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TUBERCULOSIS IN WILDLIFE IN THE RUWENZORI NATIONAL PARK, UGANDA (PART II)

M. H. WOODFORD¹

SUMMARY

The results of post-mortem examinations of 90 warthog (Phacochoerus aethiopicus) conducted in the Ruwenzori National Park, Uganda during a survey of tuberculous infection in wildlife are described. Nine per cent of warthog were found to show gross lesions on autopsy and of these organisms which could by typed, Mycobacterium bovis was isolated in 2 of 6 cases and 5 atypical mycobacterial strains were isolated from the remaining 4. The distribution and character of the lesions is described and it is concluded that the route of infection in the warthog is alimentary. A mycobacterial survey of 8 other species of mammals, 7 species of birds, 5 species of fish and 1 species of amphibian is described. None of the mammals (except possibly 1 elephant), birds, fish or amphibia was found to be infected with M. bovis but several individuals were found to harbour atypical, probably saprophytic, mycobacterial types.

The origin of tuberculosis in buffalo and warthog in the Ruwenzori National Park is discussed and is concluded to have been previous contact with domestic cattle.

INTRODUCTION

In Part I (Woodford, 1982) the gross pathology, prevalence and epidemiology of tuberculosis in African buffalo (*Syncerus caffer*) in the Ruwenzori National Park have been described. This article reports the results of a survey conducted to establish the status of the disease amongst the other wild fauna in the Park and of an attempt to find the source of pathogenic atypical mycobacterial infections of buffalo and warthog (*Phacochoerus aethiopicus*).

MATERIALS AND METHODS

Ninety warthogs shot at random for another worker who was studying their reproductive physiology, were examined at post-mortem in the same manner as the buffalo. Specimens suspected of being tuberculous were collected from 10 animals and were examined at the Animal Health Research Centre (AHRC), Entebbe by histology, stained smear, culture and animal inoculation. Since the warthog survey depended upon the research programme of another worker it was possible to sample only Sectors D and E of the Park. Seven other mammalian species were also examined *post mortem*. These comprised 10 reedbuck (*Redunca redunca*), 7 bushbuck (*Tragela-phus scriptus*), 6 Defassa waterbuck (*Kobus defassa*), 9 Uganda kob (*Kobus adenota*), 7 topi (*Damaliscus korrigum*), 2 elephants (*Loxodonta africana*) and 2 hyenas (*Crocuta crocuta*). Specimens of deep pharyngeal and mesenteric lymph nodes which in all cases except 1 of the elephants showed no visible lesions, were collected and subjected to histological, bacteriological and cultural examination and to animal inoculation at the AHRC, Entebbe.

Specimens of liver and gut content collected from 14 birds of 7 species shot at random were examined at AHRC, Entebbe by the same routine. The bird species comprised 3 white-backed vultures (*Pseudogyps africanus*), 2 yellow-billed storks

¹ Present address: c/o UNDP, PO Box 4595, Maputo, Mozambique.

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(Ibis ibis), 3 marabou storks (Leptoptilos crumeniferus), 1 white pelican (Pelecanus onocrotalus), 2 pink-backed pelicans (Pelicanus rufescens), 2 African cormorants (Phalacrocorax carbo) and 1 Hammerkop stork (Scopus umbretta).

Five fish of each of 5 different species (*Tilapia* spp., *Barbus* spp., *Bagrus* spp., *Clarius* spp. and *Protopterus* spp.) were similarly sampled as were 20 frogs (*Ptychadena* spp.) collected from buffalo wallows in Sector D and from the Kazinga Channel.

In co-operation with the Mission doctor at Kagando Hospital, Busongora County, Toro, biopsy material taken from the infected cervical lymph nodes of 4 Bakonjo children living just outside the north-western boundary of the Park was examined for evidence of tuberculosis. Where the disease was found to be present the mycobacteria were typed at the Medical Research Council (MRC) laboratory, Kampala.

A sample of 420 domestic goats in the villages of Katwe, Kazinga and Katunguru was subjected to the single intradermal comparative tuberculin test. The sample represented about 25% of the total number of goats present in these fishing villages within the Park.

RESULTS

Warthog

Tuberculosis was suspected in 10 instances and confirmed by smear and histology in 1 case and by smear, culture and animal inoculation in 7. All 8 positive animals had calcified abscesses in the submaxillary lymph nodes. In addition 4 had lesions in the thoracic cavity affecting the lungs. In 2 of these the disease was extensive and consisted of caseo-calcific consolidation of the entire lung substance due to the coalescence of masses of small haematogenous nodules. One of these infections was generalised and although no lesions were seen in the liver or spleen there were many nodules on the peritoneum and the mesenteric and carcass lymph nodes were all enlarged containing caseo-calcific granulomata scattered throughout the lung substance. Lesions were seen in the gastro-hepatic nodes in 2 instances including the generalised case described above. It is concluded that the route of infection in the warthog is alimentary.

