9

David Bruce (1855–1931): Malta fever, nagana, and East African trypanosomiasis

David Bruce (1855–1931; Figure 9.1) made significant contributions regarding brucellosis (in Malta), nagana (in Zululand), and east African trypanosomiasis (in Uganda). In all of these ventures he was accompanied by a fellow-worker – his wife, formerly Mary Elizabeth Steele (1849–1931).

Bruce was born in Melbourne, Australia, of Scottish parents. At six years of age he travelled to Scotland, and was educated at Stirling High School. At seventeen he joined the business world, but shortly afterwards entered Edinburgh University and graduated MB CM in 1881. As well as being a keen and accomplished sportsman (he excelled at boxing), he had an enthusiastic interest in natural history. Following a brief period in general practice he entered the Army Medical School at Netley, where his major interests were in pathology and research in that discipline.[1]

Malta fever (brucellosis)

Bruce's first assignment in the RAMC was at Valletta, Malta, in 1884. He had married in 1883, and his wife, a trained microbiologist, was also a most accomplished artist. Her accurate sketches and paintings were to enhance Bruce's outstanding research activities throughout his life (see Figure 9.2).

FIGURE 9.1 David Bruce (1855–1931) (reproduced courtesy of The Wellcome Library, London).

Malta was, of course, a British possession with strategically important naval and military bases. British soldiers based in Valletta, however, had a marked morbidity from Malta (Mediterranean) fever, which caused about 120 000 missed days of work each year; the average time period off-duty was 90 days. There was also significant mortality. At the time, poor hygiene and contaminated water supplies were (as was the case with most other diseases) considered causative. Bruce demonstrated a Gram-negative micrococcus (*Micrococcus melitensis*), 3 μm in diameter, in specimens of spleen, kidney and liver obtained at post-mortem. Monkey inoculation of this micrococcus cultured on peptone broth produced a death sixteen days later in one of three experiments, following a febrile illness. Post-mortem there was congestion of liver and spleen, but no involvement of Peyer's patches – making typhoid fever most unlikely. Following this, seven other monkeys were inoculated; all developed a febrile illness and four died. A report incriminating this organism (subsequently named *Brucella melitensis*) was transmitted to the Pasteur Institute, Paris.

In 1904, a team consisting of Major Horricks, Staff Surgeon E A Shaw, Dr T Zammit, Captain I Crawford Kennedy RAMC and several others set out to ascertain the mode of transmission of this causative organism. Zammit discovered

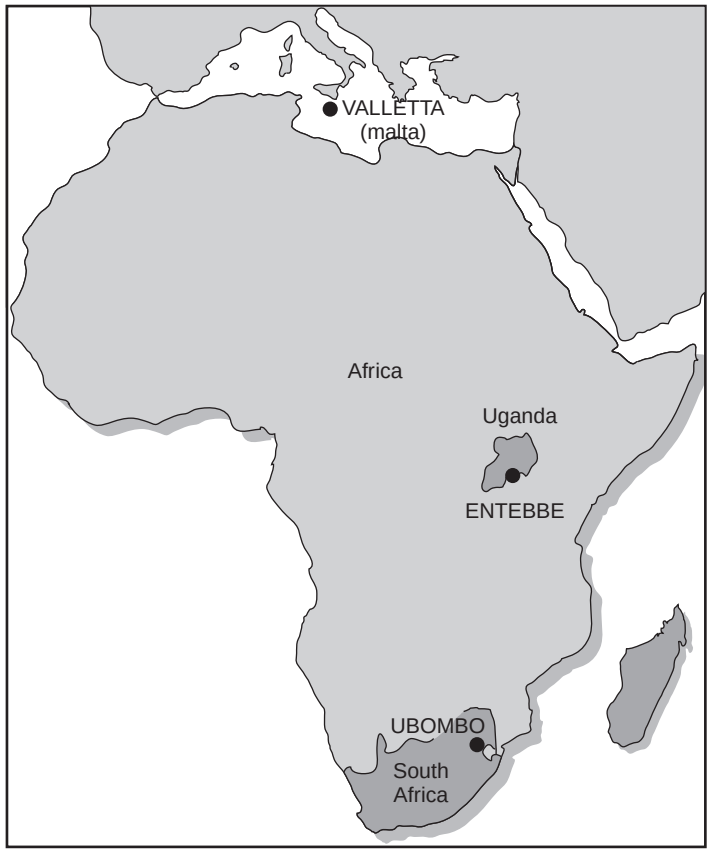

FIGURE 9.2 Map showing sites of Bruce's major researches.

