N. farcinica.

When these guinea pigs were killed and autopsied 75 days later no gross evidence of any pathological lesions was discerned.

These findings were taken as indicating, first, that a generalized N. farcinica infection did not ensue from a skin contamination gaining entry by way of a tick attachment; and, second, that feeding ticks were unlikely to acquire significant infections from a skin contamination of N. farcinica.

5. The uptake of Nocardia farcinica infection by ticks

It was regarded as important to establish that N. farcinica infection could actually be acquired by ticks during their normal process of feeding on infected animals. Accordingly, A. variegatum larvae and nymphs were fed on the ears of both infected and non-infected rabbits.

Before applying to rabbits, random samples of larvae and nymphs were examined for the presence of N. farcinica by grinding up and then culturing the macerated material in various media. None of the cultures so made showed any sign of N. farcinica growth.

The circumstances of the experiment are set out in Table 9.

That N. farcinica had been acquired by these feeding ticks was examined by culturing ground-up material from these ticks on the day that they detached replete, and on the day that they emerged as the following instars after incubation at 25°C. The results of these cultures are given in Table 10.

These results in Table 10 show clearly that N. farcinica infections were acquired by both larvae and nymphs from infected Rabbits 1, 4 and 7 during normal feeding, and that these infections were then retained by the developing ticks through to the emergence of the following instars. Similar infections were not acquired from the

non-infected control rabbit. The remaining ticks from these fed on Rabbits 1, 4 and 7 were used in the transmission trials in Chapter IV.

TABLE 3.

Summary of feeding performances of A. variegatum on rabbits

Instar	No. of hosts	Nos. applied	Nos. engorged (% of these applied)	Mean engorged weight - gms (SD)	Mean days to engorge	Range of days to engorge (interquartile range)
Larvae	8	10,900	8,202 (79·83%)	0·0031 (0·00014)	8·89	5 - 14 (8·9·10)
Nymphs	12	2,436	1,961 (80·50%)	0·0488 (0·0061)	8·62	6 - 17 (7·8·10)
Adult Females	14	110	60 (54·54%)	1·8962 (0·5261)	23·15	16 - 32 (20·22·26)

(Males and females applied simultaneously in at least equal numbers)

TABLE 4.

Summary of minimum developmental periods of A. variegatum when incubated at a constant 25°C and 86% r.h.

Developmental period	No. of ticks (or egg batches) observed	Duration of period - days	
		Mean	Range
Pre-oviposition	48	10·62	7 - 18
Oviposition - hatching	48	53·66	49 - 62
Moulting -			
larva - nymph	8702	13·14	12 - 15
nymph - adult	1961	16·77	15 - 18

TABLE 5.

Summary of feeding performance of H. anatolicum excavatum larvae on rabbits

No. of hosts	Total number of larvae applied	Detached as engorged larvae			Detached as engorged nymphs		
		Nos. engorged (%)	Mean engorged weight (g) (SD)	Mean days to engorge (range)	Nos. engorged (%)	Mean engorged weight (g) (SD)	Mean days to engorge (range)
4	1,000	243 (24·3)	0·00049 (0·00006)	6·81 (5-11)	213 (21·3)	0·0232 (0·0140)	15·63 (13-18)

TABLE 6.

Summary of feeding performance of *H. anatolicum excavatum* nymphs applied to rabbits as flat nymphs

No. of hosts	Total number of nymphs applied	Nos. engorged (%)	Mean engorged weight (g) (SD)	Mean days to engorge (range)
2	56	29 (51·78)	0·0236 (0·0115)	7·55 (6 - 9)

TABLE 7.

Summary of feeding performance of H. anatolicum excavatum females on rabbits

(Males and females applied simultaneously in equal numbers)

No. of hosts	Nos. applied	Nos. engorged (%)	Mean engorged weight (g) (SD)	Mean days to engorge (range)
4	39	32 (82·05)	0·6842 (0·3282)	11·44 (8 - 16)

TABLE 8.

Summary of minimum developmental periods of *H. anatolicum excavatum* when incubated at a constant 25°C and 86% r.h.

Developmental period	No. of ticks (or egg batches) observed	Duration of period - days	
		Mean	Range
Pre-oviposition	30	6·33	5 - 8
Oviposition - hatching	30	42·16	38 - 49
Moulting -			
larva - nymph	234	8·25	6 - 10
nymph - adult	25	25·00	22 - 28

TABLE 9.

The feeding of A. variegatum larvae and nymphs on rabbits (larvae on left ears, nymphs on the right)

Infected and treated status	No. of larvae (nymphs) applied	No. of larvae (nymphs) engorged	Days to engorge - range	Days to moult at 25°C - range
Rabbit 1				
I/V injection of 1 ml TPB culture of N. farcinica.	1800	1648	6 - 9	13 - 16
Cyclophosphamide treatment.	(400)	(391)	6 - 10	16 - 24
Rabbit 4				
I/V injection of 1 ml TPB culture of N. farcinica.	1500	1293	8 - 15	14 - 16
Betsolan treatment.	(400)	(381)	8 - 15	18 - 23
Rabbit 7				
I/V injection of 1 ml TPB culture of N. farcinica.	1500	1208	6 - 9	13 - 15
No treatment.	(400)	(355)	6 - 9	17 - 24
Control Rabbit				
Non-infected	1500	1046	6 - 10	14 - 16
Non-treated	(400)	(208)	6 - 11	17 - 24

TABLE 10.

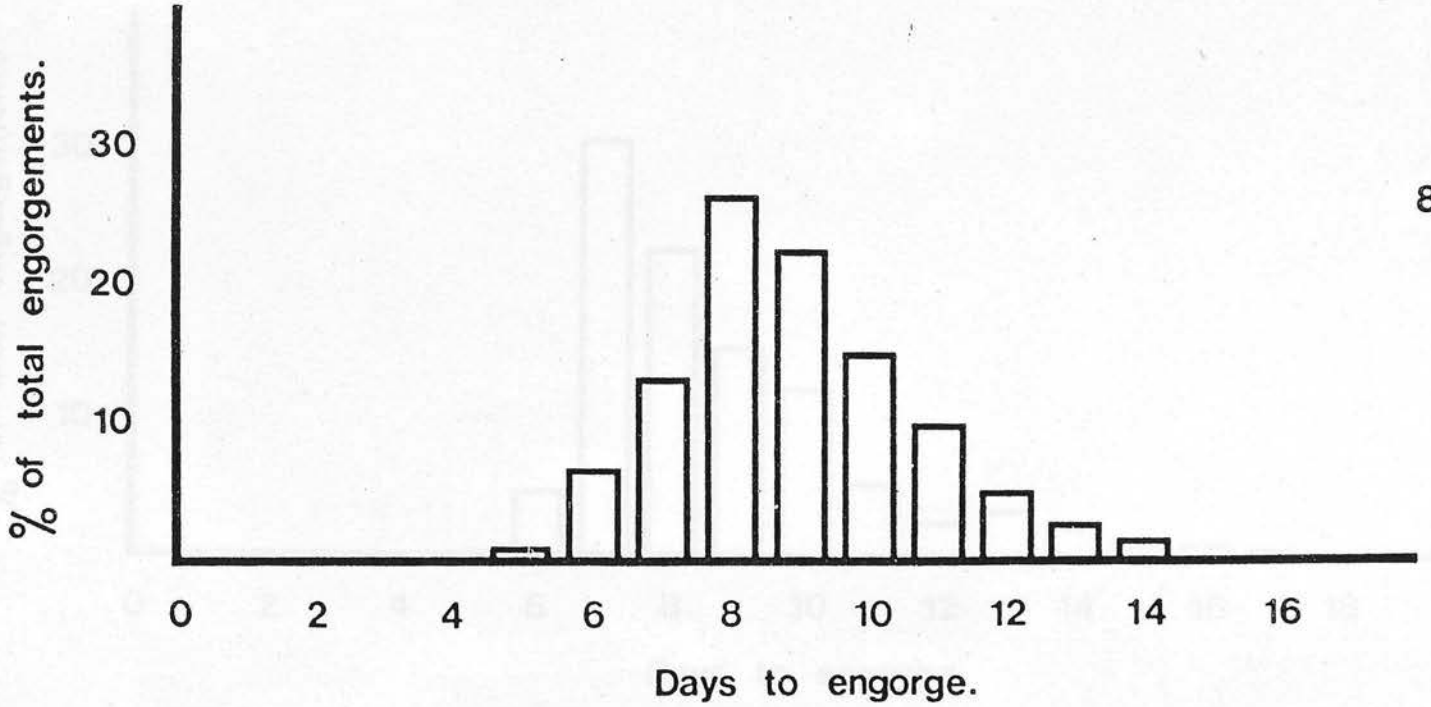
The presence of *N. farcinica* infection in ground-up tick material made on the day of detachment and on the day of emergence as the following instars

Host	No. of freshly-engorged larvae (nymphs) cultured	No. of freshly-engorged larvae (nymphs) giving positive cultures	No. of freshly-moulted nymphs (adults) cultured	No. of freshly-moulted nymphs (adults) giving positive cultures
Rabbit 1	200 (60)	112 (41)	200 (60)	81 (33)
Rabbit 4	200 (60)	118 (47)	200 (60)	93 (36)
Rabbit 7	200 (60)	47 (21)	200 (60)	32 (16)
Control	200 (60)	0 (0)	200 (60)	0 (0)

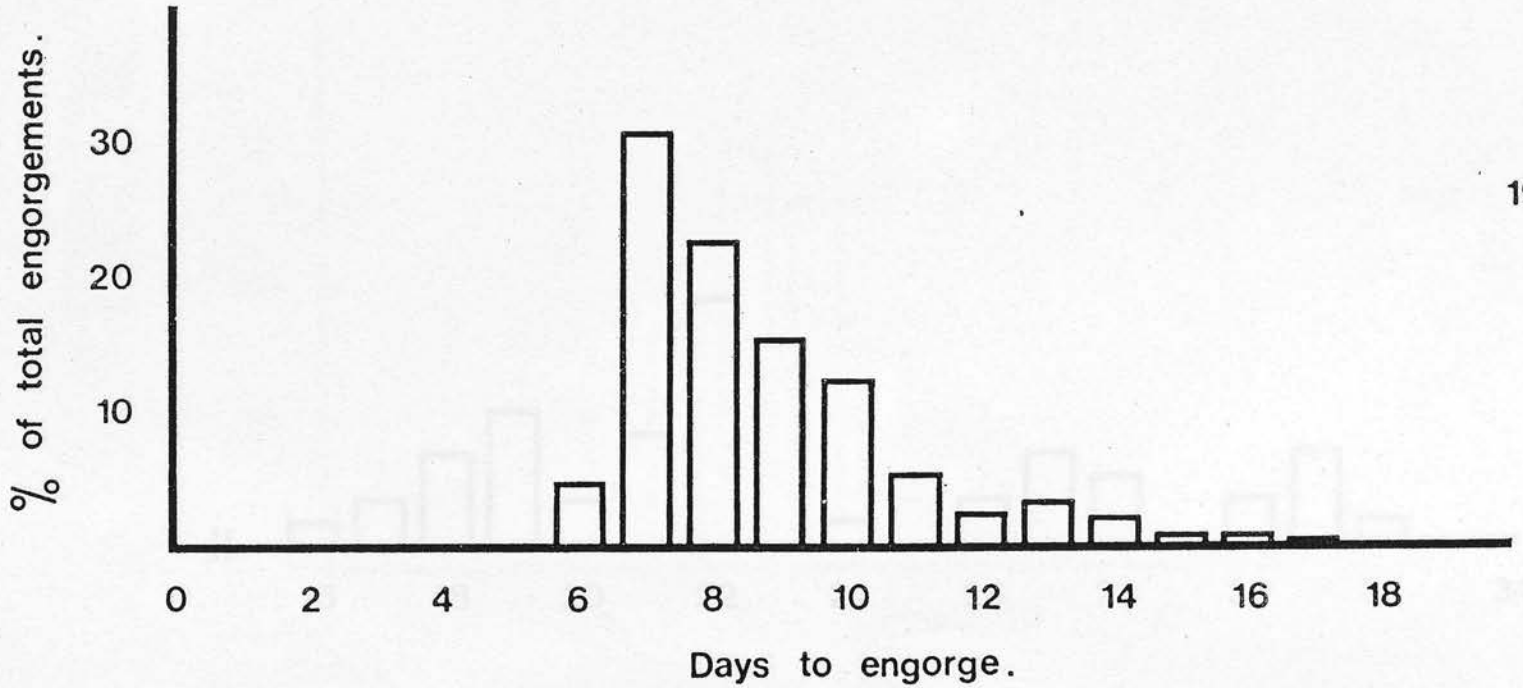
FIG. 24.

Distribution of engorgement of

Amblyomma variegatum larvae on rabbits.



Distribution of engorgement of
A. variegatum nymphs on rabbits.



1961 engorgements
on 12 rabbits.

Distribution of engorgement of
A. variegatum females on rabbits.

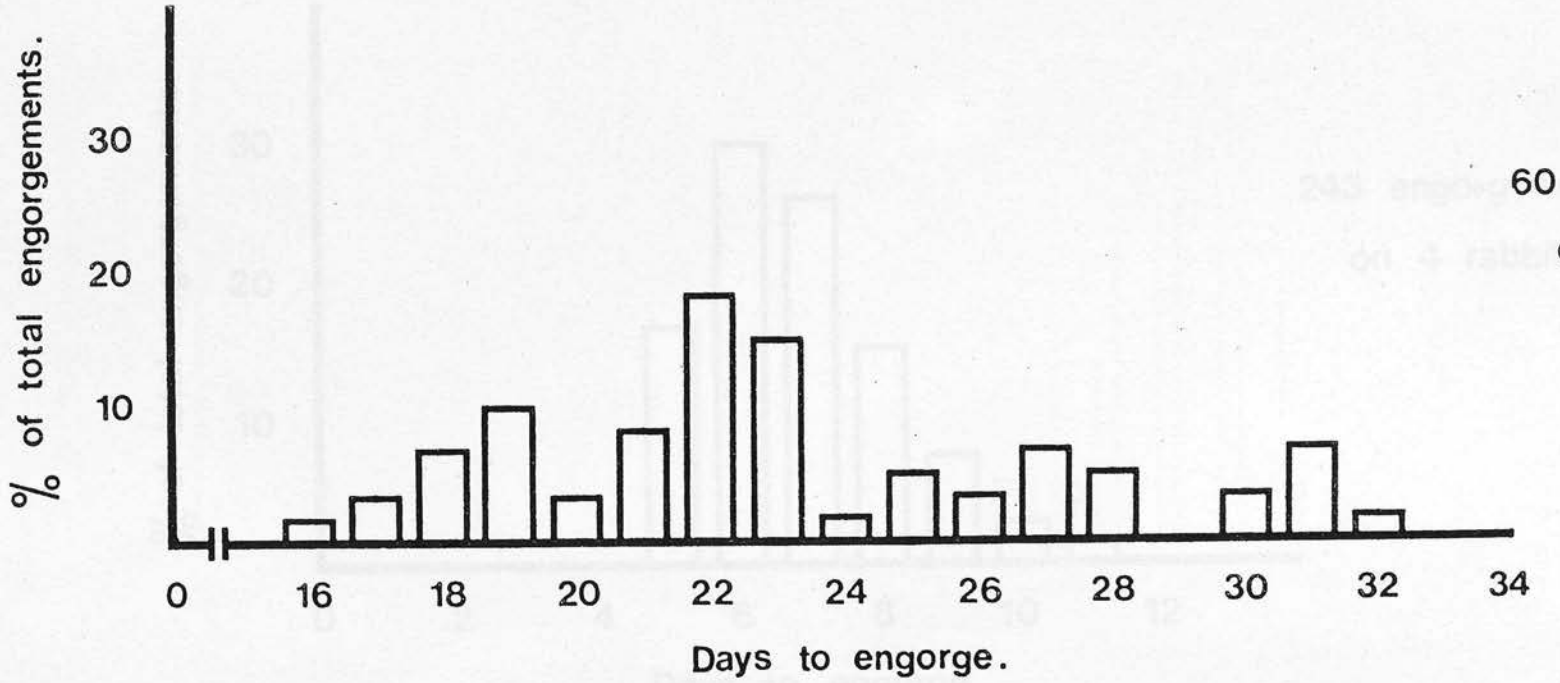


FIG. 27.

Distribution of engorgement of

Hyalomma anatolicum excavatum larvae on rabbits.