All the affected warthog were in good condition including the generalised case whose enlarged carcass lymph nodes were clearly visible before the animal was shot. Unfortunately acid-fast organisms recovered from this animal died out before they could be typed. However, from 6 cases M. bovis was isolated in 2 instances and 5 atypical mycobacterial strains were identified from the remaining 4. One of the atypical strains was considered to have the general characteristics of M. intracellulare but was not pathogenic for guinea-pigs. Unfortunately the other 4 strains died out before they could be freed of contamination and tested for pathogenicity for guinea-pigs. The data on tuberculosis in warthog in Ruwenzori National Park are summarised in Table I.

TABLE I Tuberculosis in warthog			
Sector	No. shot	No. tuberculous	% tuberculous
D	10	1	10
E	80	7	8

Elephant

Two elephants were examined *post mortem*; 1 was shot having lost its trunk in a snare and the other died following drug immobilisation. The latter was found to have 2 large $(30 \times 5 \text{ cm})$ cylindrical calcified bodies floating free in the peritoneal cavity. Several small $(5 \times 3 \text{ cm})$ caseo-calcareous heavily encapsulated abscesses were seen in the lungs. Similar lesions occurred in the substance of the spleen and a diffuse nodular peritonitis extended throughout the abdominal cavity. The uterus was grossly enlarged and contained numerous dry cheesy abscesses some of which were in the early stages of calcification. The entire uterine cavity was occupied by this caseo-calcareous material. Mesenteric and sublumbar lymph nodes contained heavily encapsulated caseo-calcareous abscesses. The mediastinal, bronchial, submaxillary and parotid nodes contained many small $(3 \times 3 \text{ cm})$ calcified granulomata. The elephant was in good bodily condition.

Smears of centrifuged deposit obtained from lung and uterine lesions revealed the presence of scanty beaded acid fast rods. Culture and guinea-pig inoculation were both negative. Histology revealed calcified foci surrounded by fibrous tissue with no cellular reaction or evidence of active tuberculosis.

Other mammals

Acid-fast rods were seen in smears from the mesenteric nodes of 2 bushbuck, 3 reedbuck, 2 waterbuck, 7 Uganda kob and 1 hyena. Scanty growth on Lowenstein-Jensen medium at 25 and 37°C (but not at 44°C) was obtained in the case of 1 bushbuck, 1 reedbuck, and 2 Uganda kob. One culture from a bushbuck and 1 from a Uganda kob were identified as being atypical mycobacteria after subculture on glycerol agar had produced sufficient growth for testing at the MRC laboratory, Kampala. None of the cultures was pathogenic for guinea-pigs.

Birds

Scanty weakly acid-fast rods were seen on stained smear of centrifuged deposits in 6 of the birds sampled. These comprised 3 white-backed vultures, 1 yellow-billed stork, and 2 pink-backed pelicans. Culture of the deposits produced growth at 25 and 37°C (but not at 44°C) in the case of specimens from 1 white-backed vulture, the yellow-billed stork and a pink-backed pelican; none of the cultures was pathogenic for guineapigs.

Fish

Scanty acid-fast organisms were seen on smear of centrifuged deposit of liver and gut contents from a *Tilapia* spp., 2 *Barbus* spp., 4 *Bagrus* spp., 1 *Protopterus* sp. and 1 *Clarius* sp; the organisms seen were few in number and beaded in appearance. Weak growth was obtained on Lowenstein-Jensen medium at 25 and 37°C (but not at 44°C) in the case of 1 *Tilapia* sp., 1 *Barbus* sp. and 1 *Protopterus* sp; none of the material proved pathogenic for guinea-pigs.

Amphibia

Three frogs from Sector D wallows yielded acid-fast organisms on smear of centrifuged deposit of liver and gut contents and in 2 cases scanty growth was obtained on Lowenstein-Jensen medium at 25 and 37°C. Subcultures on glycerol agar grew more strongly and were classified at MRC laboratory, Kampala as atypical myco-

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bacteria. However, neither of the strains isolated were pathogenic for guinea-pigs and neither grew at 44°C.

Humans

Acid-fast bacilli were seen on a smear of a cervical lymph node abscess from 1 of the Bakonjo children sampled. This specimen grew at 37° C and was identified as *M. tuberculosis* at the MRC laboratory.

Goats

The single intradermal comparative tuberculin test proved negative in all the 420 goats tested.

DISCUSSION

Origin of tuberculosis in the Ruwenzori National Park

There is evidence, mostly oral, confirming the presence in and around the area now known as the Ruwenzori National Park of large numbers of domestic cattle, sheep and goats at various times since the arrival in 1889 of the first European explorer (Stanley, 1890). Written records are available from this date and the fortunes of the people and their stock are accurately recorded. At the end of the last century the human population suffered a severe smallpox epidemic and at the same time their cattle were largely destroyed by rinderpest (Posnansky, 1965). A few years later trypanosomiasis became endemic in the area and both the human and domestic animal populations were severely affected (Morris, 1960).

