INT J TUBERC LUNG DIS 8(8):924–937

REVIEW ARTICLE

Bovine tuberculosis: an old disease but a new threat to Africa

W. Y. Ayele,* S. D. Neill,† J. Zinsstag,‡ M. G. Weiss,‡ I. Pavlik*

*** Department of Food and Feed Safety, Veterinary Research Institute, Brno, Czech Republic; † Agriculture, Food and Environmental Science, Department of Agriculture and Rural Development, Belfast, Northern Ireland, United Kingdom; ‡ Department of Public Health and Epidemiology, Swiss Tropical Institute, Basel, Switzerland**

SUMMARY

Bovine tuberculosis (TB) is a disease characterised by progressive development of specific granulomatous lesions or tubercles in lung tissue, lymph nodes or other organs. *Mycobacterium bovis* is the causative agent of the disease. Bovine species, including bison and buffaloes, are susceptible to the disease, but nearly all warm-blooded animals can be affected. All species are not equally susceptible to the disease; some are spill-over (end) hosts and others maintenance hosts. In Africa, bovine TB primarily affects cattle; however, infection in other farm and domestic animals, such as sheep, goats, pigs, dogs and cats, is not uncommon. Wild ruminants and carnivores are also affected and are the natural reservoirs of the infectious agent in the wild. Man is also susceptible to the disease, the highest risk groups being individuals with concomitant HIV/AIDS infection. In Africa, human TB is widely known to be caused by *M. tuberculosis*; however, an unknown proportion of cases are due to *M. bovis*. This infection in humans is under-reported as a result of the diagnostic limitations of many laboratories in distinguishing *M. bovis* from *M. tuberculosis*. None of the national reports submitted to the OIE and WHO by African member states mention the importance of *M. bovis* in human TB cases. Consumption of unpasteurised milk and poorly heat-treated meat and close contact with infected animals represent the main sources of infection for humans. This review attempts to examine the impact of bovine TB on the health of animals and humans.

KEY WORDS: cattle; bovine tuberculosis; milk; pasteurisation; wild animals; zoonosis

THE WORLD HEALTH ORGANIZATION (WHO) estimated that for the years 1990–1999, human tuberculosis (TB) incidence and mortality would be respectively 88 million and 30 million, with most cases occurring in developing countries.[1] The annual global incidence of TB was predicted to increase to 10.2 million by 2000, a 36% increase from 1990. In 1995, 3.3 million cases were reported to the WHO Global Tuberculosis Programme. Of these, 62% occurred in the South-east Asian and Western Pacific regions, 16% in sub-Saharan Africa, and 7–8% in each of the regions of the Americas, Eastern Mediterranean and Europe. Given the rapidly spreading global human immunodeficiency virus (HIV) epidemic in developing countries, the WHO estimated that 70% (6 million) of humans co-infected with TB and HIV live in sub-Saharan Africa.[2]

In industrialised countries, bovine tuberculosis is controlled in farm animals, as a result of which human infection is minimised, although a potential risk remains. These countries are conscious of local and international implications of the disease for trading in animals and animal products. In Africa, however, bovine TB represents a potential health hazard to both animals and humans, as nearly 85% of cattle and 82% of the human population live in areas where the disease is prevalent or only partially controlled.[1,3] In Africa, as in most developing countries, *Mycobacterium bovis* infection remains an uninvestigated problem. For this reason, the WHO, with the participation of the Food and Agriculture Organization (FAO), convened a meeting on zoonotic bovine TB in November 1993 in Geneva, Switzerland, where the public health significance of *M. bovis* in humans and animals worldwide was discussed. Data collected from most developing countries, mainly from sub-Saharan Africa, were insufficient to represent the true epidemiological picture of the disease. It was therefore recommended that collection of scientific data on human TB due to *M. bovis* should be prioritised.[4]

TB is a neglected public health problem and accounts for about 25% of all avoidable adult deaths in developing countries.[5] The epidemiology of TB has been affected in recent decades by the upsurge in HIV

Correspondence to: Wuhib Y Ayele, DVM, PhD, Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic. Tel: (+420) 533 331 634. Fax: (+420) 541 211 229. e-mail: wuhibayele@yahoo.com