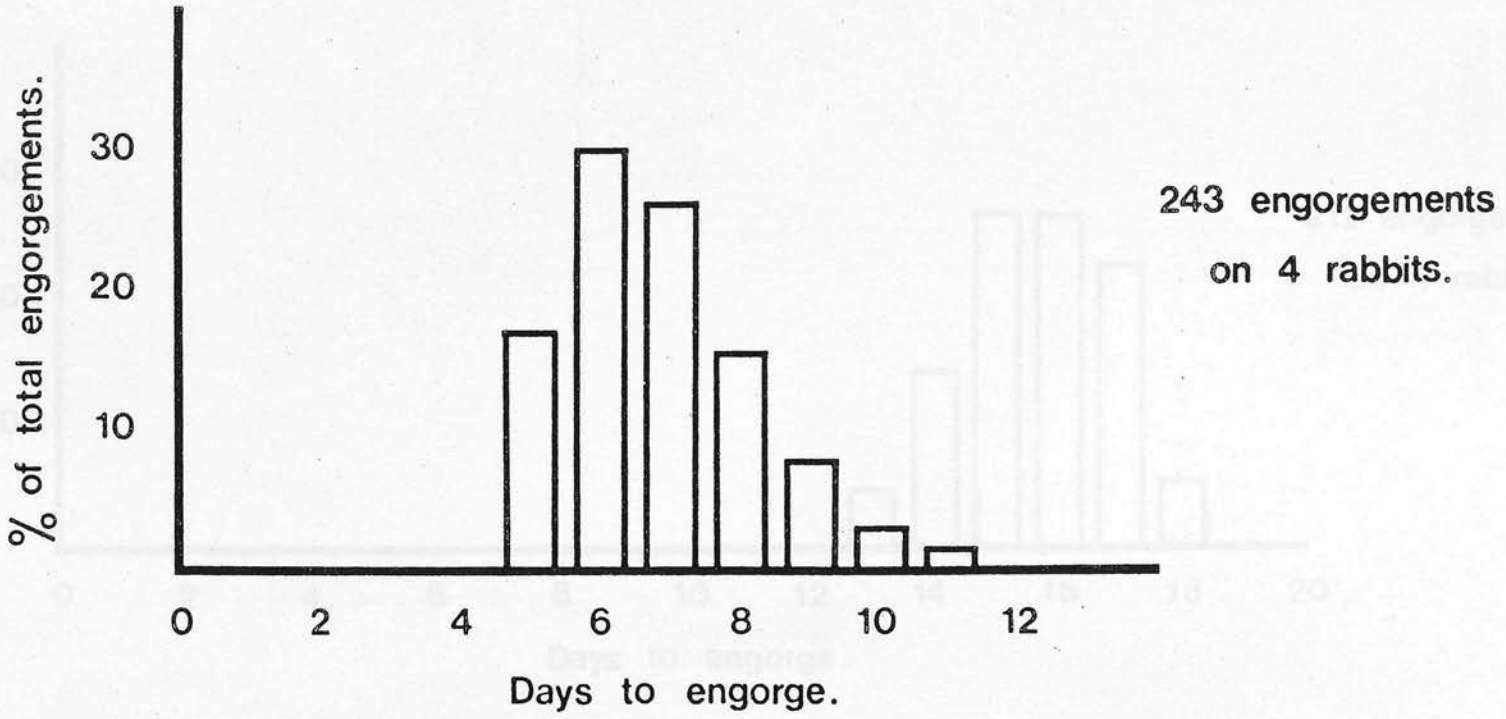


FIG. 28.

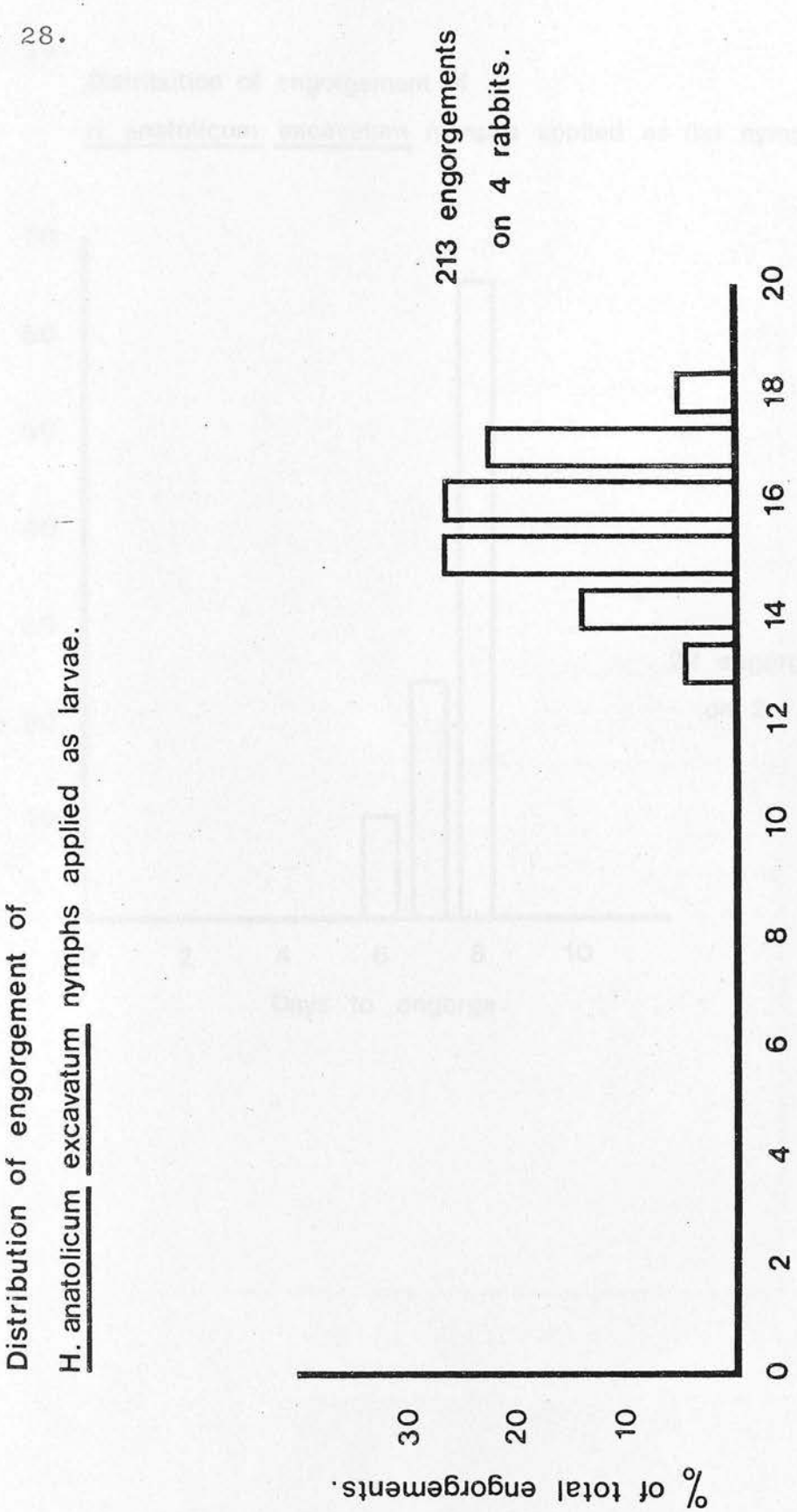

Distribution of engorgement of
H. anatolicum excavatum nymphs applied as larvae.
213 engorgements
on 4 rabbits.
% of total engorgements.
30
20
10
0
2
4
6
8
10
12
14
16
18
20
Days to engorge.

FIG. 29.

Distribution of engorgement of

H. anatolicum excavatum nymphs applied as flat nymphs.

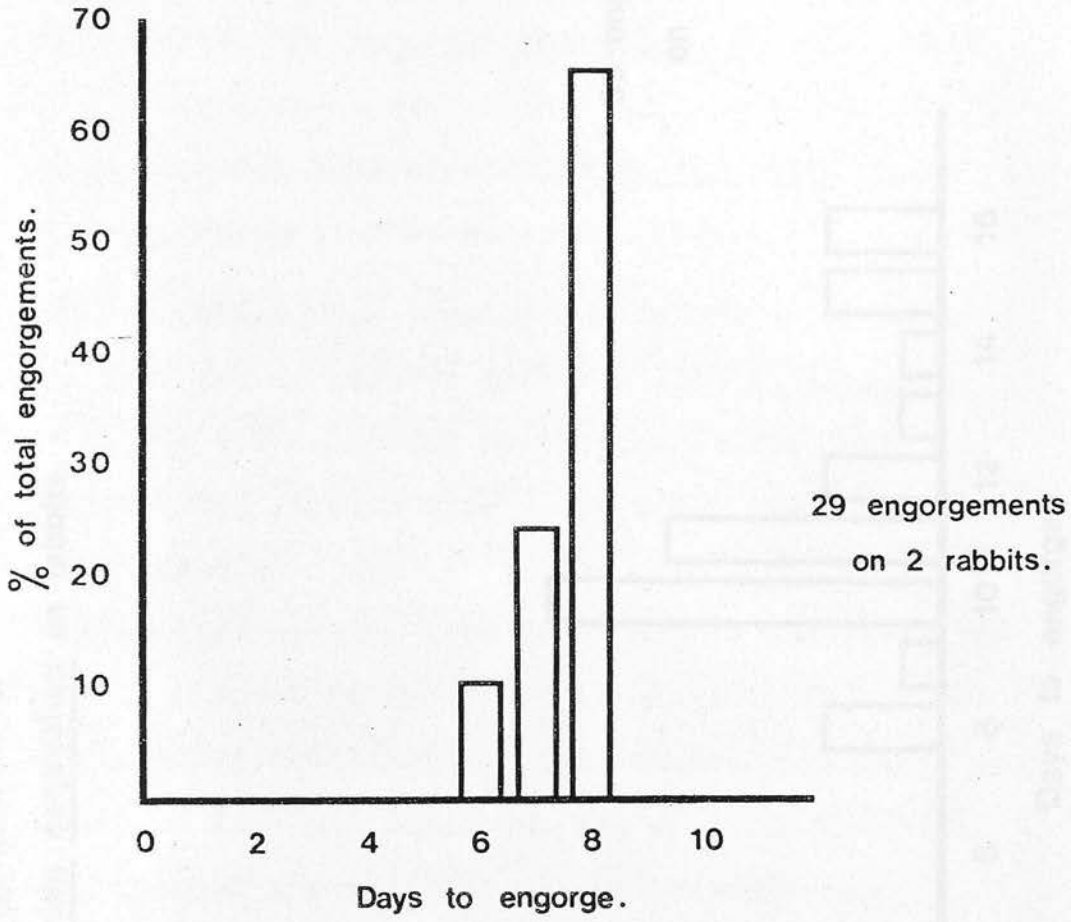
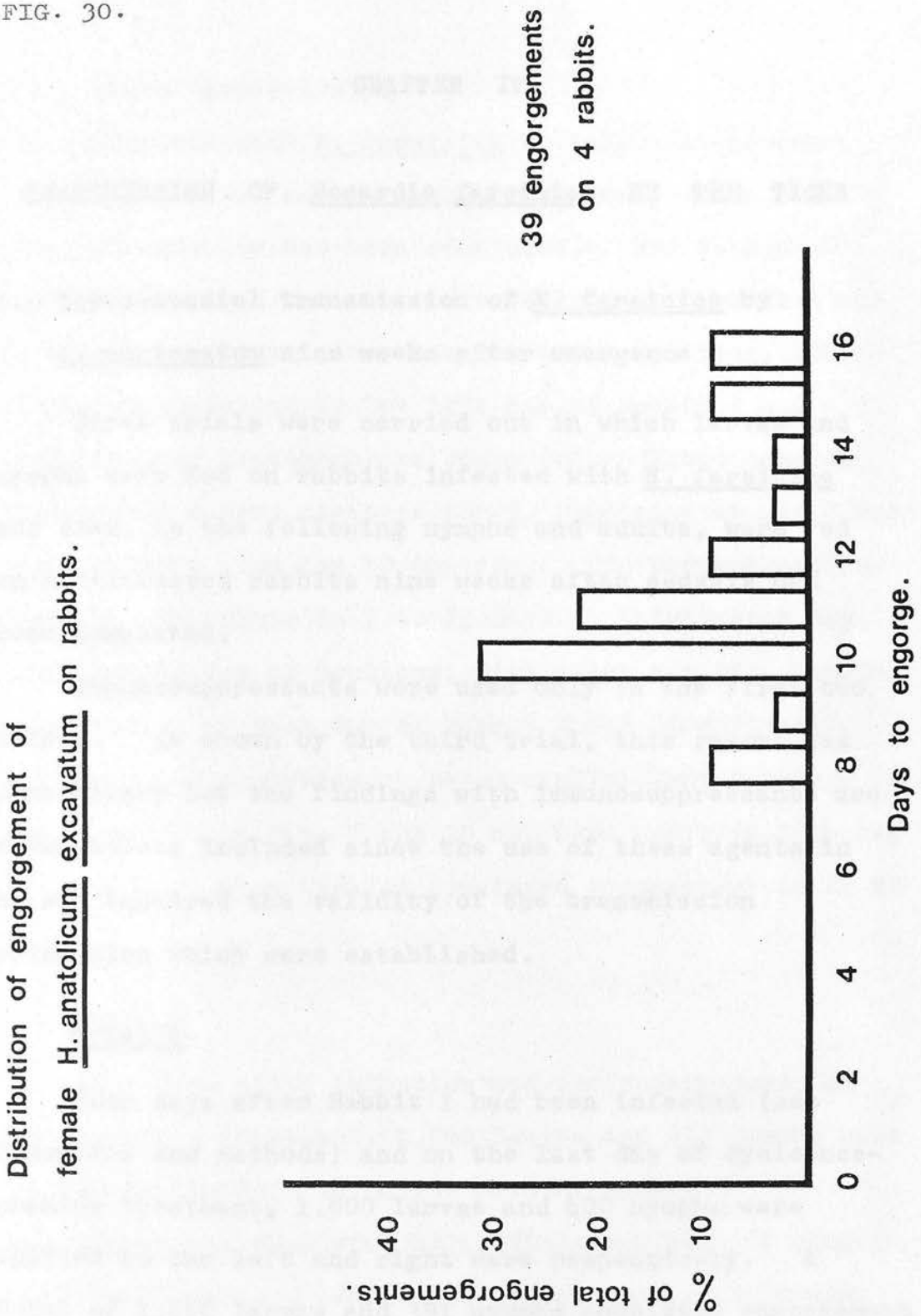


FIG. 30.


Distribution of engorgement of female H. anatolicum excavatum on rabbits.
39 engorgements on 4 rabbits.
% of total engorgements.
40
30
20
10
0
2
4
6
8
10
12
14
16
Days to engorge.

CHAPTER IV.

TRANSMISSION OF *Nocardia farcinica* BY THE TICKS

1. Trans-stadial transmission of *N. farcinica* by *A. variegatum* nine weeks after emergence

Three trials were carried out in which larvae and nymphs were fed on rabbits infected with *N. farcinica* and then, as the following nymphs and adults, were fed on non-infected rabbits nine weeks after ecdysis had been completed.

Immunosuppressants were used only in the first two trials. As shown by the third trial, this resort was unnecessary but the findings with immunosuppressants are nevertheless included since the use of these agents in no way impaired the validity of the transmission principles which were established.

Trial 1

Four days after Rabbit 1 had been infected (see Materials and Methods) and on the last day of cyclophosphamide treatment, 1,800 larvae and 400 nymphs were applied to the left and right ears respectively. A total of 1,648 larvae and 391 nymphs completed engorgement in six to nine days and six to 10 days respectively, and moulted to nymphs and adults in 13 to 16 and 16 to 24

days, again respectively.

Infection with N. farcinica in Rabbit 1 was confirmed in a blood sample taken immediately after the first infestation had been concluded. The emerged instars were used for the transmissions to Rabbits 2 and 3. Forty-one days after completion of moulting, 100 nymphs were applied to the left ear of Rabbit 2 and betamethasone treatment was commenced. Blood samples from Rabbit 2 were negative for N. farcinica at day 7 but were positive from day 10 onwards. Engorgement was completed by 79 nymphs in 7 to 11 days. Thirty-nine days after completion of moulting, five males and five females were applied to each ear of Rabbit 3 and betamethasone treatment was commenced. Blood samples from Rabbit 3 were negative at days 7 and 10 but were positive from day 14 onwards. Nine females completed engorgement in 21 to 31 days.

Trial 2

Four days after infection and the commencement of betamethasone treatment, 1,500 larvae and 400 nymphs were applied to the left and right ears of Rabbit 4. Engorgement was completed by 1,293 larvae and 381 nymphs in 8 to 15 days. Larvae and nymphs moulted to nymphs and adults in 14 to 16 and 18 to 23 days. Blood samples taken immediately after infestation had been concluded

confirmed that Rabbit 4 was infected with N. farcinica. The emerged instars were used for the transmission to Rabbits 5 and 6.

Forty-one days after completion of moulting, 100 nymphs were applied to the left ear of Rabbit 5 and betamethasone treatment was commenced. Engorgement was completed by 88 nymphs in 7 to 11 days. Blood samples from Rabbit 5 were negative at days 7 and 10 but were positive from day 14 onwards.

Forty-five days after completion of moulting, five males and five females were applied to each ear of Rabbit 6 and betamethasone treatment was commenced. Six females completed engorgement in 22 to 30 days. Blood samples from Rabbit 6 remained negative for N. farcinica until day 18 but were positive from then onwards.

Trial 3

No immunosuppressants were used in any part of this trial.

Four days after Rabbit 7 had been infected, 1,500 larvae and 400 nymphs were applied to the left and right ears respectively. Engorgement was completed by 1,208 larvae and 335 nymphs in 6 to 9 days and these moulted to nymphs and adults in 14 to 15 and 17 to 24 days respectively. Infection with N. farcinica was confirmed in Rabbit 7 in blood samples taken immediately after the conclusion of infestation. The emerged instars were

used for transmission to Rabbits 8 and 9.

Forty-five days after completion of moulting, 100 nymphs were applied to the left ear of Rabbit 8. Blood samples from Rabbit 8 were negative for N. farcinica at days 7 and 10 but were positive from day 14 onwards. Engorgement was completed by 82 nymphs in 7 to 12 days.