Rinderpest greatly reduced the numbers of buffalo and other wild fauna on at least 3 occasions between 1890 and 1952 but a recovery was always possible because the competing people and their cattle were simultaneously being decimated by trypanosomiasis. Finally in 1924 when the measures introduced to control the tsetse fly had failed the authorities closed the area to human habitation. The withdrawal of the remnants of the human and cattle populations removed all competition and the wild animals flourished. In 1931 the area now known as the Ruwenzori National Park was declared a Game Reserve and in 1952 it was gazetted as the Queen Elizabeth National Park. From that date until the present time there has been no contact between domestic cattle and the wild fauna of the Park. However, 26 fishing villages scattered along the shores of Lake George and Idi Amin Dada are the home of numerous goats which graze illegally in the Park.

The situation south of the Maramagambo Forest is different for there is no evidence that cattle were ever grazed alongside the buffalo in that part of the Park and indeed no sign of tuberculosis was found there. No reactors to the tuberculin test were found amongst the 420 goats tested in 2 villages on the Toro side of the Kazinga Channel and in 1 village on the Ankole bank.

Tuberculosis is common in the human population in Uganda and the 1 child (of 4 examined) from which organisms were recovered was infected with the human type.

That the wild fauna of the Ruwenzori National Park provide the original focus of bovine tuberculosis from which cattle became infected is unlikely. Apart from the reports of Guilbride, Rollinson, McAnulty, Alley and Wells (1963), and Thurlbeck, Butas, Mankiewicz and Laws (1965) there are no other accurate accounts of the disease occurring in wild African buffalo. Sikes (1969) is misquoted by Mohan and Gotts (1970) as saying that she found 1 out of 8 buffalo shot in Kenya to have advanced

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tuberculosis; in fact the animal in question was shot in the Ruwenzori National Park, Uganda. Mohan and Gotts (1970) state that Guilbride *et al* (1963) have misinterpreted Mammerickx (1960) as saying that tuberculosis has been reported in all parts of the former Belgian Congo (now Zaire). The source of bovine tuberculosis in buffalo and warthog in the Ruwenzori National Park is concluded to have been the local domestic cattle and the disease can now be considered endemic in these 2 wild populations.

The origin of the atypical infections in both buffalo and warthog is obscure. Waddington (1967) found that the Ankole cattle of Uganda which graze a similar environment to that of the buffalo and warthog were widely infected with atypical strains of mycobacteria and that these could interfere with the results of the comparative tuberculin test by producing a transient sensitivity to mammalian tuberculin. He also found that his experimental atypical infections in cattle were not easily transmitted to contact cattle in the field and concluded that the organisms were picked up from the environment rather than from other animals. It is probable that a similar situation exists in the case of atypical mycobacterial infections in buffalo and warthog but the ecology of the atypical organisms and the part they may play in provoking a measure of immunity in their hosts to *M. bovis* is far from clear.

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LA TUBERCULOSE CHEZ LES ANIMAUX SAUVAGES DU PARC NATIONAL DE RUWENZORI, EN OUGANDA (2E PARTIE)

Résumé--Les résultats observés à l'examen post-mortem de 90 phacochères (Phacochoerus aethiopicus) du Parc national de Ruwenzori, en Ouganda, à l'occasion d'une enquête sur la tuberculose des animaux sauvages sont donnés. 9 p. 100 des phacochères ont été trouvés à l'autopsie porteurs de lésions importantes et, parmi les organismes qui ont pu être typés, Mycobacterium bovis a été isolé dans deux cas sur six et cinq souches de mycobactéries atypiques ont été isolées dans les quatre autres cas. La répartition et les caractéristiques des lésions sont décrites et il est conclu que la voie d'introduction de la maladie est, chez le phacochère, alimentaire. Une enquête mycobactérienne a intéressé huit autres espèces de mammifères, sept espèces d'oiseaux, cinq espèces de poisson et une espèce d'amphibien. Aucune espèce de mammifère (à l'exception possible d'un éléphant) d'oiseau, de poisson et d'amphibien n'a été reconnue comme infectée par M. bovis mais plusieurs individus ont été trouvés porteurs de germes mycobactériens atypiques, probablement de nature saprophyte.

L'origine de la tuberculose chez le buffle et le phacochère du Parc national de Ruwenzori est discutée et, en conclusion, est attribuée à un contact préalable de ces animaux avec le bétail domestique.

TUBERCULOSIS EN ANIMALES SALVAJES EN EL PARQUE NACIONAL DE RUWENZORI, UGANDA (PARTE II)

Resumen—Se describen los resultados del examen postmortem de 90 cerdos salvajes (*Phacochoerus aethiopicus*) llevado a cabo en el Parque Nacional de Ruwenzori en Uganda, durante un reconocimiento de tuberculosis. En el 9% de cerdos salvajes examinados se encontraron lesiones tuberculosas, aislándose *Mycobacterium bovis* de dos animales, como también otras cepas atípicas. Se concluye que la ruta de infección es oral. Se describe también los resultados de peces y una de anfibios. Ninguno de los mamíferos (excepto un elefante), aves, peces o anfibios se encontraron infectados con *M. bovis*, aislándose sin embargo varias cepas atípicas.

Se discute el origen de la tuberculosis en búfalos y cerdos salvajes en el Parque Nacional de Ruwenzori y se concluye que hubo contacto previo con ganado doméstico.