the organism in the blood of a goat (see Figure 9.3). It was subsequently shown that more than 50 per cent of the island's goats were infected, most subclinically, and 11 per cent excreted the organism in their urine. Thus, the source of infection was identified. This fact was confirmed when Maltese goats were introduced into Rhodesia, when an epidemic of undulant fever resulted, and on the *SS Joshua Nicholson*, which was shipping goats to the USA, where everyone – including the captain – who drank goat's milk, suffered from the disease.

The part played by the Maltese bacteriologist Scicluna in unravelling this problem is difficult to determine.

Guiseppe Caruana Scicluna (1853–1921)

Scicluna had studied at the Pasteur Institute, Paris, and worked as a sanitary inspector/analytical chemist in the Police Department in Valletta. Later, he

FIGURE 9.3 Photograph, taken in Malta, depicting milking of a goat, the milk of which contained the causative organism of brucellosis (reproduced courtesy of The Wellcome Library, London).

was Superintendent of Public Health and Chief Government Medical Officer. Appreciation of his work later recorded:[2]

> His collaboration with Bruce in the isolation and cultivation of the specific bacterial cause of Undulant Fever – the micrococcus melitensis – from the spleen of patients, has never been widely enough known to connect his name with such an important discovery and to elicit recognition. The first spleen smears were made on agar plates and tubes prepared at the Public Health Department and were incubated in an improvised stove made out of a biscuit tin!

In 1887, he had apparently made several attempts to inoculate blood samples from the fingers of ten patients suffering from the disease into agar tubes. From one, colonies appeared in all the culture tubes incubated at 37ºC for 68 hours. Following this success, Bruce isolated the micrococcus from biopsy tissues. John Eyre wrote that 'Bruce carried out his cultivation and inoculation experiments in the laboratory of the Public Analyst, Scicluna'.[3] However, Scicluna prepared the agar plates and succeeded in culturing the bacteria from the spleens of four British soldiers dying of the disease. Madkour, in a book published in 1959, commented that Bruce never acknowledged Scicluna's contributions in this research.[4] It also seems likely that Scicluna suggested to Zammit that goat's milk was the source of human infection.[5]

Scicluna thus probably played a crucial role in the solution of the aetiology of Malta fever; Figure 9.4 shows a plaque on the exterior wall of the laboratory in Valletta in which Bruce and Scicluna both worked.[6]

By eliminating goat's milk from the diet, the military authorities eliminated Malta fever from British troops.

This discovery led to Bruce being elected FRS, and he was also appointed Assistant Professor in Pathology at the RAM School at Netley; here he worked with Almroth Wright, who at that time occupied the Chair of Pathology.[7]

FIGURE 9.4 Plaque situated on the exterior wall of the laboratory in Valletta, Malta, where both Bruce and Scicluna worked.

Nagana

In 1894, Bruce was posted to Pietermaritzburg (Natal, South Africa) for military field service. At the time, cattle and also horses were dying in hundreds from a disease the origin(s) of which was obscure; the Zulus gave it the name *nagana*.[8] As a result, the country was faced with ruin, and grave economic stress affected the indigenous stock-raisers and white settlers.[9] On the initiative of the Governor of Natal and High Commissioner of Zululand, Sir Walter Hely-Hutchinson, who had recently served as Lieutenant-Governor of Malta whilst Bruce was working on the Malta (undulant) fever problem (see above), Bruce was ordered to proceed on secondment from Natal to Zululand in an attempt to discern the aetiology of this mysterious disease. Nagana and (tsetse) fly disease of travellers and hunters were, at that time, considered separate entities.[10]

Africans in certain parts of the continent were already aware that nagana resulted from a bite of the tsetse fly. The inhabitants of this region of Africa (and also West Africa, where it was called *surra*) and India had been seriously bothered by the disease for at least a century,[11] and perhaps since 1742.[12] David Livingstone (1813–73) was in fact familiar with it on the banks of the Zambesi in 1847;[13] he described the disease in 1857[14] and produced an accurate drawing of *Glossina morsitans*. Many local residents, some of European origin, were of the opinion that it was caused by the bite of the tsetse fly (which elaborated a poison within itself),[15] leading to the death of the animal some ten days after

this event. An alternative theory, apparently held by most Zulus, was that game animals harboured the disease, and that it was transmitted to cattle in food and water contaminated by them – perhaps faecally.[16] We now know that both theories possessed an element of truth.[17] Livingstone was fully convinced that the disease did not affect man; he and his party lived in a heavily infected area for months without ill effect. This suggests that human strains of *Trypanosoma brucii* were not at that time present in this part of Africa.