Article submitted 28 July 2003. Final version accepted 9 February 2004.

infection. As many HIV-infected individuals are co-infected with TB, the incidence of the disease may rise in the coming years.[6] The correlation between the prevalence of *M. bovis* infection in humans and that in local cattle populations highlights the potential threat of this disease for humans.[7]

The global prevalence of human TB due to *M. bovis* has been estimated at 3.1% of all human TB cases, accounting for respectively 2.1% and 9.4% of pulmonary and extra-pulmonary TB cases.[2] In industrialised countries, human TB due to *M. bovis* is relatively rare as a result of TB control in cattle. Nevertheless, an estimated <1% of all TB cases are reported to be caused by *M. bovis*, probably due to reactivation of dormant lesions among the elderly.[8]

Bovine TB is a zoonotic disease with potential public health and socio-economic significance, as it can affect international trade in animals and animal products. Bovine TB is present in almost all African countries,[4] affecting both domestic and wild animals. Daborn and Grange reported that the disease was prevalent in 33 (80%) of 43 African member countries of the regional commission of the Office International des Epizooties (World Organisation for Animal Health, OIE).[9] Bovine species are natural hosts to the disease, but a wide spectrum of domestic and wild animals, as well as man, can be infected.[3,10] Animals reported as having been infected with *M. bovis* include North American bison (*Bison bison*), buffaloes (*Syncerus caffer*), elk (*Cervus elaphus*), domestic and wild pigs (*Sus scrofa*), goats (*Capra hircus*), camels (*Camelus bactrianus*), dogs (*Canis familiaris*), cats (*Felis catus*), sheep (*Ovis aries*), possums (*Trichosurus vulpecula*), badgers (*Meles meles*), mink (*Lutreola vison*), ferrets (*Putorius furo*) and non-human primates.[3,11,12] All species are not equally susceptible, and are often grouped into spill-over (end) hosts and maintenance hosts. Cattle and other bovine species are considered the primary and most well-known reservoirs or maintenance hosts. In countries where maintenance hosts are present endemically in the wild, infection from these populations to domestic cattle or other farm animals is difficult to avoid.

Many factors account for the failure of developing countries to control and eradicate bovine TB, many of them politico-economic. Added to the high costs of a sustainable testing programme are problems of social unrest due to political instability and ethnic wars, resulting in the displacement of large numbers of human and animal populations; lack of veterinary expertise and communication networks; insufficient collaboration with bordering countries and hence a lack of quarantine; and smuggling of live animals across state boundaries. Scarce human and financial resources are often absorbed by action against the high incidence of other acute and fatal diseases, such as contagious bovine pleuropneumonia, foot and mouth disease, African and classical swine fever and parasitic diseases.

The epidemiology and public health significance of bovine TB in Africa remain largely unknown, often for the above reasons. In addition, however, few laboratories are capable of differentiating *M. bovis* from *M. tuberculosis* and other members of the *M. tuberculosis* complex (MTC). Collins and Grange reported a lack of interest in typing human tubercle bacilli in the laboratories of several countries,[13] leading to underestimations of the incidence of *M. bovis*. The primary sources of infection for humans are consumption of unpasteurised milk and close association between humans and animals.[9,14] Rural inhabitants and some urban dwellers in Africa still consume unpasteurised and soured milk potentially infected with *M. bovis*. Milk-borne infection is the main cause of non-pulmonary TB in areas where bovine TB is common and uncontrolled.[7]

The current problem of *M. bovis* in developing countries may to some extent mimic the pre-eradication period in Europe before the 1960s, where the prevalence of bovine TB in the human population was relatively high.[15] Lee and Mills underline the urgent need to develop and build scientific capacity in developing counties to improve health worldwide and curb the global spread of infectious diseases, and cite poor governance, poor planning, poor accountability and failure to conduct research as the main obstacles to controlling this global disease burden in developing countries.[16]

The purpose of this review is to highlight the potential danger of bovine TB for domestic and wild animals in Africa and to emphasise the emerging public health threat of this disease for humans, exacerbated by the current upsurge in HIV infection. The influence of cultural beliefs about TB control in some societies is also discussed.