Forty-three days after completion of moulting, five males and five females were applied to each ear of Rabbit 9. Blood samples from Rabbit 9 were negative for N. farcinica until day 25 but were positive from then onwards. Five females completed engorgement in 22 to 33 days.

Rabbits 1, 2, 3, 4, 5, 7, 8 and 9 were killed and autopsied at different times within the period 87 days to eight months of infected tick application and no gross evidence of any pathological process could be discerned.

Rabbit 6 was in poor condition and died eight months after infected tick application. At autopsy there was hydronephrosis and pelvic calculi with cortical fibrosis. It was suggested that the cause of death was renal failure - possibly due to congenital abnormality.

As a routine cross-check, guinea pigs were inoculated with the cultures used for original infections of Rabbit 1, 4 and 7 and also with material prepared from random samples of the batches of infected ticks to be used in transmission. These animals were all found to have

necrotic lesions in the liver when killed and autopsied 48 days after infection and N. farcinica was consistently recovered from material taken from these lesions (see Figs. 31, 32, 33 and 34). Histological examination showed encapsulated areas of necrosis with little calcification. A collection of giant cells was seen within the granulation tissue around the lesion and, in the centre, coagulative necrosis. The lungs of one guinea pig had one pleural lesion which was solid, with some granulation tissue and necrosis.

The results of serum and Nocardin tests are given in Tables 11, 12, 13 and 14.

2. Trans-stadial transmission of Nocardia farcinica by Amblyomma variegatum thirty-three weeks after emergence

Trial 4

The first three trials showed that N. farcinica infection could be acquired from rabbits by A. variegatum larvae and nymphs and could then be transmitted to other rabbits by the following nymphs and adults nine weeks after they had moulted. These next trials were set up to establish if A. variegatum infected in this way would transmit N. farcinica at a much longer interval (e.g. 33 weeks) after moulting. A fourth trial was then set up to prove whether the emerged instars are able to transmit

FIG. 23. Guinea pig, 3 days after subcutaneous injection of N. farcinica culture, showing swelling and erythema at the site of injection.

FIG. 31. Guinea pig liver showing sharply defined areas of pallor without encapsulation. Typical coagulation with necrosis.

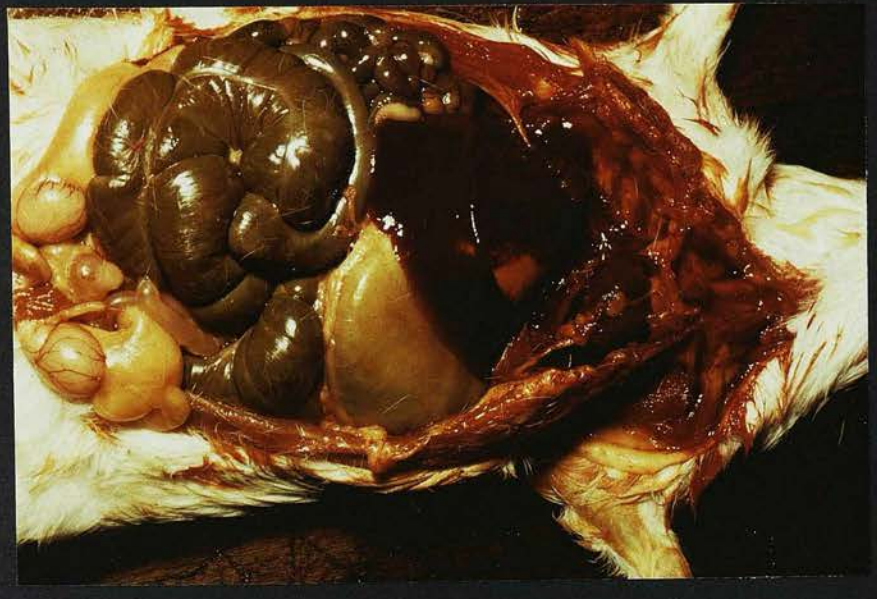
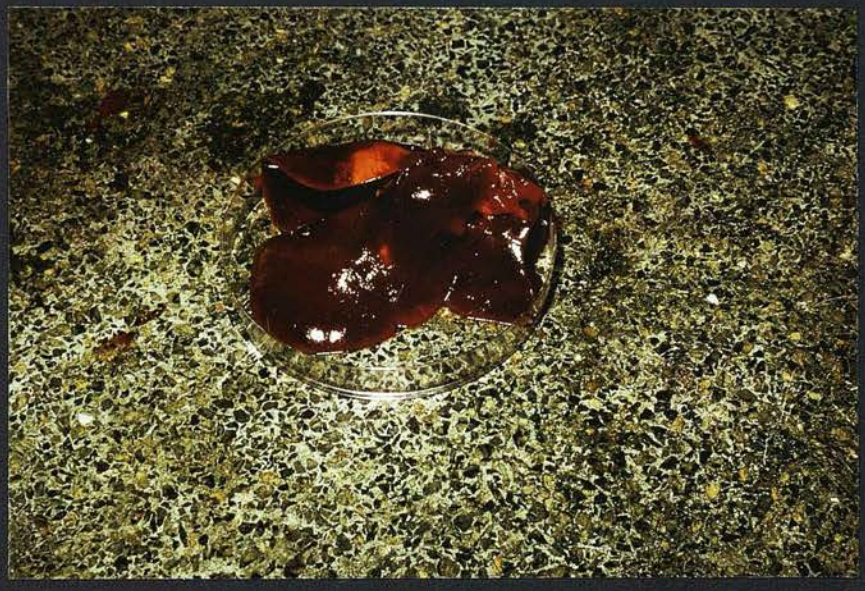
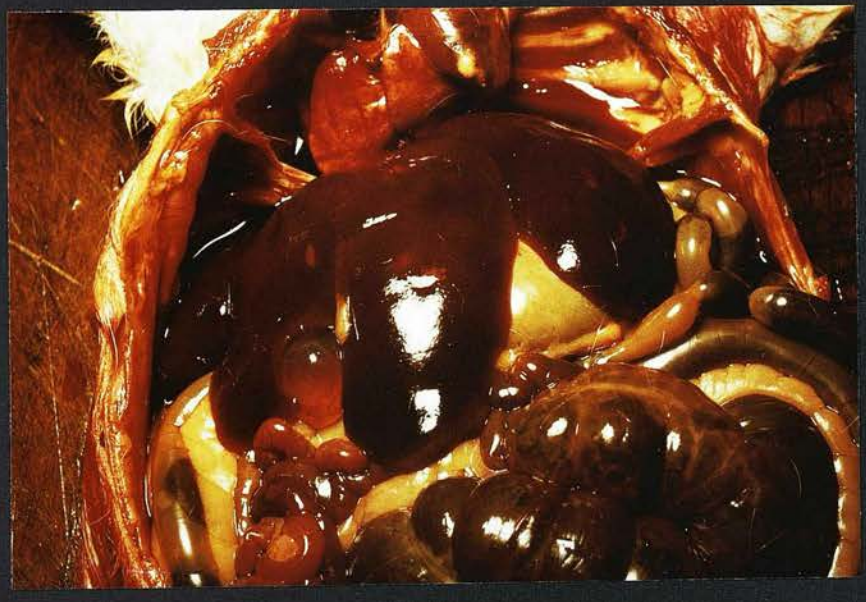


FIG. 32. Guinea pig liver showing sharply defined areas of pallor without encapsulation. Typical coagulation with necrosis.

FIG. 33. Guinea pig liver with small foci of necrosis and inflammation scattered through the liver substance.





the infection when feeding was commenced at longer periods. No immunosuppressants were used in this trial. All ticks used in this trial were derived from the same batches of emerging instars used for transmission of infection to Rabbits 2 and 3 of the first trial.

Two hundred and thirty seven days after the completion of moulting, 140 nymphs were applied to the left ear of Rabbit 10. Blood samples from Rabbit 10 were negative for N. farcinica at day 7 but were positive from day 10 onwards. Engorgement was completed by 92 nymphs in 7 to 11 days.

Two hundred and thirty four days after the completion of moulting, five males and five females were applied to each ear of Rabbit 11. Blood samples from this rabbit were negative at day 7 but were positive from day 10 onwards.

Six females completed engorgement in 17 to 24 days.

Both Rabbits 10 and 11 were killed and autopsied 82 days after infected tick application. At autopsy no gross evidence of any pathological lesion was revealed.

3. Trans-stadial transmission of Nocardia farcinica by Amblyomma variegatum under various conditions

The ticks used in the previous four trials were all stored as emerged instars at 18°C and relative humidity was maintained throughout constantly at 85 - 87 per cent.

Studies were then done on the ability of A. variegatum to retain and transmit N. farcinica infections to rabbits under various environmental conditions.

The ticks used in transmission of infections in the following trials (Trials 5, 6, 7, 8, 9 and 10) were all derived from the same batches of ticks which acquired the infection as larvae and nymphs from Rabbit 4.

Trial 5

After having completed moulting 177 and 178 days previously, 114 nymphs and 55 adults were kept in jars in which relative humidity was maintained at 85 to 87 per cent, but in which temperature was adjusted daily as in the following programme in order to simulate possible natural conditions -

0900 hours to 1000 hours - room temperature (15-16°C)

1000 hours to 1630 hours - 37°C

1430 hours to 1630 hours - room temperature (15-16°C)

1630 hours to 0900 hours - 10°C.

After 17 days of this regime, three nymphs and eight adults were dead. The remaining ticks then were used in transmission trials. One hundred and one nymphs were applied on the left ear of Rabbit 13. Blood samples from Rabbit 13 were negative for N. farcinica at days 7 and 10 but were positive from day 14 onwards.

Ninety-three nymphs completed engorgement in 7 to 11 days.

Five males and five females were applied on each ear of Rabbit 14. Blood samples were again negative for N. farcinica at days 7 and 10 but were positive from day 14 onwards.

Five females completed engorgement in 18 to 23 days.

Both Rabbits 13 and 14 were killed and autopsied 130 days after infected tick application. Autopsy revealed no gross evidence of any pathological lesion.

Trial 6

One hundred and ten nymphs and 36 adults, after completing moulting 173 and 175 days previously, were kept in closed culture jars with a relative humidity maintained constantly at 44 per cent. The temperature was adjusted in the same way as used in Trial 5.

A total of 31 nymphs and 13 adults were dead at the 17th day of this regime. The remaining ticks then were used for feeding.

Seventy-nine nymphs were applied to the left ear of Rabbit 15. Blood samples from Rabbit 15 were positive from day 7 onwards. Engorgement was completed by 19 nymphs only, in 9 to 11 days.

Five males and five females were applied to each ear of Rabbit 16. Blood samples from Rabbit 16 remained

negative for N. farcinica until day 18 but were positive from then onwards.

Only five females completed engorgement in 22 to 23 days.

Both Rabbits 15 and 16 were killed and autopsied 130 days after infected tick application. At autopsy no gross evidence of any pathological lesion was discerned.

Trial 7

One hundred and fourteen nymphs and 36 adults, after completing moulting 173 and 175 days previously, were kept in closed culture jars with a relative humidity maintained constantly at 20 per cent. Again the temperature was adjusted in the same way as in Trials 5 and 6.

After three days of this regime six nymphs and five adults were found dead. The remainder were all dead by the 10th day and hence transmission trials were not possible. On the other hand, when cultures were made from these dead ticks in TPB, there was clear evidence of growth of N. farcinica.

Trial 8

One hundred and sixteen nymphs and 32 adults, after completing moulting 156 and 151 days previously, were put in a closed culture jar in which the relative humidity was maintained constantly at 85 - 87 per cent

and the temperature was adjusted continuously at 25°C. They were left under this regime for 45 days. After 5 days of this regime, five nymphs and one adult were dead, and after 10 days a further two nymphs and four adults were dead. By the 45th day, one more nymph and a further nine adults had died. The remaining ticks were used as follows:

One hundred and eight nymphs were applied on the left ear of Rabbit 17. Blood samples from Rabbit 17 were positive from day 7 onwards. Engorgement was completed by 90 nymphs in 9 to 16 days.

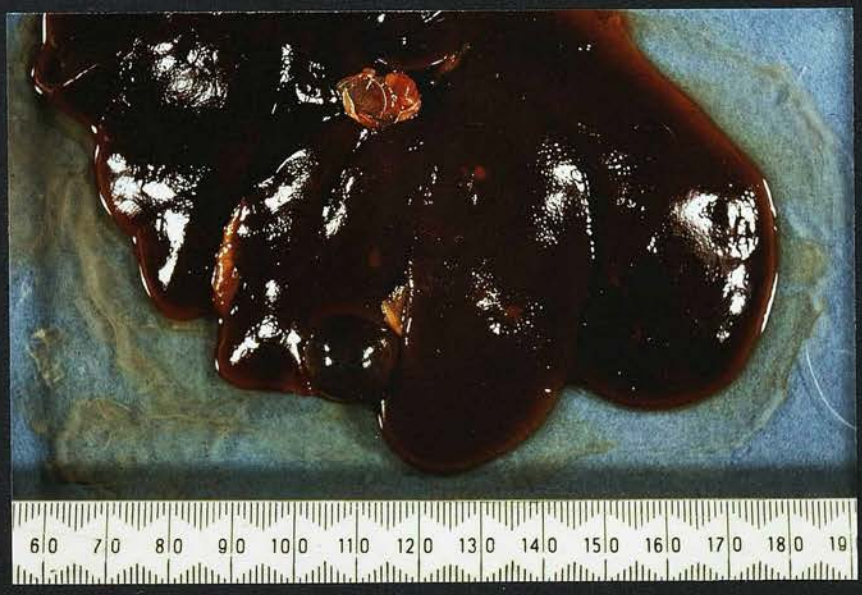
Five males and four females were applied on the right ear of Rabbit 18. On its left ear, six males and three females were applied. Blood samples were only positive on day 10. Six females completed engorgement in 19 to 26 days.

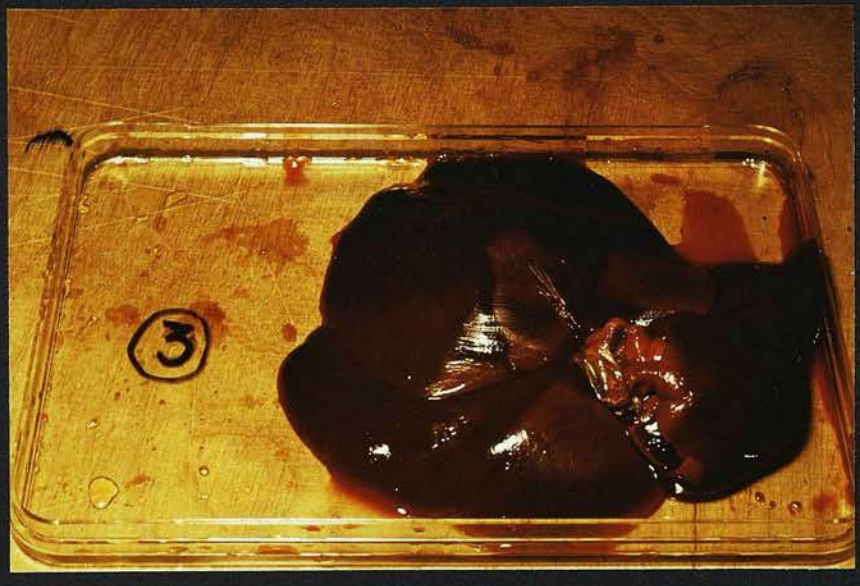
When Rabbit 17 was autopsied 100 days after the application of infected ticks, lesions measuring between 2 mm and 2 cm in diameter were seen on the dorsal and ventral surfaces of the liver (see Fig. 35). Culture of material from these lesions established the presence of N. farcinica.

Rabbit 18 was killed and autopsied 99 days after infected tick application. There was only one lesion 2 mm in diameter found in the liver. Cultures of this material in TPB also established the presence of N. farcinica.

FIG. 34. Guinea pig liver with small foci of necrosis and inflammation scattered through the liver substance.

FIG. 35. Rabbit liver showing pale defined areas of necrosis without encapsulation.


6 0 7 0 8 0 9 0 10 0 11 0 12 0 13 0 14 0 15 0 16 0 17 0 18 0 19


3

Histological examination showed a low level of chronic inflammation with necrosis.

Trial 9

Sixty-two nymphs and 34 adults, after completing moulting 156 and 151 days previously, were put in a closed culture jar in which the relative humidity was maintained constantly at 44 per cent. The temperature was again constant at 25°C. After 5 days of this regime four nymphs and three adults had died and, at day 10, another two nymphs and two adults were dead. By the 45th day, no nymphs were alive and a further 25 adults had died.

The remaining three males and six females were applied on the left ear of Rabbit 19. Blood samples from Rabbit 19 were positive for N. farcinica only on days 10 and 14. This rabbit was killed and autopsied but there was no gross evidence of any pathological lesion discerned.

Trial 10

Eighty-three nymphs and 40 adults, after completing moulting 156 and 151 days previously, were put in a closed culture jar in which the relative humidity was maintained constantly at 20 per cent and temperature again was constant at 25°C. After 5 days, five

nymphs and four adults had died and, at day 10, a further 10 nymphs and four adults had died. Seven days later, all nymphs had died and, at day 40, no adults survived and hence transmission trials were not possible.