Bruce and his wife[18] thus set off on a long trek to Ubombo Hill, some 650 metres (2000 feet) above sea level in northern Zululand, by mule and ox wagon, to take up their challenge on 27 October 1894.[19] However, from notes written at Pietermaritzburg before they set off it is clear that Bruce was already intellectually involved in the problem, for he made an attempt to infect animals by injecting into them watery and alcoholic tsetse-fly extracts.

Following his work which had unravelled the cause of Malta (undulant) fever, Bruce felt that a bacterium was the most likely candidate. Furthermore, he had spent the year 1888 at Robert Koch's institution in Berlin – 'a Mecca for budding bacteriologists'.[20] At Ubombo Hill, where they arrived on 24 November 1894, the Bruces took over a wattle-and-daub hut that had belonged to a 'squatter' married to a Zulu woman. Figure 9.5 shows Bruce at Ubombo while he was researching nagana.[21] His work began by undertaking daily blood examinations (including cell counts) on a 'brown cow'. Each examination revealed micrococci and slender, poorly-staining bacilli; however, on the sixth day he recorded in his notes 'also Haematozoa.'[22]

FIGURE 9.5 Bruce in Zululand while researching the cause of nagana (reproduced courtesy of The Wellcome Library, London).

Bruce had very little knowledge of trypanosomes, and 'at first thought that the wriggling object might possibly be a small filaria'.[23] The observation was repeated on a 'black and white cow'. Healthy calves were shown to develop nagana when taken down to the low country;[24] their blood also contained 'haematozoa' – in one case numbering 10 000 per mm^3. Whilst in the low country, two of the Bruces' dogs – pointers – were bitten by tsetse flies; shortly after returning to the hill, both developed acute, fatal nagana with 'haematozoa' in their blood. One of them, 'John Keats' apparently had four organisms ('wriggling about like little snakes') per erythrocyte. When the splenic pulp and blood of this dog were cultured, they were shown to be bacteriologically sterile. Bruce also examined large numbers of healthy cattle, and noted that 'haematozoa' were invariably absent. Within two months, and probably a mere five to six weeks[25] of his arrival in Zululand, Bruce seems to have clearly established a positive correlation between nagana and blood 'haematozoa', and furthermore that the 'organism' was responsible for both nagana and tsetse fly disease.[26] He recorded these observations in his *Preliminary Report on the Tsetse Fly Disease or Nagana, in Zululand*;[27] this was later expanded in his *Further Report on the Tsetse Fly Disease or Nagana, in Zululand*.[28] Later, in 1915, he described this work in considerable detail in four Croonian lectures delivered to the Royal College of Physicians.[29]

At this exciting point in the saga, Bruce was recalled, on 26 January 1895, to Natal.[30] However, on reaching Pietermaritzburg he discovered that there had been no compelling reason for this order and, following communications between the Governor and the War Office (who were seemingly pretty uncooperative), the Bruces were able to return to Ubombo on 8 September 1895. However, by the time they arrived, seven months had been wasted. Bruce was by this time fully convinced that the 'haematozoa' were causatively related to nagana, but this had to be confirmed scientifically. Therefore, he infected healthy animals by inoculating the blood of diseased ones. A minor setback came when a dog fed on coagulated, infected blood developed nagana – a surprise observation which seemed to support the 'Zulu theory' (see above); in retrospect, the only reasonable explanation must be that trypanosomes entered via an oral abrasion(s).[31]