ETIOLOGY

Bovine TB is caused by *M. bovis*. Although cattle are considered to be the primary hosts of *M. bovis*, the disease has an exceptionally wide mammalian host range, which includes humans.[11,17] *M. bovis* is a member of a closely related group of mycobacteria referred to as MTC,[18] which comprises *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. microti* and *M. bovis* bacille Calmette-Guérin (BCG)[19] as well as the newly characterised bacteria *M. canetti*[20] and *M. caprae comb.* nov., sp. nov.[21] Isolates with characteristics intermediate between *M. tuberculosis* and *M. bovis* have also been reported.[22] MTC bacteria are usually regarded as subspecies and are characteristically 99.9% similar at the nucleotide level, with identical 16S rRNA sequences.[23] However, there are distinct phenotypic differences between the subspecies, and not least their host range and pathogenicity.

M. bovis closely resembles *M. tuberculosis*, and precise identification of and distinction between the

two can be established by biochemical and molecular biology techniques. In the public health centres of most developing countries, Löwenstein-Jensen (LJ) medium, a medium on which *M. bovis* may grow poorly or not at all, is commonly used for the isolation of *M. tuberculosis*. Inoculated media are often incubated insufficiently for *M. bovis* cultures to appear. This may help to explain the low number of bovine-type human TB cases reported in developing countries.[8] *M. bovis* is a robust pathogen and may survive in the environment, in buildings, on transport vehicles, on pasture and in slurry. The organism has been reported to survive in cow faeces for more than 5 months in winter, 4 months in autumn, 2 months in summer, and in soil for up to 2 years.[24] Manure fertilisation of arable land is common practice in developing countries; survival of *M. bovis* in soil and slurry therefore implies pasture and vegetable contamination, representing a potential source of infection to animals and humans, respectively.

PATHOGENESIS

There are numerous ways in which cattle can become infected with *M. bovis*; these can be affected by animal age and behaviour, environment and climate, and prevailing farming practices.[25] Under natural conditions, the main route of *M. bovis* infection in cattle is by inhalation. This mode of transmission is dominant in industrialised countries, where intensive farming is practised. In field case studies of bovine TB in these countries, lesion distribution and pathology show predominant involvement of the upper and lower respiratory tract and associated lymph nodes.[26,27] Confirmed tuberculin reactors frequently appear to have an absence of lung lesions; however, lesions when present within the lung parenchyma are usually too small (<1 cm) to be easily detected during meat inspection.[28]

A generally accepted concept is that infection with *M. bovis* can become established in cattle by inhalation of tubercle bacilli, possibly a single bacillus, in an aerosol droplet[29] that lodges within the respiratory tract, probably the alveolar surface of the lung.[30] Bacilli are phagocytosed by macrophages, and subsequently interact with cells involved in innate and acquired immune responses in tissue or draining lymph nodes. This often results in nonvascular nodular granulomas known as 'tubercles'. Characteristic tuberculous lesions occur most frequently in lungs and retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, serous membranes, pleura and other organs.[11,26] The role of activated mononuclear macrophages is considered most important in protecting the host against *M. bovis*. Macrophages are involved in processing mycobacterial antigens and presenting them to T-lymphocytes, which are considered a key recognition unit in the immune response to mycobacteria.[31] The characteristic lesion caused by *M. bovis* in cattle is described as having a centre of caseous necrosis, usually with some calcification, with a boundary of epithelioid cells, some of which form multinucleated giant cells and few to numerous lymphocytes and neutrophils.[3,26] Primary lesions in cattle, unlike man, are rarely contained by the immune response, and dissemination from a lesion may occur by natural ducts such as bronchi, by lymphatic spread or by haematogenous spread when massive miliary TB occurs.[32]

It is worth noting that despite the many studies on bovine TB over the years, it was recently concluded that a better understanding of the dynamics of the events following *M. bovis* exposure of cattle and subsequent infection would be of significant benefit to diagnosis and disease control.[25]

TRANSMISSION

Inhalation of *M. bovis* is the most probable and principal route to bovine infection and is facilitated by close, prolonged contact between infected and healthy animals. Ingestion of *M. bovis* directly from infected animals or from contaminated pasture, water or utensils may also be very common in some regions. While congenital infections and vertical transmission have been recorded, these routes, like genital transmission, which occurs when reproductive organs are infected,[26] are now rarely seen in regions that have intensive eradication programmes.