4. Trans-ovarian transmission of Nocardia farcinica by Amblyomma variegatum

Trial 11

Trans-stadial transmission having been established, it was obviously of importance to test the possibility of trans-ovarian transmission. An eleventh trial was set up by examining the eggs laid by A. variegatum females which had transmitted N. farcinica infections to Rabbits 3, 6 and 9 in the first three trials. Neither the cultures which were made in different media after grinding the eggs, nor the direct stained smears of crushed eggs showed any evidence of the presence of N. farcinica. A number of larvae which had emerged from the same batches of eggs were then fed on rabbits. Twenty days after hatching, 600 larvae were applied to the left ear of Rabbit 20. Blood samples from Rabbit 20 were negative on days 7, 10, 14, 18, 22, 25, 30 and 36. Engorgement was completed by 442 larvae in 7 to 13 days.

Rabbit 20 was killed and autopsied 104 days after tick feeding. No gross evidence of any pathological

lesion was detected.

5. Trans-stadial transmission of *Nocardia farcinica* by *Hyalomma anatolicum excavatum* eight weeks after emergence

Trial 12

In this trial two rabbits - Rabbits 21 and 22 - were used as sources of infection.

Four days after Rabbit 21 had been infected (see Materials and Methods) 100 larvae were applied to the left ear. A total of 34 larvae and 53 nymphs completed engorgement in 6 to 8 and 15 to 19 days respectively and moulted to nymphs and adults in 6 to 9 and 11 to 34 days - again respectively. Twelve flat nymphs were also recovered - these having completed engorgement and moulting in 10 to 11 days after application as larvae.

Infection with *N. farcinica* in Rabbit 21 was confirmed in blood samples taken during the infestation and after it had been completed. The emerged instars were used for transmissions to Rabbits 23 and 24.

Four days after Rabbit 22 had been infected, 200 larvae were applied on each ear. A total of 80 larvae and 68 nymphs completed engorgement in 6 to 9 and 15 to 19 days respectively and moulted to nymphs and adults in 7 to 10 and 13 to 21 days, again respectively. Infection with *N. farcinica* in Rabbit 22 was confirmed

immediately after the infestation had been completed.

Some of the emerged instars were also used for transmissions to Rabbits 23 and 24, and the remainder were used for transmission in other trials.

Fifty-six days after the completion of moulting, 46 nymphs were applied to the left ear of Rabbit 23. Blood samples from Rabbit 23 were positive for N. farcinica infection from day 7 onwards. Engorgement was completed by 28 nymphs in 7 to 8 days.

Thirty days after completion of moulting, seven males and seven females were applied on each ear of Rabbit 24. Blood samples from Rabbit 24 were negative on day 7 but were positive from day 10 onwards. Engorgement was completed by 12 females in 11 to 16 days.

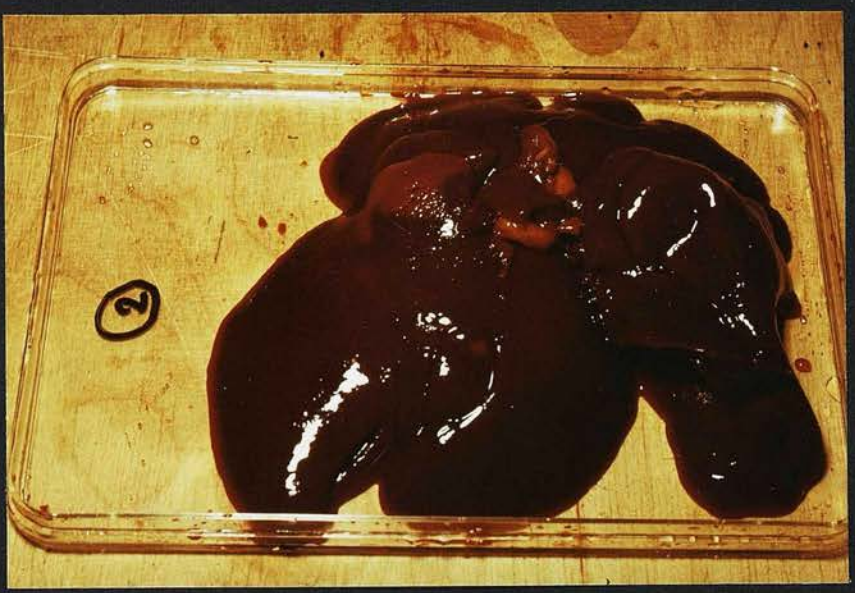
Rabbits 21 and 22 were killed and autopsied 122 days after infection.

Autopsy of Rabbit 21 revealed the presence of one necrotic lesion in the liver. The lesion measured 1·3 x 1·6 cm (see Fig. 36). Cultures of materials from this lesion in TPB established the presence of N. farcinica. Histological examination of this lesion showed necrotic and calcareous material with many polymorphs and surrounded by a zone of epithelial, mononuclear and polymorphonuclear cells. Giant cells were also seen in the granulation tissue which surrounded the lesion.

FIG. 36. Rabbit liver with pale defined areas of necrosis without encapsulation.

FIG. 37. Rabbit liver with pale defined areas of necrosis without encapsulation.


①


②

Autopsy of Rabbit 22 revealed no gross evidence of any pathological lesion.

Rabbits 23 and 24 were killed and autopsied 44 days after infected tick feeding. Autopsy of Rabbit 23 revealed the presence of one necrotic lesion in the liver, (see Fig. 37). Cultures of material from this lesion in TPB established the presence of N. farcinica. Histological examination of this lesion showed a low level of chronic inflammation with necrosis.

6. Trans-stadial transmission of Nocardia farcinica by Hyalomma anatolicum excavatum twenty-nine weeks after emergence

Trial 13

It had been demonstrated in the ~~13th~~ 12th trial that larvae and nymphs of H. a. excavatum could acquire N. farcinica infection from rabbits and transmit these infections to other rabbits for periods up to 8 weeks after emergence. A further trial was set up to ascertain whether these instars are able to retain and transmit the infection for longer periods after emergence. The adults used in the transmission in Trial 13 were derived from the batch fed as nymphs on Rabbit 22 in the last trial (all nymphs which fed as larvae on Rabbit 22 were found dead after 190 days of storage in standard conditions).

One hundred and ninety three days after the completion of moulting, six males and six females were applied on the right ear of Rabbit 25, and on its left ear six males and seven females were also applied. Blood samples from Rabbit 25 were negative at days 7, 10, 14, 18, 22, 25, 30 and 36. Engorgement was completed by 12 females in 8 to 14 days.

Rabbit 25 was killed and autopsied 43 days after tick application. At autopsy no gross evidence of any pathological lesion was discerned.

7. Transmission of Nocardia farcinica by infected Hyalomma anatolicum excavatum larvae moulted to nymphs and then further to adults

Trial 14

The adults of H. a. excavatum which were used in Trial 14 had acquired N. farcinica infection from Rabbit 21 as larvae and transmitted this infection to Rabbit 23 when fed as nymphs. Eighty-two days after the completion of moulting, eight males and six females were applied on the right ear of Rabbit 26 and five males and six females were applied to its left ear. Blood samples from Rabbit 26 were negative for N. farcinica at days 7, 10, 14, 18, 22, 25, 30 and 36. Engorgement had been completed by eight females in 9 to 13 days.

Rabbit 26 was killed and autopsied 43 days after

tick feeding. At autopsy no evidence of any pathological lesion was discerned.

8. Trans-ovarian transmission of *Nocardia farcinica* by *Hyalomma anatolicum excavatum*

Trial 15

It was again important to ascertain whether *N. farcinica* could be transmitted trans-ovarially by *H. a. excavatum*. Accordingly, eggs from females which had transmitted the infection to Rabbit 24 were examined first by searching stained material from ground-up eggs for direct evidence of the organism and, second, by culturing this material in TPB. There was no evidence of the presence of *N. farcinica* in these eggs.

Further, larvae which emerged from these eggs were applied to Rabbit 27 (200 on each ear). As confirmation, a number of larvae which had hatched from the same batch of eggs were fed on rabbits. Two hundred larvae were applied on each ear of Rabbit 27. Blood samples were negative from Rabbit 27 at days 7, 10, 14, 18, 22, 25, 30 and 36. Engorgement was completed by 109 larvae and 92 nymphs in 5 to 8 and 18 to 24 days respectively.

Rabbit 27 was killed and autopsied 55 days after the application of ticks. Autopsy revealed no lesions.

Results of serum tests

Sera from all rabbits and guinea pigs infected during the first three trials were checked for the degree of inhibition imposed on N. farcinica growth and thus attributable to the presence of antibodies. The sera were collected 30 days after Rabbits 1, 4 and 7 were injected with N. farcinica cultures and 30 days after tick application to Rabbits 2, 3, 5, 6, 8 and 9.

Also 30 days after the guinea pigs were injected with tick material - these guinea pigs were used as routine cross-checks - some of the rabbits were treated with immunosuppressants. A control rabbit and a control guinea pig were also included. The summary of growth inhibitory properties of these sera is shown in Tables 11 and 12.

TABLE 11.

Summary of growth inhibitory properties of sera from rabbits infected in various circumstances

Rabbit	Circumstances of infection	Degree of inhibition
1	Culture inoculation; Cyclophosphamide treatment.	Marked
2	Infected A. variegatum nymphs; Betsolan treatment.	Slight
3	Infected A. variegatum adults; Betsolan treatment.	Very slight
4	Culture inoculation; Betsolan treatment.	Marked
5	Infected A. variegatum nymphs; Betsolan treatment.	None
6	Infected A. variegatum adults; Betsolan treatment.	Marked
7	Culture inoculation only.	Marked
8	Infected A. variegatum nymphs.	Slight
9	Infected A. variegatum adults.	Slight
Control		None

TABLE 12.

Summary of growth inhibitory properties of sera from guinea pigs infected by tick material

Guinea pig	Circumstances of infection	Degree of inhibition
1	S/C inoculation of 1 ml tick material prepared from nymphs with transmitted N. farcinica infection to Rabbit 2	Slight
2	I/P inoculation of 1 ml of the same material used for guinea pig 1	Marked
3	S/C inoculation of 1 ml tick material prepared from adults which transmitted N. farcinica infection to Rabbit 3	Slight
4	I/P inoculation of 1 ml of the same material used for guinea pig 3	Slight
5	S/C inoculation of 1 ml tick material prepared from nymphs which transmitted N. farcinica infection to Rabbit 5	Slight
6	I/P inoculation of 1 ml of the same material used for guinea pig 5	Very slight
7	S/C inoculation of 1 ml tick material prepared from adults which transmitted N. farcinica infection to Rabbit 6	None
8	I/P inoculation of 1 ml of the same material used for guinea pig 7	Marked

TABLE 12 (Cont'd).

9	I/P inoculation of 1 ml tick material prepared from nymphs which transmitted N. farcinica infection to Rabbit 8	None
10	I/P inoculation of 1 ml of the same material used for guinea pig 9	Slight
11	S/C inoculation of 1 ml tick material prepared from adults which transmitted N. farcinica infection to Rabbit 9	Slight
12	I/P inoculation of 1 ml of the same material used for guinea pig 11	Slight
Control		None

Results of Nocardin Tests

The responses to Nocardin were tested on rabbits and guinea pigs which had been infected during the first three trials. Results of reactions judged by the degree of swelling at the site of inoculation and by the extent of the area of erythema around the site of inoculation.

Two batches of Nocardin 1 and 2 were injected on one side of each animal, approximately two inches apart. Nocardin tests were made 40 days after Rabbits 1, 4 and 7 had been injected with N. farcinica culture, 40 days after infected tick application on Rabbits 2, 3, 5, 6, 8 and 9, and 40 days after the guinea pigs had been injected with tick material.

Control rabbits and control guinea pigs were also tested.

The summary of the reactions due to Nocardin inoculation of the rabbits and guinea pigs infected in various circumstances is shown in Tables 13 and 14.

TABLE 13.

Summary of reaction to Nocardin inoculations

into rabbits infected in various circumstances

Rabbit	Circumstances of infection	Nocardin inoculation i/d	Degree of swelling	Degree of Erythema
1	Culture inoculation; Cyclophosphamide treatment	Batch 1 Batch 2	1·9 mm 1·9 mm	2·5 mm 2·0 mm
2	Infected A. variegatum nymphs; Betsolan treatment	Batch 1 Batch 2	None None	None None
3	Infected A. variegatum adults; Betsolan treatment	Batch 1 Batch 2	2·5 mm 2·1 mm	3 mm 3 mm
Control		Batch 1 Batch 2	None None	None None
4	Culture inoculation; Betsolan treatment	Batch 1 Batch 2	2·4 mm 2·1 mm	4 mm 3 mm
5	Infected A. variegatum nymphs; Betsolan treatment	Batch 1 Batch 2	1·2 mm 1·1 mm	3 mm 3 mm

TABLE 13 (Cont'd)

6	Infected *A. variegatum* adults; Betsolan treatment	Batch 1 Batch 2	2·0 mm 2·1 mm	5 mm 4 mm
Control		Batch 1 Batch 2	None None	None None
7	Culture inoculation; No treatment	Batch 1 Batch 2	2·2 mm 2·1 mm	3 mm 2·5 mm
8	Infected *A. variegatum* nymphs	Batch 1 Batch 2	1·6 mm 1·6 mm	None None
9	Infected *A. variegatum* adults	Batch 1 Batch 2	1·4 mm 1·4 mm	None None
Control		Batch 1 Batch 2	None None	None None

Nocardin Batch 1 was prepared from original culture of *Nocardia farcinica* and inoculated into all infected and control rabbits.

Nocardin Batch 2 was either prepared from positive blood culture drawn from Rabbit 1 and inoculated into Rabbits 1, 2, 3 and their control, or from positive blood culture drawn from Rabbit 4 and inoculated into Rabbits 4, 5, 6 and their control, or from positive blood culture drawn from Rabbit 7 and inoculated into Rabbits 7, 8, 9 and their control.

TABLE 14.

Summary of reaction to Nocardin inoculations into guinea pigs infected in various circumstances

Guinea pig	Circumstances of infection	Nocardin inoculation i/d	Degree of swelling	Degree of Erythema
1	S/C inoculation of 1 ml tick material prepared from nymphs which transmitted N. farcinica infection to Rabbit 2	Batch 1	2·2 mm	3·0 mm
		Batch 2	2·0 mm	5·0 mm
2	I/P inoculation of 1 ml of the same material used for guinea pig 1	Batch 1	2·6 mm	9 mm
		Batch 2	2·6 mm	10 mm
3	S/C inoculation of 1 ml tick material prepared from adults which transmitted N. farcinica infection to Rabbit 3	Batch 1	2·5 mm	7 mm
		Batch 2	2·3 mm	8 mm
4	I/P inoculation of 1 ml of the same material used for guinea pig 3	Batch 1	2·6 mm	7 mm
		Batch 2	2·1 mm	8 mm
Control		Batch 1	1·3 mm	4 mm
		Batch 2	1·0 mm	4 mm

TABLE 14 (Cont'd)

5	S/C inoculation of 1 ml tick material prepared from nymphs which transmitted N. farcinica infection to Rabbit 5 (Died)	-	-	-
6	I/P inoculation of 1 ml of the same material used for guinea pig 5	Batch 1 Batch 2	1·8 mm 2·1 mm	5 mm 5 mm
7	S/C inoculation of 1 ml tick material prepared from adults which transmitted N. farcinica infection to Rabbit 6	Batch 1 Batch 2	2·6 mm 2·4 mm	8 mm 9 mm
8	I/P inoculation of 1 ml of the same material used for guinea pig 7	Batch 1 Batch 2	2·7 mm 2·5 mm	9 mm 10 mm
Control		Batch 1 Batch 2	1·6 mm 1·1 mm	5 mm 3 mm
9	S/C inoculation of 1 ml tick material prepared from nymphs which transmitted N. farcinica infection to Rabbit 8	Batch 1 Batch 2	2·0 mm 2·1 mm	9 mm 12 mm
10	I/P inoculation of 1 ml of the same material used for guinea pig 9 (Died)	-	-	-

TABLE 14 (Cont'd)

11	S/C inoculation of 1 ml tick material prepared from adults which transmitted N. farcinica infection to Rabbit 9 (Died)	-	-	-
12	I/P inoculation of 1 ml of the same material used for guinea pig 11	Batch 1	2·5 mm	5 mm
		Batch 2	2·2 mm	5 mm
Control		Batch 1	1·3 mm	4 mm
		Batch 2	1·5 mm	6 mm

Nocardin Batch 1 was prepared from original culture of N. farcinica and inoculated into all infected and control guinea pigs.

Nocardin Batch 2 was either prepared from positive blood culture drawn Rabbit 1 and inoculated into guinea pigs 1, 2, 3, 4 and their control, or from positive blood culture drawn from Rabbit 4 and inoculated into guinea pigs 5, 6, 7, 8 and their control, or from positive blood culture drawn from Rabbit 7 and inoculated into guinea pigs 9, 10, 11, 12 and their control.

CHAPTER V.

DISCUSSION AND CONCLUSIONS

Although many workers from Neumann (1888) to Kuseltan (1967) have commented on an apparently close association between tick infestations and bovine farcy, no record could be found of any detailed examination of the relationship. Hence, the primary object of this study was to make such a detailed examination and to establish whether or not N. farcinica could be acquired by ticks from one host and could then be transmitted to another host when feeding as the following instars. The epidemiological implications of this are obvious.

The study fell naturally into different sections and hence it is as well if these are discussed in turn.