When healthy cattle were taken to the low country, muzzled, and fed on fodder brought down from the hill, they still contracted nagana; those kept on the hill and fed herbage brought from the low country remained fit and well. In an epidemiological study carried out over several miles of country, he found that in some kraals the Zulus no longer kept cattle because they knew they would die of nagana, whereas in others they flourished; the former were, he noted, in bush (scrub), with game animals nearby, and the latter in open country. The role of game animals in relation to nagana remained unclear until Bruce succeeded in 'infecting' a dog by inoculating the blood of an antelope; he repeated this experiment many times, but results were inconsistent. He later concluded that about one-quarter of the local herbivorous game animals (tolerant to infection) harboured 'haematozoa'

in their blood, and therefore served as reservoirs of infection.[32] He then proceeded to demonstrate, by inoculation experiments, that game animals can be infected, but remain asymptomatic.[33]

At an entomological level, Bruce also carried out tsetse-fly breeding experiments[34] and noted that larvae were retained in the abdomen of the parent fly. He showed that *G. palpalis* was responsible. He proceeded to demonstrate living 'haematozoa' in the proboscis of tsetse flies fed on infected animals.[35] One hour after feeding, they were present in the stomach in clumps of a dozen or so, and remained very active; after four hours, activity was undiminished. Bruce's notes recorded that the longest survival period of the organism(s) in the tsetse-fly intestine was 118 hours. From these experiments Bruce concluded that the fly can transmit the organisms for up to 48 hours after feeding on an infected animal, and that after 72 hours infection must be unlikely. In order to establish whether or not a later developmental cycle existed within the fly, he carefully dissected the gut of infected specimens; although he recorded 'disc-like bodies, spirilla, and so forth',[36] he decided that these were independent of the 'haematozoa'.

At this point, the South African war intervened and he was unable to keep the flies for a full three weeks in order to establish the developmental cycle within the insects' salivary glands. This was ultimately unravelled by F K Kleine (1861–1950) in 1908, more than a decade later, who demonstrated that the responsible trypanosome develops in the tsetse fly in a similar way to that of the malarial parasite and filarial sp. in the mosquito, and that infection is transferred in saliva when the fly bites. At the siege of Ladysmith (which lasted from October 1899 until March 1900), in a battle with the Boers, Bruce was compelled to work as a surgeon (his prime objective was to investigate an outbreak of enteric fever amongst British troops) while his wife was a sister with the Red Cross. Of 563 deaths during the siege, 393 resulted from typhoid. For their work there, Bruce both received a medal and was promoted in rank, while his wife was awarded the Royal Red Cross Medal.[37]

Bruce thus established beyond doubt, by a series of elegant experiments, that nagana is caused by a 'haematozoa', later named *Trypanosoma brucii (brucei)* by H G Plummer (1850–1918) and J R Bradford (1863–1935);[38] this is conveyed by an infected tsetse fly, which is itself infected by feeding on game animals, which form the major reservoir of infection. Bruce was therefore the first investigator to demonstrate transmission of a protozoan parasite by an insect bite;[39] he was also the first to demonstrate the developmental cycle within the tsetse fly. He satisfied himself, furthermore, that dosing with arsenic – first established to be efficacious by David Livingstone in a mare afflicted by nagana[40] – had an inhibitory effect on trypanosomes in the blood of animals, but did not prevent a subsequent infection.[41]

Bruce's researches that led to the solution of the *nagana* problem are regarded by most historians of medicine/science as his greatest contribution.[42] This was scientifically more challenging than the brucellosis, and east African trypanosomiasis research that was to follow.

East African trypanosomiasis

The first Royal Society sleeping sickness expedition (consisting of Low, Castellani and Christy; see Chapter 8), which set out to unravel the aetiology of the 'negro lethargy' which in 1901 was ravaging the local population on the northern shores of Lake Victoria Nyanza, failed to identify the cause of the disease. Bruce, with his research ability now firmly established (he had already solved the causes of brucellosis and nagana), was at this point sent by the Royal Society to determine the origin of yet another disease which was causing a great deal of local morbidity and mortality. He was accompanied in this venture by David Nabarro (1874–1958), described as a 'quiet Portuguese' pathologist, and, of course, his wife.

On 12 March 1903, the Bruces joined Castellani and Christy at Entebbe, Uganda (Low had already left for England). Castellani had by then demonstrated 'haematozoa' in the cerebrospinal fluid (CSF) of five cases (and in the peripheral blood of one), although, being a bacteriologist and highly impressed (and influenced) by a Portuguese report, implicated a diplo-coccus.