Animal-to-animal transmission

Infectious animals may shed *M. bovis* in a number of ways: in faeces, milk, discharging lesions, saliva and urine.[29] Intensive livestock farming promotes close contact between animals, favouring the spread of *M. bovis*. Extensive livestock farming, however, especially transhumance with no housing system, raises the question as to how bovine TB transmission can take place. Close contact between animals occurs for example at water points such as ponds, wells and streams. In Africa, grazing animals usually gather at night for protection from predators. Vaccination and artificial insemination centres, dipping tanks, auction stations, market places and transportation are the commonest animal gathering places, and again are sites where transmission of infection could easily occur. Due to the high ambient temperature in tropical zones, animals tend to concentrate under trees or other shaded areas for parts of the day, preferring to graze early in the morning and late in the afternoon. Possibly the most dangerous spots for nose-to-nose or mouth-to-mouth contact between animals are salt supplementing points. Therefore, while extensive farming is safer than zero level grazing systems to prevent disease transmission, some of the above situations simulate the dangers of intensive farming in relation to disease transmission.

Animal-to-human transmission

Human TB due to *M. bovis* in developing countries today is analogous to conditions in the 1930s and 1940s in Europe, where more than 50% of cervical lymphadenitis cases in children were caused by *M. bovis* infection.[33] This is exacerbated by the added burden of HIV/acquired immune-deficiency syndrome (AIDS). In industrialised countries, the incidence of TB due to *M. bovis* in humans is almost at zero level as a result of pasteurisation of milk and milk products and eradication of bovine TB in cattle populations.[13] However, in developing countries, bovine TB in animals can be widely distributed in regions where control measures are not applied or are conducted sporadically and pasteurisation is rarely practised.[2] In industrialised countries, the direct correlation between *M. bovis* in cattle and TB due to *M. bovis* in humans has been well documented, whereas little information is available from developing countries.[13,33] Pulmonary TB due to *M. bovis* is more common in rural dwellers, as a result of inhalation of dust particles or bacteria-containing aerosols shed by infected animals, while urban dwellers acquire the infection via the gastrointestinal route and develop extra-pulmonary TB.[7] In countries with a relatively high prevalence of bovine TB in cattle, abattoir and farm workers are the groups most exposed to infection.

Current economic and social globalisation has created greater opportunities for the spread of zoonotic diseases such as TB. When considering the revival of TB in countries previously declared to be free of the disease, it is worth noting the statement by Grange: 'we are now learning the hard way that none are safe until all are safe'.[8]

Human-to-animal transmission

The role of humans in infecting cattle with bovine TB was reviewed by Torning in 1965.[34] Sjögren and Hillerdal cited several examples of human-to-cattle transmission, and stressed the potential danger that patients with smear-positive pulmonary TB due to *M. bovis* may pose to animals.[35] However, reports of human infection of cattle are rare.[17] The genitourinary tract in humans is a site of non-pulmonary TB due to *M. bovis*; genitourinary TB may appear to be of little importance to epidemiologists in studying human infection, but this route of infection from man to cattle is well-documented. Grange and Yates reported that farm workers urinating in cowsheds may represent a source of infection for animals.[36] An analogous situation is thought to occur in rural Africa, where patients with genitourinary TB may urinate on pasture: animals craving salt preferentially graze on this grass and may succumb to infection.

Human-to-human transmission

Human TB caused by *M. bovis* as a result of human-to-human transmission was reported in The Netherlands in 1994.[37] Evidence of transmission of *M. bovis* between humans is considered rare and largely anecdotal, and the rate of transmission seems insignificant compared to animal-to-animal or animal-to-human infection.[17]

Human-to-human transmission of *M. bovis* is considered less efficient than that of *M. tuberculosis*;[38] however, transmission among HIV-infected humans, where immunosuppression increases the susceptibility of the host organism to infection, may be different. *M. bovis* has been isolated from HIV-infected individuals in some industrialised countries, with an additional serious complication of high primary resistance to isoniazid, streptomycin and pyrazinamide.[39]

FACTORS CONDUCIVE TO THE PERSISTENCE OF TB IN AFRICA

The neglect of TB control by many governments—poorly managed control programmes, poverty, high population growth, population dislocation and the rise of HIV-endemic areas in Africa—predisposes to the persistence of TB. In many developing countries, TB control programmes, and especially the WHO-recommended DOTS strategy, do not work properly often due to cultural beliefs, socio-economic factors and poor infrastructures.