1. The organism

The organism used in this work was positively identified as N. farcinica (see Materials and Methods). The precise identity of some of the organisms described in the literature as causing bovine farcy is, however, much in doubt. For this reason, the cultural, morphological and pathogenic characteristics of N. farcinica as observed in this study are compared with the findings of other authors.

N. farcinica grew aerobically and the optimum temperature for growth was found to be 37°C on various solid and liquid media. This is a generally acceptable finding since Nocard & Leclainche (1903) mentioned that the organism grew aerobically on various media at 30 to 40°C. Almost all other workers state that 37°C is the most suitable for the growth of N. farcinica, but Gray (1935) recorded that the strain of N. farcinica (which he isolated from infected cow's milk) grew at a temperature of 23 to 40°C - again aerobically. The organism grew rapidly on Lowenstein-Jensen's media on which the colonies appeared on the third day of incubation as small, pale yellow nodules. After eight or more days these colonies adopted a pronounced "button-shaped" appearance and were raised from the surface of the medium. They varied in colour from greyish-white to pale yellow. Growth of the organism on the same medium has been tried by Mostafa (1967b), who observed the appearance of the colonies on day six after incubation as small, pale yellow nodules, which reached maximum growth in about three to four weeks.

Sabouraud's, nutrient and blood agars were also used for the cultivation of the organism. Growth was slower on these media than on Lowenstein-Jensen's medium. The colonies appeared on the fourth or fifth day of incubation. They were small, and yellow to yellowish-

brown. Typical cauliflower colonies were observed when these media were incubated for ten or more days and became larger, raised from the surface of the medium with irregular edges. This is recorded only by Mostafa (1967b), who observed cauliflower colonies on Lowenstein-Jensen's medium and on inspissated serum. He also used blood-agar for the isolation of the organism but considered it to be unsatisfactory and described inconspicuous growth as colonies of variable size and folded appearance. No record could be found in the literature of the use of Sabouraud's agar by other workers. Awad & Karib (1958) found that the best growth of the organism was on glycerine - potato agar. On the other hand, Nocard (1888) stated that the organism grew well on potato culture, in serum with agar, and in agar culture (gélose) - the best growth being obtained from the last medium.

Traum (1919) mentioned that blood serum or blood media are essential for the initial culture of N. farcinica. In the present study, several liquid media were also tried and the presence of blood did not appear to be critical. Very satisfactory results, for example, were obtained from TPB, in which the organism appeared on the third or fourth day as small, yellowish-white pellicle-like colonies on the bottom of the universal containing the medium. At the seventh day of

incubation, the colonies started to grow on the surface of the medium. They were at first flaky and yellowish-white, and coalesced later to form one wrinkled layer. This is referred to as surface growth. Mostafa (1962) mentioned the same type of growth when he cultivated the organism in broth and in serum broth. Colonies started at the bottom of the tube and later colonies were observed on the surface. The surface colonies looked like tiny droplets of grease on cold water. They increased rapidly in size and became wrinkled as the culture aged. In contrast to Mostafa (1967b), who did not observe any enhancement in the growth of the organism when he added glycerine to his ordinary media at the rate of five per cent, I observed a slight enhancement of N. farcinica growth by the addition of glycerine to TPB at the rate of 1·2 per cent.

All the liquid media used in this work remained clear after the surface growth took place except for a few flakes. This finding is similar to that of Traum (1919) when he observed that N. farcinica grew at first in the bottom of the tube and later on the surface as a membrane. The membrane became wrinkled as the culture aged but the medium - which was either glycerine broth or Loeffler's broth serum - remained clear.

N. farcinica was also cultivated in Sabouraud's and nutrient broths. Growth was similar to that in TPB but

slower, i.e. bottom growth started at day five and surface growth did not appear before nine days of incubation.

As shown by positive results from subcultures, N. farcinica cultures remained viable for two and a half years at 4°C on Lowenstein-Jensen's medium and for approximately eight months at room temperature on both Lowenstein-Jensen's and Sabouraud's media. No further growth was observed in either media, either at room temperature or 4°C. Also, cultures on Lowenstein-Jensen's medium and in TPB remained viable for two months at 37°C and, when subcultures were made from these cultures, a discernible growth was observed at the third day of incubation, reaching maximum growth at 10 to 12 days. According to Topley & Wilson (1964) cultures of N. farcinica remain viable for four months at 37°C, and are killed by 70°C in 10 minutes. Mostafa (1967b) observed that animal tissues remained infective if kept in a refrigerator. He also mentioned that cultures retained their virulence and viability for years in the refrigerator and for months in the incubator (the exact time was not mentioned). It was also noted in the present work that, when the relative humidity was increased to 85 per cent, the growth of N. farcinica on nutrient agar was faster and the number of colonies increased in comparison with cultures kept at lower

relative humidities (i.e. 15 per cent and 44 per cent) at the same incubating temperature. Cultures of N. farcinica were readily destroyed by 15 minutes immersion in a 70 per cent or absolute alcohol. Cultures did not survive heating at 70°C for 20 minutes. Similar observations were made by Mostafa (1962), in which he found that the organisms could be killed by caustic soda, by five per cent oxalic acid, by absolute alcohol, and by heating at 65°C or above for 15 minutes. As was shown in some preliminary work, when Staphylococcus epidermidis was seeded with N. farcinica on the same agar, the growth of the latter was markedly affected and even sometimes inhibited completely. This was ascribed to the faster-growing S. epidermidis tending to starve the slower growing N. farcinica of nutrients. For that reason it was decided to add a penicillin/streptomycin solution of a strength of 50,000 IU and 50,000 mg respectively at the rate of 250 IU penicillin and 250 mg streptomycin per ml broth in cases of suspected contamination or in cases of the isolation of the organism from tick tissues. This amount of antibiotic was enough to kill the contaminants but did not affect N. farcinica growth. Mostafa (1962 and 1967b) emphasized the role of contaminants which occur in natural farcy lesions; he stated that these contaminants were mostly Staphylococcus or Streptococcus spp. Though he recorded that he added

penicillin to culture media, he did not specify the concentration.

As seen in stained smears prepared from young and old cultures grown on various media, the morphological character of N. farcinica is that of tangled masses of closely-interwoven, thin, branching filaments. These were Gram-positive and acid-alcohol-fast. Nocard's (1888) first isolation agrees with this description, and so does that of Traum (1919) regarding an organism isolated from cases of bovine lymphangitis in California. It should be noted here that a clinically similar disease to bovine farcy was investigated in Sumatra by Vryburg (1907), in India by Holmes (1908), Raymond (1910) and Sheather (1920). Vryburg, Raymond and Sheather have incriminated a Gram-negative bacilli as a cause of this disease. These findings were at variance with others elsewhere. On the other hand, the findings of Holmes (1908), who is stated to have investigated the same outbreak as Raymond, differ from those of Raymond, Vryburg and Sheather. Holmes described branching filaments and referred to the development of small "button-shaped" colonies with crenated edges, but he failed to maintain these in pure culture. Daubney (1927) examined pus from natural cases of bovine lymphangitis in Kenya. In stained smears he was able to demonstrate acid-fast Actinomyces which proved to be identical to Actinomyces

farcinicus (Nocard, 1888). Similar descriptions were given by Gray (1935) who isolated the organism for the first time from infected cow's milk. The branching filamentous organism was again Gram-positive and acid-fast. Awad & Karib (1958) mentioned that the organism isolated from natural cases of bovine farcy in Sudan appeared as branching filaments which were again Gram-positive and acid-alcohol-fast, and thus similar to Nocard's organism. A similar description was also given of an organism isolated from the testes and udders of infected cattle in the Sudan (Awad, 1960). From pus and lesions, Mostafa (1962 and 1967b) isolated the organism with the same characteristics as given by Awad & Karib (1958). It was found in the present work that the organism took Ziehl-Neelsen stain in a more even way. The filaments were red in colour with a few granules which were deep violet. On the other hand, it did not take Gram-stain uniformly and had a beaded appearance with darkly-stained Gram-positive granules distributed irregularly along the lightly stained filaments. These findings agree entirely with Mostafa (1962 and 1967b) who illustrated the beaded form in smears prepared from cultures, lesions or pus. In the present study, it was observed that in young and old cultures the filaments break down into short hyphae, rods, bacillary form, diphtheroid or coccoid. Similar variations have been

observed by other authors. Schlegel (1913) made sections from nodules in the lung and pleura of a nine-month-old calf. In these sections, he demonstrated a branching filamentous organism as well as small coccoid bodies, both of which were acid-fast and alcohol-fast. On the other hand, Traum (1919) mentioned that, in the pus and tissue collected from natural cases of bovine lymphangitis, there were small acid-fast bacilli and coccoid forms as well as the branching filaments. He found it difficult to differentiate the bacillary forms from tubercle bacilli. In cultures, he also mentioned that, in addition to these forms, some bacilli were curved. A similar observation was made by Awad & Karib (1958). They mentioned that, in some lesions which had undergone caseation and partial calcification, the filaments had broken down into small rods resembling tubercle bacilli. I have also noticed that in old cultures, tick-infected material, pus and lesions many filaments were broken down into the above-mentioned forms, but these forms were less common in new cultures. This finding could support Awad & Karib (1958), who mentioned that these changes were found especially in old lesions with caseation or calcification. This point was not emphasised by Mostafa (1967b), who merely said that fragmentation into short rods, bacillary and coccoid forms had been observed in both

young and old cultures. Topley & wilson (1964) stated that N. farcinica could break down into smaller bacilli or rods. In many stained smears prepared from cultures, pus, blood cultures and tick-infected material, it was possible to see single filaments which were detached from the mother filament. In blood cultures, the filaments were thickened. This was perhaps due to a long incubation in the liquid medium which could have allowed the filaments to absorb liquid. The organism has also been isolated from the salivary glands, intestines and faeces of ticks. There is no such record in the literature. Although Kuseltan (1967) stated that larvae and nymphs of Hyalomma asiaticum were found to be infected with Nocardia sp., he did not describe the details of his examination. Budding was also observed in stained smears prepared from cultures, blood cultures and tick-infected materials which were cultured in TPB. This character has been mentioned by Awad & Karib (1958) and Mostafa (1967b). In his article "Observation on the life cycle of Nocardia", Morris (1951) considered that the growth of Nocardia occurs by budding.

N. farcinica was more pathogenic in guinea pigs than in rabbits. Subcutaneous inoculation of N. farcinica cultures into guinea pigs gave rise to hard local swellings at the sites of inoculation. Intradermal inoculation gave rise to pustular lesions from which pus

containing N. farcinica erupted. On the other hand, the subcutaneous or intradermal inoculation of cultures into rabbits produced lesions in only one case - a small erythematous area at the site of intradermal inoculation. No lesions were found at the post-mortem examination of rabbits which had been inoculated with N. farcinica cultures by either the intradermal or the subcutaneous route. In guinea pigs, which had received subcutaneous inoculations, however, small, hard abscesses were seen at the sites of inoculation.

The susceptibility of different animals to N. farcinica injection was first discussed by Nocard & Leclainche (1903), who found that the organism was pathogenic to guinea pigs and cattle but not to rabbits, horses, dogs and cats. On the other hand, Traum (1919), inoculating rabbits subcutaneously and guinea pigs subcutaneously and intradermally, produced local swellings in both species and subsequently demonstrated the presence of the organism in pus drawn from these swellings. (The organism being acid-fast and similar to that found in natural cases of bovine farcy.)

Similar findings with guinea pigs were recorded by Daubney (1927) and one of these guinea pigs developed an orchitis three months later. Daubney did not record if he inoculated rabbits. Local swellings and abscesses also resulted from N. farcinica - isolated from cow's

milk - when Gray (1935) injected cultures subcutaneously into guinea pigs but whether or not the organism was recovered from these lesions is not recorded. Similar observations were made by Awad & Karib (1958). Mostafa (1967c) gave injections of N. farcinica cultures subcutaneously into rabbits and provoked either no reaction or a very small swelling but guinea pigs, identically inoculated, developed local swellings. Some of these swellings erupted and N. farcinica was easily isolated from the pus.

In this study, six guinea pigs - used as routine cross-check - were injected subcutaneously with infected tick materials and all developed local swellings at the site of inoculation. These lesions persisted from the third day to the seventh or eighth day and they measured 10 to 18 mm in diameter. The surrounding erythema exceeded 30 mm in diameter in some of these cases. Thereafter, these swellings contracted and finally disappeared around the 14th to 16th day. In one guinea pig, the lesion erupted on the sixth day after inoculation and N. farcinica was demonstrated from the cultured pus.

At autopsy, guinea pigs, which had been inoculated subcutaneously and were subsequently killed or had died, showed no evidence of pathological lesions except in one instance in which there was a small abscess at the site of inoculation. Broken, acid-fast filamentous organisms

were demonstrated in pus from this lesion. Another six guinea pigs were injected with similar material but by the intraperitoneal route. All of them showed lesions in the liver after death or killing at various periods. The lesions consisted of necrotic or infarctive areas, yellowish and variable in size. Similar findings following intraperitoneal injection of cultures or pus were made by Traum (1919), Bernard (1924 and 1927) and Awad & Karib (1958). Daubney (1927) also stated that intraperitoneal injections of N. farcinica cultures into guinea pigs were followed by peritonitis and lesions in the liver and spleen.

In the present work, it was decided to produce a generalized N. farcinica infection in rabbits. Three rabbits were inoculated intravenously with 1 cc of TPB culture of N. farcinica. The first rabbit was treated with cyclophosphamide and the second with betsolan. The third rabbit was not treated but was considered as an infected control. Positive blood cultures were obtainable from the first and second rabbits on day 7, 10, 14, 18 and 22, while from the third rabbit (non-treated) positive blood cultures were obtainable on day 10, 14, 18, 22 and 25. No previous record could be found in the literature of recovering the organism from rabbits in cultures from whole blood, or of using immunosuppressants to assist in this process.

Traum (1919) injected rabbits intravenously with N. farcinica cultures or pus drawn from nodules from natural cases of the disease but did not comment upon the fate of these rabbits.

Mostafa (1967c) inoculated one rabbit intravenously and another rabbit intraperitoneally with N. farcinica cultures. In an autopsy made 30 and 40 days later, the rabbit injected intraperitoneally showed a fibrinous shred in the peritoneal cavity, and a smear of this showed an amorphous mass of degenerated organisms which retained their acid-fast staining quality.

According to Waksman (1957), strains of N. farcinica are transmissible to guinea pigs, cattle and sheep but not to rabbits, cats, dogs and horses.

Two isolations of an organism found to be transmissible to rabbits by Fey, Holm & Teuscher (1954) were identified as N. farcinica by Magnusson & Mariat (1967). Fey and his colleagues recorded that these organisms were pathogenic to rabbits. They had inoculated four rabbits intravenously with cultures, and two of these rabbits died seven and 10 days later. Post-mortem examination showed small tuberculosis-like lesions in some of the internal organs. A third rabbit was killed and post-mortem examination revealed similar lesions. The fourth rabbit appeared healthy but, when killed 30 days later, a small lesion was found in the kidney.

In the present study, results obtained by the intravenous inoculation of N. farcinica culture into three rabbits (two of which received immunosuppressants) showed they gave positive blood cultures. Further, sera from all three were markedly inhibitory for N. farcinica growth in culture. This was attributed to the presence of antibodies.

The first rabbit which had received cyclophosphamide died eight months later. Autopsy revealed the presence of profuse, creamy-white pus in the pleural cavity and a dissemination of the same material in parts of the lungs. This material was of lymphocytic origin and cultures in TPB established the presence of N. farcinica. The other two rabbits were killed 293 days later. Autopsy revealed no evidence of any pathological lesions.

From the results obtained in the whole process of infected tick feeding on rabbits, it was found that 22 out of 25 rabbits on which infected A. variegatum and H. a. excavatum were fed gave positive blood cultures. Also, four out of these 22 rabbits which showed positive blood cultures also developed infarctive or necrotic lesions in the liver. Material from these lesions were cultured in TPB and gave positive N. farcinica growth.

From the above-mentioned results and also from the comments made by Fey, Holm & Teuscher (1954), we could

conclude that rabbits can easily acquire a generalized infection with N. farcinica but only a small proportion of those so infected develop lesions.

2. The ticks

The colonies of A. variegatum and H. a. excavatum were both maintained under similar conditions - i.e. all developing stages at 25°C and 85 per cent relative humidity, and all emerged instars at 18°C and 87 per cent relative humidity. Emerged ticks were retained in storage for at least a month to allow hardening before applying to a host. From the results obtained as regards the proportion of successful hatching and moulting, and as regards the yield of successfully engorged ticks from a given number applied, there was some indication that these standard maintenance conditions might be more favourable for A. variegatum than for H. a. excavatum. The minimum developmental periods of A. variegatum when incubated at a constant 25°C and 86 per cent relative humidity were as follows:

Pre-oviposition	7 to 8 days
Oviposition to hatching	49 to 62 days
Larva - nymph	12 to 15 days
Nymph - adult	15 to 18 days.

Since developmental periods are not always described in precisely the same way, it should be noted that

"pre-oviposition", as used here, refers to the period which elapses between the detachment of the replete female and the first appearance of eggs; "oviposition to hatching" refers to the period between the first appearance of eggs and the first emergence of larvae; and "larva - nymph" and "nymph - adult" both refer to the period between the detachment of replete instars on a particular day and the first emergence of the following instars in that batch.