Bruce, largely as a result of his nagana work, felt it pertinent to research the possible aetiological role of the trypanosome. Using centrifuged specimens of CSF, he detected the protozoan in 70 per cent of 34 cases of 'negro lethargy', but in none of 12 controls. Subsequent work revealed trypanosomes in 100 per cent of 40 cases examined. Extrapolating, Bruce concluded that these cases and 'trypanosome fever' (already investigated in West Africa; see Chapter 11) represented different stages of a single disease.

Injection of 'infected' CSF into a monkey produced a sleeping sickness-like illness, but since the animal was found at post-mortem to have co-existent tuberculosis, this result was discounted. Subsequent inoculation of blood from a sleeping-sickness sufferer subcutaneously was followed by a similar illness, and at post-mortem trypanosomes could be demonstrated in the central nervous system. An epidemiological study throughout the area showed a close correlation between the proportion of *Glossina palpalis* infected and the prevalence of sleeping sickness. Extensive studies on animals revealed that dogs and rats are partially susceptible to sleeping sickness, but that guinea-pigs, donkeys, goats and sheep are refractory. A preliminary report was published in the *British Medical Journal*.[43]

On 25 May, Bruce and Nabarro were joined by E D W Greig (1874–1950) and they wrote a 'Further Report' which cleared up many outstanding problems. In 1909, Kleine (see above), an associate of Koch and a member of the German Sleeping Sickness Commission, showed that flies bred in the laboratory only become infective after about twenty days after their infecting feed; this was subsequently confirmed by Bruce and his co-workers.

On 28 August 1903, the Bruces left Africa with the intention of continuing their work on brucellosis in Malta. In 1908 Bruce was, however, appointed Director of the third Royal Society Commission on Sleeping Sickness. This was basically an epidemiological investigation, the results of which were reported in his Croonian Lectures for 1915.[44]

Bruce concluded that there were three distinct patterns of trypanosomal disease:

1. Nagana, which affected a vast region from Sudan to Zululand
2. *Trypanosoma rhodesiense*, responsible for sleeping sickness in Nyasaland (now Malawi) and Rhodesia (now Zambia and Zimbabwe) and transmitted by *Glossina morsitans*
3. *T gambiense*, responsible for 'trypanosoma fever' in the Congo and Uganda and transmitted by *Glossina palpalis*.

In 1910, J W W Stephens (1865–1946) and H B Fantham (1875–1937) in fact discovered *Trypanosoma rhodesiense* in Nyasaland (now Malawi) and northern Rhodesia (now Zambia); furthermore, Allan Kinghorn (1880–1955) and Warrington Yorke (1883–1943) confirmed that the transmitting fly was *G morsitans.*

BRUCE'S LATER LIFE

In 1914, the year of the outbreak of the Great War, Bruce was appointed Commandant of the Royal Army Medical College. Here, he reviewed the efficacy of typhoid and tetanus inoculation.[45] He continued in this capacity until his retirement in May 1919, when he was appointed a representative of the Royal Society on the governing body of the Lister Institute.

In his latter years, Bruce received numerous honours. He was a staunch advocate of prevention, as this quotation from his Presidential Address to the British Association for the Advancement of Science shows:[46]

> It must be no longer said that the man was so sick that he had to send for the doctor. The medical practitioner of the future must examine the man while he is apparently well, to detect any incipient departure from normal and to teach and urge modes of living comfortable to the laws of personal health, and the public health authorities that man's environment is in accordance with scientific teaching.

BRUCE'S PRESIDENTIAL ADDRESS TO THE SOCIETY OF TROPICAL MEDICINE

Bruce did not speak, surprisingly, about any of his major discoveries – the causes of brucellosis, nagana or east African trypanosomiasis – in this address, but instead he analysed 1000 cases of tetanus; he was still (in 1917) Chairman of the War Office Committee for the Study of Tetanus. At the beginning of his lecture, he apologized to his audience for the fact that he was not addressing a strictly *tropical* disease, but one which 'in a time of war [the Great War was still in progress] has an important place among war diseases'.

Sir David Bruce died on 27 November 1931 while the funeral service for his wife was in progress at Christ Church, Westminster.[47]

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