These results agree with Walker's as quoted by Hoogstraal (1956) and Branagan's unpublished data, but Lewis (1932-1939) recorded 86 days as the period for oviposition to hatching of A. variegatum. Lewis, however, did not specify the temperatures at which these periods were observed.

It has to be noted here that all emerged instars were fed on rabbits, whereas Branagan and Walker both fed larvae and nymphs on rabbits and adults on rams. On the other hand, Lewis (1932; 1939) used chicken, hare and sheep as hosts.

The minimum developmental periods of H. a. excavatum incubated again at constant 25°C and 86 per cent relative humidity were as follows:

Pre-oviposition	5 to 8 days
Oviposition to hatching	38 days
Larva - nymph	6 to 10 days

Nymph - adult 22 to 28 days

Larva - nymph - adult 11 to 34 days.

It is seen that the developmental periods of H. a. excavatum were much shorter than those of A. variegatum except in the case of nymph - adult development.

The data obtained on the developmental periods of H. a. excavatum agree with those given by Serdyukova (1946b) but agree with only part of the data given by Feldman-Muhsam (1948). She gave no record of the pre-oviposition, oviposition and hatching periods.

A. variegatum is strictly a three-host tick, i.e. each instar feeds on one animal, drops off and moults, then feeds on another animal in following instar. The results in this study show that only 79 to 83 per cent of larvae applied completed engorgement, the mean engorged weight of which was 0·0031 gm. The time to engorge was five to 14 days (mean 8·89). This agrees entirely with Branagan's findings in which six to 14 days is recorded as the range for larval engorgement but does not agree with Lewis' (1932-1939) record which was five to eight days and neither with Walker's five days as quoted by Hoogstraal (1956). Only 80·50 per cent nymphs applied completed engorgement and the mean engorged weight was 0·0488 gm. Range of engorgement period was six to 17 days (mean 8·62). This again

agrees entirely with Branagan's record which was five to 12 days. Walker's findings, as quoted by Hoogstraal (1956), was five days - presumably only a minimum time was recorded. Only 54·54 per cent of females applied completed engorgement. The mean engorged weight was 1·8962 gm, achieved in 16 to 32 days (mean 23·15). These results agree with Branagan's findings, i.e. 14 to 31 days as a range for engorging A. variegatum females. The figure 54·54 per cent represents only the females which achieved full repletion allowing them to lay eggs.

As found in this study, H. a. excavatum could behave either as a two-host or three-host tick. Larvae, nymphs and adults were all fed on rabbits. Only 24·3 per cent larvae applied completed their engorgement. The mean engorged weight was 0·00049 gm, achieved in a period of five to 11 days (mean 6·81 days). These findings are higher than the record of Serdyukova (1946b) which was two to four days. This disparity might be due to the fact that Serdyukova's findings were from natural H. a. excavatum feeding on their usual hosts whereas, in the present study, the rabbits used for feeding cannot be regarded as normal hosts. Serdyukova (1946a) mentioned that, when larvae of H. a. excavatum were fed on calves (their usual host), they all engorged and detached as replete larvae; when, however, he fed larvae on rabbits some detached, moulted on the host and

re-attached, then finally detached as replete nymphs. Similar findings were obtained in this study - i.e. when H. a. excavatum larvae were fed on rabbits, some dropped as engorged larvae, some were collected as flat nymphs, while others again were collected as engorged nymphs. Moulting to nymphs definitely took place inside the ear-bags, and this would support Delpy's (1946) suggestion that, if some larvae remain on the host throughout the nymphal stage they first detach and wander to another place, moult and then re-attach.

The number of engorged nymphs recovered from those applied as flat larvae during this study was 21·3 per cent. The mean engorged weight was 0·0232 gm achieved in a time of 13 to 18 days (mean 15·63). But when 56 flat nymphs were fed on another rabbit, a total of 51·78 per cent completed engorgement. The mean engorged weight was 0·0236 gm in six to nine days (mean 7.55).

H. a. excavatum applied as flat nymphs would engorge in nine to 12 days. Though this is longer than the previous findings, Feldman-Muhsam (1948) did not specify the host species. In contrast, Serdyukova (1946b) recorded a period of only four and a half to six days. As shown in a previous result, 82·05 per cent of females applied completed their engorgement. The mean engorged weight was 0·6842 gm achieved in a period of eight to 16 days (mean 11·44). Neither Serdyukova nor Feldman-Muhsam

gave any record of the engorgement period of H. a. excavatum females.

3. Transmission

As has been mentioned before, an associated between bovine farcy and tick infestations has been noted since the earliest recognition of the disease (e.g. Neumann, 1888). It is, therefore, somewhat surprising that the true nature of this association does not appear to have been previously subjected to any critical examination, even though the closeness of the relationship had been described so well by such workers as Daubney (1927). The central purpose of this study was to make such a critical examination - i.e. were generalized N. farcinica infections caused by means of a skin contamination of the organism gaining entry through the traumata of tick attachments, or were they brought about by infective material being introduced during the processes of tick feeding? Further, if the latter case was true, could the infection be acquired from infected hosts by feeding ticks and then transmitted to another host by the following instar?

In this study, generalized N. farcinica infections did not result from minor skin traumata (pin-pricks and scarification) made in rabbits' ears and guinea pigs' backs which had previously been painted with cultures of

the organism, and neither did they result when ticks were fed on surfaces so treated. Though these findings have no more value than any other negative result, they did appear to indicate - in the light of other findings - that this route of infection is unlikely to be of significance. These findings must, however, be set against the expressed conviction of Sudanese nomads (Mostafa, 1962) that bovine farcy is introduced into cattle by means of traumata caused by thorns. This, of course, falls into the category of folklore, but it must be reckoned that such pastoral people are usually very precise observers and live in close association with their livestock, so that it would be unwise at this stage to dismiss entirely the possibility that bovine farcy infections gain entry through plain skin traumata. If a reason has to be sought why N. farcinica infections seldom became established through such skin traumata, it would seem quite likely that the organism does not normally compete successfully with the growth of other skin contaminants.

These studies showed that both A. variegatum and H. a. excavatum can acquire N. farcinica infection while feeding as larvae and nymphs; that both can retain this infection for considerable periods and that both can thereafter transmit this infection when feeding as the following nymphs and adults. Since the infection can

thus be retained through trans-stadial development, bovine farcy can thus assume the character of a true tick-borne disease when either of these species acts as an associated vector.

The period for which N. farcinica is retained in transmissible form by unfed ticks is obviously of primary epidemiological importance. In a first report (Al-Janabi, Branagan & Danskin, 1975), it was shown that N. farcinica could be transmitted by both nymphs and adults of A. variegatum at periods of up to nine weeks after its acquisition by the preceding larvae and nymphs. As shown by subsequent trials, this period of retention of transmissible infection can extend up to 33 weeks. On the other hand, while similar transmissions were effected by H. a. excavatum nymphs and adults eight weeks after acquisition as larvae and nymphs, no transmissions were effected after 29 weeks.

While this gave a superficial indication that A. variegatum might be a more efficient vector of N. farcinica than H. a. excavatum, some reservation was retained over this assumption in view of the impression - as stated above - that the standard maintenance conditions might not be entirely favourable to the latter species. For this reason, transmission was studied under variously modified environments.

Nymphs and adults of A. variegatum showed a varying

ability to transmit N. farcinica under different environmental conditions.

Transmission was achieved in both developmental stages of A. variegatum in Trials 5 and 6 in which ticks were kept in relative humidity of 87 per cent and 44 per cent respectively and the temperature was adjusted to make an approximate simulation of natural conditions. In Trial 5, three nymphs and eight adults were dead by day 17 and in Trial 6, 31 nymphs and 13 adults were dead by day 17. No transmission was achieved in Trial 7 in which the relative humidity was maintained at 20 per cent and temperature was similar to those in Trials 5 and 6 since all nymphs and adults were dead by day 10 of that regime. The period at which ticks transmitted infections in Trials 5 and 6 was 25 weeks. From these results it could be concluded that a high relative humidity favours the efficiency of A. variegatum as a vector for N. farcinica.

In the following three trials (8, 9 and 10) the temperature was maintained at a constant 25°C. Transmission was achieved in both developmental stages in Trial 8 in which the nymphs and adults were kept at a relative humidity of 85 to 87 per cent. There were eight nymphs and 14 adults dead by day 45.

The results from Trial 9 in which nymphs and adults were kept at a relative humidity of 44 per cent, showed

that all nymphs were dead by day 10 and only the surviving nine adults were able to transmit infections. However, no transmission was achieved in Trial 10 in which nymphs and adults were kept at a relative humidity of 20 per cent. In this trial all nymphs and adults were dead by day 40. Periods at which ticks transmitted infections in Trial 8 and 9 were 23 weeks post-emergence.

Again, these results show that higher relative humidities favour the efficiency of A. variegatum as a vector but only by means of prolonging the survival of the ticks themselves. In short, as long as the ticks survived under these various conditions, they were able to transmit the infections.

It is, of course, recognised that these laboratory conditions are not representative of the large variations in temperature and humidity which occur in nature. Within the Kenyan distribution of A. variegatum, for example, the mean maximum and mean minimum temperatures at Kisumu are, respectively, 30·5°C and 17·5°C in February, and 27·5°C and 16·0°C in July. At Nairobi, the corresponding figures are 28·0°C and 14·5°C, and 22·5°C and 12·0°C. There will thus be significant differences in the developmental periods - i.e. the range of larva - nymph and nymph - adult developmental periods in A. variegatum is, respectively, 16 to 26 days and 18 to

27 days at 25°C, and 46 to 67 days and 102 to 120 days at 18°C (Branagan, unpublished data). Hence, a relatively small variation in mean temperature will result in a considerable extension of the period which elapses between the acquisition of N. farcinica infection by A. variegatum and the first opportunity for the following instars to transmit this infection. The biology of H. a. excavatum has not been subjected to similar studies, but it seems likely that the temperature variations in the distribution of this species will produce similar differences in developmental periods.

Further, these variations in temperature levels will obviously produce differing survival expectancies as they operate in different humidities, so that a given humidity will result in a more prolonged survival at a lower temperature than at a higher one. Though the results obtained in this study suggest that N. farcinica infections are retained in transmissible form by A. variegatum for as long as these ticks survive, the demonstration that H. a. excavatum outlives such infections indicate a possibility that environmental factors may be influential in this aspect - i.e. there was some reason to believe that the laboratory conditions were not entirely suitable for H. a. excavatum and, thus, this unsuitability might be extended into reducing the capacity to retain transmissible infections. An examination

of these possible environmental influences on vector efficiency would, of course, require a prolonged and intricate study.

It was noted that infections transmitted by the adults of A. variegatum and H. a. excavatum were slower in becoming patent than those transmitted by the nymphs of both species. This was attributed to the difference in feeding behaviour which exists between immature and adult ixodids - i.e. after attachment, the engorgement of larvae and nymphs progresses steadily until repletion is achieved. On the other hand, adult females suspend the engorgement process at an early stage until they are mated, whereupon full feeding is resumed and progresses until repletion is achieved. Given that N. farcinica infections are transmitted by way of secreted saliva, the main bulk of the pathogens will thus not be transmitted by female ticks until after they have been mated. This event, in turn, will not occur until after males have completed a blood meal and, hence, transmission of N. farcinica by adult females will be delayed by an appropriate period - probably at least four days.

Even though there was a continuing failure to make a critical examination of the true nature of relationship between ticks and bovine farcy, the presence of the disease has long been regarded as dependent on the co-existence of a suitable population of ticks. For

example, Neumann (1888) concluded that the presence of bovine farcy in France and Guadeloupe was due to the introduction of tick-infested Zebu cattle from Senegal, and a similar opinion was held by Besson (1913). Again, though he considered the association to be mechanical rather than truly tick-borne, Bernard (1924) considered that the freedom of certain areas - e.g. Martinique - from the disease could be ascribed to an absence of a suitable tick population. Recently, the same opinion has been put forward by Mostafa (1962) concerning the presence or absence of A. variegatum populations in different areas in the Sudan.

This evidence is largely circumstantial, but its possible implications should, nevertheless, be considered as of epidemiological importance in circumstances where cattle suffering from bovine farcy are being moved from one area to another. When taken in conjunction with the findings of this study, the movement of animals infected with bovine farcy presents a danger of disease spread if the animals are infested with ticks at the time of transfer and, particularly so, if the receiving area is either capable of supporting a suitable tick population or does so already. Hence, when moving cattle from an area where bovine farcy is known to occur, it would be advisable to treat with suitable ixodicides either before movement or at the time of arrival, and preferably on

both occasions. If suitable means of applying ixodicides were not available then a period of quarantine is recommended in order to ensure that all immature ticks present on these animals will have completed their engorgement and will have detached. To be effective, this period of quarantine would have a minimum period decided by the maximum time taken by nymphs to complete their engorgement and would thus - in the case of A. variegatum - be about 20 days. A maximum period in the quarantine area would, however, be set in consideration of the possibility that larvae engorging at the beginning of the period could complete their development and re-attach as emerged nymphs at the end of the period. This development period at 25°C is 16 to 26 days (Branagan, 1970), and may well be not too much at variance with that in the hotter sectors of the A. variegatum distribution. Hence, effective quarantine arrangements would probably consist of a total period of three weeks with facilities for movement to entirely fresh ground after the first 12 days of the period. Since H. a. excavatum does not invariably follow a two-host feeding behaviour pattern, it would be as well if similar quarantine arrangements were imposed when this species is involved.

When considering the overall distribution of bovine farcy in relation to tick populations, one is tempted to

find patterns determined by various climatic factors. This is probably an oversimplified approach in view of the complicated array of physical influences on tick populations which operate at a microclimatic level and which could be at considerable variance with values expressed as crude meteorological data. A valid analysis of climatic patterns would, therefore, depend upon extensive field studies on the development and survival of populations of tick vectors in a representative variety of habitats.

This study showed that N. farcinica could be transmitted trans-stadially by both A. variegatum and H. a. excavatum in the manner of a true tick-borne disease. The failure to demonstrate trans-ovarial passage, however, suggests that N. farcinica will have to be acquired afresh by each successive generation if a tick population is to remain permanently infected.

It is noted that bovine farcy occurs in countries where neither of these ixodid species are known to occur - e.g. California, Colombia and the East Indies. It is thus likely that other ixodid species are capable of acting as vectors for this disease. Species of the genus Amblyomma that come to mind are, for instance, A. americanum, A. cajennense and A. maculatum in the New World, and A. testudinarium in the Far East. One might also suspect a similar association with Hyalomma detritum and H. marginatum. These possibilities offer a wide field for future epidemiological studies.

BIBLIOGRAPHY

AL-JANABI, B. M. (1969). Intersuchungen zur Ixodidae - Faun der landwirtschaftlichen Nutztiere (Shaf, Ziege, Rind und Kamel) in der Region Bagdad. Dissertation Dr. Med. Vet. Karl-Marx Universität Leipzig, DDR.

AL-JANABI, B. M., BRANAGAN, D. and DANSKIN, D. (1975). The trans-stadial transmission of the bovine farcy organism, Nocardia farcinica, by the Ixodid Amblyomma variegatum (Fabricius, 1794). Tropical Animal Health and Production, 7, 205-209.

AWAD, F. I. (1960). Nocardiosis of the bovine udder and testes. Veterinary Record, 72, 341-342.

AWAD, F. I. and KARIB, A. A. (1958). Studies on Bovine farcy among cattle in Sudan. Zentralblatt für Veterinärmedizin, 5, 265-271.

BAILEY, K. P. (1960). Notes on the rearing of Rhipicephalus appendiculatus and their infection with Theileria parva for experimental transmission. Bulletin of Epizootic Diseases of Africa, 8, 33-43.

BERGEY'S manual of determinative Bacteriology (1957) by Robert S. Breed, E. D. G. Murray, Nathan R. Smith. 7th edition, London. Baillière, Tindall and Cox

Limited.

BERNARD, M. (1924). La medicine veterinair a la Guadeloupe. Maladies des Bovides, 100, 732-736.

BERNARD, M. (1927). Thesis, France, Imprimerie, Ouvriere, Toulouse. Cited by Mostafa, I. E. (1966). Bovine Nocardiosis (Cattle farcy). A Review. Veterinary Bulletin, 36, 189-193.

BESSON, A. (1913). Practical Bacteriology, microbiology and serum therapy. (Medical & Veterinary.) A textbook for Laboratory use. Translated from the 5th French edition by H. J. Hutchens, p. 667.

BISHOP, L. M. and FENSTERMACHER, R. (1933). An acid-fast Actinomyces from lesions resembling bovine tuberculosis. Cornell Veterinarian, 23, 288-296.

BLANC, C., BRUNEAU, J. and CHABAUD, A. (1950). Presence de Rickettsia (Coxiella) burnetti chez des tiques de l'espece Amblyomma variegatum recueillies en Afrique Equatoriale Francaise. Compte rendu hebdomadaire des Seances de l'Academi des Sciences.

BONCIU, C., BONCIU, O. and PETROVICI, M. (1964). Nocardiose des Cobayes et des Lapins. Archives

roumaines de pathologie expérimentale et de microbiologie, 2, 365-378.

BRANAGAN, D. (1970). The development and survival of Rhipicephalus appendiculatus Neumann, 1901 (Ixodidae) in laboratory and field. Ph.D. Thesis, University of Edinburgh.

BRANAGAN, D. (1974). The feeding performance of the Ixodid Rhipicephalus appendiculatus Neum. on rabbits, cattle and other hosts. Bulletin of Entomological Research, 64, 387-400.

BUXTON, P. A. and MELLANBY, K. (1934). The measurement and control of humidity. Bulletin of Entomological Research, 25, 171-175.

CAPPONI, M., FLOCH, H., CHAMBON, L., CAMICAS, J. L., CARTERON, B. and GIROUD, P. (1969). Amblyomma variegatum D'Origine Africaine ou Antillaise Et Rickettsies Du Genre Dermacentroxenus. Bulletin de la Societe de pathologie exotique, 62, 1011-1017.

COUZIN, M. (1879). Etude sur la farcin de la Guadeloupe. Revue Veterinaire, 1st series, 4, 400-405.

CRUICKSHANK, R. (1969). Medical Microbiology. A guide to the laboratory diagnosis and control of

infection. pp. 647-655, eleventh edition, E. & S. Livingstone Ltd., Edinburgh and London.

CRUZEL, A. (1869). Traite des maladies de l'espéce bovine. 1re edition. Cited by Nocard, M. (1888). Note sur la maladie des Bouefs de la Guadeloupe connue sous le nous de farcin. Annals de l'institute Pasteur, 6, 293-302.

CURASSON, G. (1942). Traite des pathologie exotique veterinaire et comparee, 2, 139. Maladies microbiennes. Paris: Vigot Fréres.

DAUBNEY, R. (1927). Bovine lymphangitis or Tropical Actinomycosis. Journal of Comparative Pathology and Therapeutics, 40, 195-216.

DAUBNEY, R. (1930). Natural transmission of heart-water of sheep by Amblyomma variegatum. Parasitology, 22, 260-267.

DELPY, L. P. (1946). Révision par des voies experimentales, du genre Hyalomma C. L. Koch (1844) (Acarina, Ixodoidea. Note preliminaire). Annales de parasitologie humaine et comparee, 21, 265-293.

DELPY, L. P. (1949). Révision par des voies experimentales, du genre Hyalomma C. L. Koch (1844)

(2e partie). Annales de parasitologie humaine et comparee, 24, 97-109.

DONATIEN, A. and LESTOQUARD, F. (1936). Rickettsia bovis, nouvelle espece pathogene pour le bouef. Bulletin de la societe de pathologie exotique, 29, 1057-1061. Cited by Neitz, W. O. and Du Toit, P. J. (1938). Tick borne diseases. Journal of South African Veterinary Medical Association, 9, 85-124.

DONATIEN, A. and LESTOQUARD, F. (1937). Transmission naturelle d'Eperythrozoon wenyoni par une tique du genere Hyalomma. Bulletin de la Societe de pathologie exotique, 30, 459-460. Cited by Neitz, W. O. and Du Toit, P. J. (1938). Tick borne diseases. Journal of South African Veterinary Medical Association, 9, 85-124.

DRURY, R. A. and WALLINGTON, E. A. (1967). Carleton's Histological Technique, p. 129. Fourth edition. Oxford University Press, New York/Toronto.

EDELSTEN, R. M. (1975). The distribution and prevalence of Nairobi sheep disease and other tick-borne infections of sheep and goats in Northern Somalia. Tropical Animal Health and Production, 7, 29-34.

EL-NASRI, M. (1961). Some observations on Bovine farcy. Veterinary Record, 73, 370-372.

FELDMAN-MUHSAM, B. (1948). On larvae and nymphs of some species of palastinean Hyalomma. Parasitology, 39, 138-147.

FELDMAN-MUHSAM, B. (1954). Revision of the genus Hyalomma. I. Description of Koch's types. Bulletin of Research Council, Israel, 4 (2), 150-170.

FEY, H., HOLM, P. and TEUSCHER, E. (1954). Nocardiosen. Kasuistische Mitteilung über einen Fall von septischer Nocardiose beim Hund und zwei Fälle von Nocardia-Abortus beim Rind. Schweizer Archiv für Tierheilkunde, 96, 642-651.

GIROUD, P., CAPPONI, M., ESCUDE, A., FAURAN, P. and MOREL, P. (1966). 1. Solement d'une souche de R. conori de larves d'Amblyomma variegatum de la Guadeloupe. Bulletin de la Societe de pathologie exotique, 59, 283-289.

GRAY, J. D. A. (1935). An aerobic acid-fast Actinomyces in cows milk due to an udder infection. Journal of Dairy Research, 6, 49-55.

HEISCH, R. B., McPHEE, R. and RICKMAN, L. R. (1957). The epidemiology of tick typhus in Nairobi.

East African Medical Journal, 34, 459-477.

HENNING, M. W. (1949). Animal disease in South Africa. Central news agency limited; South Africa. Cited by Arthur, R. (1962). Ticks and diseases, page 379.

HOLMES, J. D. (1908). A peculiar form of Streptothricosis among cattle. Journal of Tropical Veterinary Science, Calcutta, 3, 289-300.

HOOGSTRAAL, H. (1956). African Ixodoidea. 1. Ticks of the Sudan, with special reference to Equatoria Province and with preliminary reviews of the genera Boophilus, Margaropus, and Hyalomma, Washington, D.C., United States Department of the Navy, Bureau of Medicine and Surgery. Research Report No. NM 005 050.29.07.

HOOGSTRAAL, H. and KAISER, M. N. (1959). Observation on Egyptian Hyalomma ticks (Ixodoidea, Ixodidae). Five. Biological notes and differences in identity of H. anatolicum and its subspecies anatolicum Koch and excavatum Koch among Russian and other workers' identity. Annales of the Entomological Society of America, 52 (3), 243-261.

JARVIS, E. M. (1918). Report on Ixodid lymphangitis.

(German East Africa Campaign 1916-1917.) Made to the A.D.V.S. Dar-es-Salaam. Veterinary Journal, 74, 44-53.

KUSEL'TAN, I. V. (1967). Nocardiosis of lambs in the Tadzhik SSR. In "Maloizuchennie zabolevaniya sel'skokhozyaistvennykh zhivotnykh". Eds. Kovalenko, Ya. R., Laktionov, A. M., Zotov, A. P. and Ostapenko, K. A. In Russian. Izd. "Kolos"; Moscow, pp. 110-117.

LAFOSSE, B. (1849). Farcin du boeuf. Journal des vétérinaires du Midi, 12, 452.

LAFOSSE, B. (1853). De la gourme des bétes bovines. Journal des vétérinaires du Midi, 16, 481-494.

LEWIS, E. A. (1932). Some tick investigations in Kenya colony. Parasitology, 24 (2), 175-182.

LEWIS, E. A. (1939). The ticks of East Africa. Part II. Tick-borne diseases and their control. Empire Journal of experimental agriculture, 7 (28), 299-304.

LOTOTSKY, B. V. and POKROVSKY, S. A. (1946). An experiment in the organization of a minimum complex of measures against haemosporidiasis in

Northern Tadzhikistan (In Russian). Izvestiya Tadzhikskogo filiada Akademii nauk SSSR, 6, 66-74.

LUQUE, F. (1946). Streptothricosis Bovina en Colombia, Revista de Medicina Veterinaria, XV, 1-19.

MACADAM, I. (1962a). Bovine streptothricosis. Production of lesions by the bite of the tick Amblyomma variegatum. Veterinary Record, 74, 643-646.

MACADAM, I. (1962b). Tick transmission of bovine pasteurellosis. Veterinary Record, 74, 689-690.

MAGNUSSON, M. and MARIAT, F. (1967). Delineation of Nocardia farcinica by Delayed Type Skin Reaction on Guinea Pigs. Journal of general Microbiology, 51, 151-158.

MAILLET, M. (1837). Du farcin considéré dans l'espece du bouef. Recueil de médicine vétérinaire, 14, 57-63.

MEMERY, G., MORNET, P. and CAMARA, A. (1958). Premiers cas authentiques de farcin de bouef en A.O.F, Revue ó élévage et de médicine vétérinaire des pays tropicaux, 1, 11-15.

MORRIS, E. O. (1951). Observations on the life cycle of Nocardia. Journal of Hygiene, 49, 175-180.

MOSTAFA, I. E. (1962). Studies on cattle nocardiosis in the Sudan. Ph.D. Thesis, University of London.

MOSTAFA, I. E. (1967a). Studies of bovine farcy in the Sudan. I. Pathology of the disease. Journal of Comparative Pathology, 77, 223-229.

MOSTAFA, I. E. (1967b). Studies on bovine farcy in the Sudan. II. Mycology of the disease. Journal of Comparative Pathology, 77, 231-237.

MOSTAFA, I. E. (1967c). Studies on bovine farcy in the Sudan. III. The pathogenicity of Nocardia farcinica. Journal of Comparative Pathology, 77, 347-352.

MOUSIS, J. (1837). Memoire sur le farcin des betes a grosses cornes. Recueil de médicine vétérinaire, 14, p. 113. Cited by Nocard, M. and Leclainche, E. (1903). Farcin du Bouef. Les maladies microbiennes des Animaux, II, 363-368.

NEITZ, W. O. (1956). A consolidation of our knowledge of the transmission of tick-borne diseases. Onderstepoort Journal of Veterinary Research, 27, 115-163.

NEUMANN, L. G. (1888). Traite des maladies parasitaires non microbiennes des animaux domestiques. Paris, 674 pp.

NOCARD, M. (1888). Note sur la maladie des Bouefs de la Guadeloupe. Connue sous le Nom de farcin. Annales de l'institut Pasteur, 6, 293-302.

NOCARD, M. and LECLAINCHE, E. (1903). Farcin du bouef les maladies microbiennes des Animaux, II, 363-368.

PAVLOVSKY, E. N., PERVOMAISKY, G. S. and CHAGIN, K. P. (1954). Intensity of feeding of one (species) and simultaneous feeding of two species of pasture tick (Ixodidae) on rabbits (In Russian). Zoologicheskii zhurnal, 33 (3), 497-506.

PEGRAM, R. G. (1973). An unusual form of lymphandenitis in sheep and goats in the Somali democratic republic. Tropical Animal Health and Production, 5, 35-39.

PERPEZAT, A., MARIAT, F., DESTOMBES, P. and THOME, M. (1963). Importance Du farcin Chez le Zebu du Tchad. Bulletin de la societe de pathologie exotique, 3, 375-385.

PERVOMAISKY, G. S. (1949). Pathogenetic development in ticks of the family Ixodidae. Zoologicheskii zhurnal, 28, 523-526. (Moscow in Russian.) Summary in Review of applied Entomology, 41, 67. 1953.

PIRANI, A. (1930). Contributo alla conoscenza di alcune infezioni del bastiame un Eritrea. Clinica veterinaria, 4, 216-222.

PLOWRIGHT, W. (1956). Cutaneous streptothricosis. I. Introduction and epizootological features in Nigeria. Veterinary Record, 68, 350-355.

POMERANZEV, B. I., MATIKASHVILY, N. V. and LOTOTSKY, B. (1940). An ecological and faunistic outline of Ixodidae ticks occurring in Transcaucasia. (In Russian, English summary.) Parasitologiceskij sbornik. Zool. Inst. Akademii Nauk SSSR, 7, 100-103; (1939).

RAYMOND, F. (1910). Lymphangite infectieuse du bétail. Journal of Tropical Veterinary Science, Calcutta, 5, 213-232.

REISS-GUTFREUND, R. J. (1956). Un nouveau reservoir de virus pour Rickettsia prowazeki les animaux domestique et leur tiques. Bulletin de la Societe de pathologie exotique, 49, 946-1023.

ROBINSON, L. E. (1926). The Genus Amblyomma; Being part IV commencing volume II. Ticks: A monograph of the Ixodidae. By George, H. F. Nuttall, F.R.S., C. Warburton and L. E. Robinson. **Cambridge University Press, London.**

SCHLEGEL, M. (1913). Bericht über die tatigkeit des Tierhygienischen Institut der Universität Freiburg. Zeitschrift für Tiermedizin, 17, 369-399.

SERDYUKOVA, G. V. (1946a). Instances of the locas Mass increase of the tick Hyalomma anatolicum anatolicum Koch in Tadzhikistan and their causes. (In Russian.) Izvestiya Tadzhikskogo filiada Akademii Nauk SSR, 6, 60-63. Summary in the Review of applied Entomology, 35, 73-74, (1947).

SERDYUKOVA, G. V. (1946b). On the cycle of development of the tick Hyalomma anatolicum anatolicum Koch. (In Russian.) Bulletin de l'Academie des sciences de L'URSS, Séries Biologique, 2-3, 199-202. Summary in the Review of applied Entomology, 35, 140, (1947).

SFORZA, M. (1940). Piuralita di specie del cosidetto Actinomyces farcinicus. Archivio italiano di scienze mediche coloniali, 21, 406-418 et Veterinary Bulletin, 1942, 12, 406.

SHEATHER, A. L. (1920). Bovine lymphangitis. Journal of Pathology and Therapeutics, 33, 158-185.

SORILLON, M. (1829). Example du farcin dans le bouef. Recueil de medicine veterinaire, 6, p. 631.

THEILER, G. (1959). Biological notes: ticks and their host preferences. Journal of the South African Veterinary Medical Association, 30, 195-203.

TOPLEY, W. W. C. and WILSON, G. S. (1964). Principles of Bacteriology and Immunity. Edited by T. & W's Principles of Bacteriology and Immunity. 5th ed. by Sir G. S. Wilson and A. A. Miles with the help of R. Knox, A. O. Macrae, etc. 2 Vols. London 1964.

TRAUM, J. (1919). Further Report on lymphangitis in cattle caused by acid-alcohol-fast organism. Journal of the American Veterinary Medical Association, 55, 639-652.

TREVISAN, V. (1889). I. Generi e le specie delle Bacteriacee. Milano: Zanaboni e Gabuzzi.

Reproduced in the International Bulletin of Bacteriological Nomenclature and Taxonomy (1952), 2, 13.

VRYBURG, A. (1907). Une espéce particuliére du farcin du bouef sévissant en Deli (Sumatra). Recueil de Medicine Veterinaire, 84, 31-39, 171-177 and 241-248.

WAKSMAN, S. A. (1957). Actinomycetaceae Buchanan. In Bergey's manual of Determinative Bacteriology, 7th ed., p. 718. Baltimore: The Williams and Wilkins Company.

WAKSMAN, S. A. and HENRICI, A. T. (1943). The nomenclature and classification of the Actinomycetes. Journal of Bacteriology, 46, 337-341.

WALKER, J. B. (1974). The Ixodid ticks of Kenya. A Review of present knowledge of their hosts and distribution. London. Commonwealth Institute of Entomology.

WILSON, S. G. (1948). A method of assessing the scaricidal properties of DDT and "gamaxane" preparations in field trials. Bulletin of Entomological Research, 39 (2), 260-276.

WILSON, S. G. (1950). A check-list and host-list of

Ixodoidea found in Nyasaland, with description and biological notes on some of the rhipicephalids. Bulletin of Entomological Research, 41 (2), 415-428.

WILSON, S. G. (1953). A survey of the distribution of the tick vectors of East Coast fever in East and Central Africa. Proceedings 15th of the International Veterinary Congress, 1, 287-290.

XIROUDAKIS, N. (1935). Lymphangite epizootique (farcin du bouef). Recueil de Médicine Vétérinair exotique de l'Ecole d'Alfort, 8, 112-117.

YEOMAN, G. H. and WALKER, J. B. (1967). The Ixodid Ticks of Tanzania. A study of the zoogeography of the Ixodidae of an East African Country. London. Commonwealth Institute of Entomology.

APPENDIX I.

The preparation of smears and sections

For the identification of *N. farcinica* in material from cultures or lesions, smears were air-dried, fixed with heat and then stained either by Gram's or Ziehl-Neelsen's methods. The precise details of the two methods are as follows -

Gram's method:

1. Stain with 0·5% crystal violet for 1 minute.
2. Pour off, and stain with Gram's iodine for 1 - 2 minutes.
3. Differentiate with absolute alcohol for 15 - 30 seconds.
4. Counterstain with basic fuchsin (1 in 20) or with 1% neutral red for 30 seconds.
5. Wash with water, and blot dry.

Modified Ziehl-Neelsen's method:

1. Stain with heated, filtered carbol fuchsin for 5 minutes.
2. Wash with water.
3. Flood with 1% acid alcohol for 5 - 10 seconds.
4. Wash with water.
5. Counterstain with 1% methylene blue for 30 seconds.
6. Wash with water, and allow to dry.

Tissues for histological examination were fixed in

buffered formol saline for about 48 hours - or slightly shorter or longer according to the thickness of the specimen. They were then prepared in an automatic tissue processor (Histokinette) in which they were subjected to the following treatments -

1. Two periods of 3 hours each in 70% alcohol.
2. Three hours in 90% alcohol.
3. Three periods of 2 hours each in absolute alcohol.
4. Four hours in methyl benzoate.
5. Four hours in methyl benzoate with celloidin.
6. Fifteen minutes in benzene.
7. Two periods of 2 hours each in molten paraffin wax.
8. One hour in paraffin wax in a vacuum-embedding bath.
9. Final embedding in wax.

Histological sections were cut with a Leitz microtome (Model Minot-Mikrotom Typ 1212) at 6 μ thickness, and were stained by either of two methods - as follows -

Haematoxyline-eosin method:

1. Two 2-minute rinsings with xylol.
2. Two 1½-minute rinsings with absolute alcohol.
3. Ditto with 90% alcohol.
4. Ditto with 70% alcohol.
5. Wash in water.
6. Stain with haematoxyline for 20 - 30 minutes.
7. Rinse in running water.
8. Differentiate with acid alcohol for 5 - 30 seconds.

9. Rinse in running water.
10. Wash in Scott's tap-water substitute for 3 - 5 minutes.
11. Stain with 1% eosin for 3 minutes.
12. Rinse in water.
13. Two periods of 1½ minutes each in 70% alcohol.
14. Ditto in 90% alcohol.
15. Ditto in absolute alcohol.
16. Ditto in xylol.
17. Mount in DePeX or in Canada balsam.

Gram's method:

1. Two periods of 2 minutes each in xylol.
2. Two periods of 1½ minutes each in absolute alcohol.
3. Ditto in 90% alcohol.
4. Ditto in 70% alcohol.
5. Wash in water.
6. Stain for 2 minutes in 0·5% crystal violet.
7. Stain with Gram's iodine for 2 minutes.
8. Differentiate in absolute alcohol for 2 minutes.
9. Counterstain with 1% neutral red for 2 minutes.
10. Rinse in water.
11. Two periods of 1½ minutes each in 70% alcohol.
12. Ditto in 90% alcohol.
13. Ditto in absolute alcohol.
14. Ditto in xylol.
15. Mount in DePeX or in Canada balsam.

APPENDIX II.

The preparation of culture media

Blood agar was prepared by suspending 19·5 g of Columbia agar base (Oxoid) in 500 ml of distilled water, which was then brought to the boil. The whole was then autoclaved at 121°C for 15 minutes and then cooled to 50°C before adding 5% of blood. Defibrinated horse blood was used - this being supplied at monthly intervals in 25 ml bottles (Wellcome Foundation). The medium was then poured into petri dishes and incubated at 37°C for 24 hours as a check on sterility before storing at 10°C for use as and when required.

Nutrient agar was prepared and stored in the same way except that no blood was added.

Sabouraud's agar was prepared according to the following formula -

Glucose	20 g
Mycological peptone	5 g
Agar	10 g
Water	500 ml.

The solid ingredients were dissolved in the water while heating slowly. The whole was then autoclaved at 121°C for 15 minutes before pouring as slopes in universal vials. Sterility tests and storage conditions were as above. Sabouraud's broth was prepared in the same way but without the agar, and was contained in universal

vials.

Tryptose phosphate broth (TPB) was prepared by mixing 14·5 g of proprietary medium (Oxoid) in 500 ml of heated water. The whole was then distributed into individual universals in 6 ml volumes. These were then autoclaved at 121°C for 15 minutes before cooling, testing and finally storing at 10°C as above.

Tryptose phosphate broth with glycerine (TPB + glycerine) was prepared in the same way but with the addition of 1·2% glycerine.

Trop. Anim. Hlth Prod. (1975) 7, 205–209

THE TRANS-STADIAL TRANSMISSION OF THE BOVINE FARCY ORGANISM, *NOCARDIA FARCINICA*, BY THE IXODID *AMBLYOMMA VARIEGATUM* (FABRICIUS, 1794)

B. M. Al-Janabi, D. Branagan and D. Danskin

Centre for Tropical Veterinary Medicine, Easter Bush, Roslin, Midlothian, Scotland

SUMMARY

Larvae and nymphs of A. variegatum *acquired* N. farcinica *infections from rabbits and, at periods of up to 9 weeks, transmitted these infections to other rabbits when feeding as the following instars. This clear demonstration of the trans-stadial transmission of the bovine farcy organism confirms the existence of an epidemiological feature which has previously only been suspected.*

INTRODUCTION

Though numerous authors from Neumann (1888) to Mostafa (1962) have commented on the frequent association between bovine farcy and infestations of the genus *Amblyomma*, no record could be found of a precise examination of the nature of this relationship. It was not clear, for instance, whether the causal organism (*Nocardia farcinica*) was merely a skin contaminant which gains entry through infestational traumata, or whether the pathogen is transmitted in a truly tick-borne manner, i.e., being acquired by ticks while feeding on an infected host and then passed on to another host during the feeding of the following instar.

This paper describes trans-stadial transmissions of *N. farcinica* from one rabbit to another by *Amblyomma variegatum* (Fabricius, 1794). Circumstances prevented the use of cattle in these experiments but it was considered that the principles of transmission at issue could as well be demonstrated in rabbits, which are eminently suitable as hosts for tick feeding. The work forms part of a much larger study and these particular results are presented at this juncture since they give the first clear evidence of this form of transmission with the bovine farcy organism.

MATERIALS AND METHODS

Original cultures of *N. farcinica* were obtained from the Medical School, University of Glasgow through the kindness of Dr. John Gordon. Stock cultures were stored on Lowenstein–Jensen medium at 4°C after incubation at 37°C for 7 days and tryptose phosphate broth (TPB) cultures at 37°C were used for propagation and identification.

The organism was identified in smears from TPB cultures by the characteristic morphology (Mostafa, 1967) when stained by standard Gram and Ziehl–Neelsen methods and by subculture in Sabouraud's medium, where typical "cauliflower" colonies could be observed after about 10 days' incubation at 37°C. These "cauliflower" colonies could also be seen on blood agar and nutrient agar.

The presence of circulating infection in rabbit hosts was tested by culturing 1 ml of whole blood (drawn with full sterile precautions from an ear vein) in 6 ml of TPB at 37°C. This incubation of an original culture was continued for 21 days unless a positive result was obtained in a subculture made at the 7th day of incubation or again at the 14th day. In turn, each subculture was incubated for 8 days and examined at intervals for typical surface growth and by means of stained smears. When seeded on to blood–agar and Lowenstein–Jensen medium, material from positive whole blood

cultures produced colonies with characteristics identical to those produced by the original cultures supplied by Dr. Gordon. As far as is known, no specific biochemical test has yet been described for *N. farcinica.*

Of diverse breed, the rabbit hosts were all between 4 and 6 months old, and culture of blood samples—as above—was used to confirm the absence of *N. farcinica* in each animal before use. Original infections were transmitted to rabbits by the intravenous inoculation of 1 ml of a 7-day TPB culture. Strict aseptic precautions were taken before and after the inoculation of these original infections. Before commencing the tick transmission experiments, preliminary trials had demonstrated that *N. farcinica* could be reliably isolated in blood samples taken 7 days after a rabbit had been infected in this manner.

This finding was of value in timing the application of ticks to the source rabbits, for the presence of large numbers of either larvae or nymphs on each ear did not permit satisfactory blood sampling. Infection in these rabbits was confirmed after tick engorgements had been completed. Infestations in the transmissions by nymphs were confined to one ear, leaving the other clear for sampling. Though both ears were occupied in the adult transmissions, these infestations were sufficiently sparse to allow a suitable area of skin to be cleaned for sampling. Evidence of tick-transmitted infection was sought in blood samples taken every third or fourth day from the 7th day after infestation until the 25th day, and thereafter at weekly intervals until the 63rd day.

Since cattle could not be used in these experiments, rabbits were chosen as hosts because of their outstanding merits in tick-feeding procedures. In the past, however, rabbits have had a somewhat unsatisfactory reputation as hosts for *N. farcinica.* Immunosuppressants were therefore used in the first two experiments with a view to, first, raising the pathogen levels in the blood acquired by the ticks; and, second, emphasising the pathogen's presence in rabbits after transmission by ticks. In Experiment I, cyclophosphamide ("Endoxana"; Ward, Blenkinsop) treatment was commenced on the day that Rabbit I was infected and was continued for a total of four consecutive days at the rate of 72·5 mg per kilo body weight. In the other two rabbits in Experiment I and all three rabbits in Experiment 2, betamethasone ("Betsolan"; Glaxo) treatment by injection of aqueous solution was commenced either on the day of inoculation of culture or when ticks were applied, and was continued for a total of 18 consecutive days at the rate of 1·6 mg per kilo body weight. As shown by the third experiment, this resort was unnecessary but the findings with immunosuppressants are nevertheless included since the use of these agents in no way impairs the validity of the transmission principles which were established.

Ticks were drawn from the colony of *A. variegatum* which has been maintained on rabbit hosts at the Centre for Tropical Veterinary Medicine (CTVM) since 1969 and which was founded from East Africa stock. All developing stages are incubated at 25°C; all emerged instars are stored at 18°C; and relative humidity is maintained throughout at a constant 85–86 per cent in closed culture jars by means of saturated potassium chloride solutions (Buxton and Mellanby, 1934).

Prior to use in transmission experiments, the absence of *N. farcinica* in ticks was confirmed by washing random samples of each batch in 70 per cent alcohol for 30 minutes to remove surface contaminants; by grinding up the washed samples in 6 ml TPB to which antibiotics had been added; and then incubating the whole at 37°C for 7 days. In no case was *N. farcinica* isolated from the stock of *A. variegatum.* This same process was used to confirm the presence of *N. farcinica* in ticks after they had completed engorgement on infected rabbits.

Ticks were applied to rabbits according to the method of Bailey (1960).

EXPERIMENTS AND RESULTS

Experiment 1

Four days after Rabbit 1 had been infected and on the last day of cyclophosphamide treatment, 1800 larvae and 400 nymphs were applied to the left and right ears respectively. A total of 1648 larvae and 391 nymphs completed engorgement in 6–9 and 6–10 days respectively and moulted to nymphs and adults in 13–16 and 16–24 days—again respectively. Infection with *N. farcinica* in Rabbit 1 was confirmed in a blood sample taken immediately after the infestation had been completed. The emerged instars were used for the transmissions to Rabbits 2 and 3.

Forty-one days after the completion of moulting, 100 nymphs were applied to the left ear of Rabbit 2 and betamethasone treatment was commenced. Blood samples from Rabbit 2 were negative for *N. farcinica* at day 7 but were positive from day 10 onwards. Engorgement was completed by 79 nymphs in 7–11 days.

Thirty-nine days after completion of moulting, five males and five females were applied to each ear of Rabbit 3 and betamethasone treatment was commenced. Blood samples from Rabbit 3 were negative at days 7 and 10 but were positive from day 14 onwards. Nine females completed engorgement in 21–31 days.

Experiment 2

Four days after infection and the commencement of betamethasone treatment, 1500 larvae and 400 nymphs were applied to the left and right ears of Rabbit 4. Engorgement was completed by 1293 larvae and 381 nymphs in 8–15 days. Larvae and nymphs moulted to nymphs and adults in 14–16 and 18–23 days. Blood samples taken immediately after infestation had been completed confirmed that Rabbit 4 was infected with *N. farcinica*. The emerged instars were used for the transmissions to Rabbits 5 and 6.

Forty-one days after completion of moulting, 100 nymphs were applied to the left ear of Rabbit 5 and betamethasone treatment was commenced. Engorgement was completed by 88 nymphs in 7–11 days. Blood samples from Rabbit 5 were negative at days 7 and 10 but were positive from day 14 onwards.

Forty-five days after completion of moulting, five males and five females were applied to each ear of Rabbit 6 and betamethasone treatment was commenced. Six females completed engorgement in 22–30 days. Blood samples from Rabbit 6 remained negative for *N. farcinica* until day 18 but were positive from then onwards.

Experiment 3

No immunosuppressants were used in any part of this experiment.

Four days after Rabbit 7 had been infected, 1500 larvae and 400 nymphs were applied to the left and right ears respectively. Engorgement was completed by 1208 larvae and 335 nymphs in 6–9 days and these moulted to nymphs and adults in 14–15 and 17–24 days respectively. Infection with *N. farcinica* was confirmed in Rabbit 7 in blood samples taken immediately after the completion of infestation. The emerged instars were used for the transmissions to Rabbits 8 and 9.

Forty-five days after completion of moulting, 100 nymphs were applied to the left ear of Rabbit 8. Blood samples from Rabbit 8 were negative for *N. farcinica* at days 7

and 10 but were positive from day 14 onwards. Engorgement was completed by 82 nymphs in 7–12 days.

Forty-three days after completion of moulting, five males and five females were applied to each ear of Rabbit 9. Blood samples from Rabbit 9 were negative for *N. farcinica* until day 25 but were positive from then onwards. Five females completed engorgement in 22–33 days.

Though no gross evidence of any pathological process could be discerned when Rabbit 9 was killed and autopsied 87 days after the application of infected ticks, one rabbit died 8 months after being infected by intravenous inoculation during the preliminary trials mentioned above. Autopsy revealed the presence of profuse, creamy-white pus in the pleural cavity and a dissemination of the same material in parts of the lung tissue. This material was of lymphocytic origin and cultures in TPB established the presence of *N. farcinica*. This finding suggests that the unsatisfactory reputation of rabbits as hosts for *N. farcinica* may have arisen from a convention of examining at an earlier stage of infection.

As a routine cross-check, guinea pigs were inoculated with the cultures for original infections and also with material prepared from random samples of the batches of infected ticks to be used in transmissions. These animals were all found to have liver abscesses when killed and autopsied 48 days after infection and *N. farcinica* was consistently recovered from material taken from these lesions.

DISCUSSION

This clear demonstration of trans-stadial transmission of *N. farcinica* by *A. variegatum* through both developmental stages implies that the epidemiology of bovine farcy could assume the characteristics of a tick-borne disease, so that the infection could persist in an area for considerable periods in the absence of affected cattle. The maximum period between acquisition and transmission of *N. farcinica* was about 9 weeks in these experiments but it is presumed that this interval is potentially very much longer. In general terms, arthropod vectors tend to outlive the infections they carry, but, even then, the vigorous survival of unfed *A. variegatum* nymphs and adults for—respectively—9–12 and 20–26 months at 18°C and 87 per cent r.h. (Branagan, unpublished data) indicates the possible scale of this epidemiological feature. Studies are continuing at the CTVM on the retention of transmissible *N. farcinica* by *A. variegatum* under various environmental conditions. Trans-ovarial transmission has not yet been effected but this aspect is being pursued further in view of the important implication of a permanently infected tick population.

It will be noted that infections transmitted by adults were slower in becoming patent than those transmitted by nymphs. This disparity is probably due to the longer time normally taken by most Ixodid females to commence their true feeding processes.

These findings support Daubney's opinion (1927) that *A. variegatum* can act as a vector of bovine farcy in Africa. It is not unlikely that the possibility of similar vector status will be demonstrated in other *Amblyomma* spp and also in some members of the genus *Hyalomma*—as suggested by Xiroudakis (1935) in Guadeloupe and by Kusel'tan (1967) in Russia.

ACKNOWLEDGEMENTS

The authors' thanks are due to Mr. W. G. MacLeod and Mrs. Susan Hood for their assistance and to Professor Sir Alexander Robertson for provision of facilities.

Accepted for publication February 1975

REFERENCES

BAILEY, K. P. (1960). Notes on the rearing of *Rhipicephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. *Bulletin of Epizootic Diseases of Africa*, **8**, 33–43.

BUXTON, P. A. & MELLANBY, K. (1934). The measurement and control of humidity. *Bulletin of Entomological Research*, **25**, 171–175.

DAUBNEY, R. (1927). Bovine lymphangitis or tropical actinomycosis. *Journal of Comparative Pathology and Therapeutics*, **40**, 195–216.

KUSEL'TAN, I. V. (1967). Nocardiosis of lambs in the Tadzhik SSR. In **Maloizuchennie zabolevaniya sel'skokhozyaistvennykh zhivotnykh.** Eds. Kovalenko, Ya. R., Laktionov, A. M., Zotov, A. P. & Ostapenko, K. A. In Russian. Izd. "Kolos"; Moscow, pp 110–117.

MOSTAFA, I. E. (1962). Studies on cattle nocardiosis in the Sudan. PhD thesis, University of London.

MOSTAFA, I. E. (1967). Studies of bovine farcy in the Sudan. II. Mycology of the disease. *Journal of Comparative Pathology*, **77**, 231–236.

NEUMANN, L. G. (1888). **Traite des maladies parasitaires non microbiennes des animaux domestiques.** Paris, 673 pp.

XIROUDAKIS, N. (1935). Lymphangite epizootique (farcin du boeuf). *Recueil de Médicine Vétérinaire Exotique de l'Ecole d'Alfort*, **8**, 112–117.

LA TRANSMISSION TRANS-STADIALE DE L'AGENT DU FARCIN DES BOVIDES. *NOCARDIA FARCINICA* PAR *AMBLYOMMA VARIEGATUM* (FABRICIUS, 1794)

Résumé—Les larves et les nymphes de *A. variegatum* s'infectent à partir des lapins et dans des délais pouvant atteindre 9 semaines et transmettent l'infection à d'autres lapins en se nourrissant aux stades successifs. Cette claire démonstration de la transmission trans-stadiale de l'agent du farcin des bovidés confirme l'existence d'un fait épidémiologique qui n'a été jusqu'ici que suspecté.

LA TRANSMISION A TRAVES DE ESTADIOS, DE LOS ORGANISMOS DE LA FARCINOSIS BOVINA, *NOCARDIA FARCINICA*, POR EL IXODICO *AMBLYOMMA VARIEGATUM* (FABRICIUS, 1794)

Resumen—Larvas y ninfas de *A. variegatum* se contaminaron con *N. farcínica* de conejos infectados experimentalmente y en diferentes períodos hasta las 9 semanas transmitieron la infección a otros conejos. Esta demostración clara de la transmisión a través de los estadios del ixódico *A. variegatum* de organismos de la Farcinosis bovina, confirma la existencia de uno de los aspectos epidemiológicos de la enfermedad, el cual se había sospechado anteriormente.