Culture and customs

In developing countries, particularly Africa, patients' beliefs and cultural factors are obstacles to TB control strategies. TB is stigmatised in many cultures and TB control may be further complicated by patients hiding their TB status due to discriminatory views about TB sufferers. A recent study in a rural area of South Africa reported the strong local community belief that TB results from breaking cultural rules and that the disease can be treated by traditional healers.[40] Here the disease is locally known as 'tindzaka', 'mafulaar' or 'makhuma'—all denoting a disease indistinguishable in presentation from TB. In addition to the use of traditional healers, factors such as stigma, difficulties in accessing health services, long waits at health centres and the sometimes negative attitudes of health workers can adversely influence patient adherence to allopathic treatment. Patients claimed to be unaware of the need to take a full course of treatment, and that the red colour of the urine due to rifampicin treatment adversely affected their partners, resulting in sexual abstention during treatment.[40]

The stigma of TB remains as powerful as that of HIV/AIDS. Sharing food and eating utensils among family members and friends is common in many African countries. Persons known to suffer from TB are often prevented from sharing and are completely ostracised. This fear of persisting social isolation tends to encourage patients to hide their disease until intervention is too late. In a close-knit community,

where two or three families live under one roof, people are as reluctant to provide information about a confirmed diagnosis of TB as they would be about HIV/AIDS. Targeted patient education needs to address misconceptions about TB and ensure adherence to treatment. Health workers should develop a knowledge and understanding of common beliefs and perceptions about illness in the communities in which they work.

Responding to public health imperatives and minimising stigma remain in a delicate balance. The profile of risk for bovine TB, however, is based on animal-to-human, rather than human-to-human transmission. Social discrimination based on TB status is thus more a matter of stigma than of appropriate public health precautions. Risk factor assessment and identification of this infectious agent in both humans and animals will be the first step towards adopting dependable preventive, therapeutic and control measures.

Illiteracy

Another, yet unsolved social problem in most rural communities of Africa is illiteracy. Inability to read and write, and failure to utilise modern methods of communication, makes prevention and control programmes difficult and often impossible to apply.

Demography, eating habits, living and socio-economic status of families

For most urban dwellers, milk is considered the main vector for infection by bovine TB, while farmers and abattoir workers are mostly exposed to aerosol infection by close contact with infected animals.[4] For most rural African populations, consumption of raw milk and milk products and close association between animals and farmers are common, and encourage exposure to both alimentary and respiratory infection by *M. bovis*.

The following factors contribute to the acquisition of infection in farmers and urban dwellers: 1) family ownership of cattle; 2) previous livestock ownership; 3) history of working with animals; 4) living with a relative who owns cattle; and 5) consumption of unpasteurised milk and raw or poorly cooked meat.[41–43]

Occupations related to acquisition of infection are: 1) abattoir workers, veterinarians and laboratory technicians; 2) animal caretakers in zoos; and 3) workers in animal reservations and national parks.[36,43,44]

Demographic factors also contribute to the epidemiology of bovine TB. These include income, education, age, number of families per dwelling, number of individuals per m^2 in a dwelling, sanitation, etc. Families in low-income countries such as Africa often experience malnutrition. This, associated with the burden of HIV/AIDS infection, increases susceptibility to various infectious diseases such as TB by impairing the immune system, particularly lymphocyte function, which plays an important role in containing mycobacterial infections.[45] Infants are more vulnerable to food-borne *M. bovis* infection, whereas in older individuals overt TB may occur as a result of endogenous reactivation.[3,8,36,43] Poor sanitation, lack of access to clean water, crowding, poor housing and the absence of health care play an important role in the epidemiology of TB in developing countries.[46,47]

HIV/AIDS-ASSOCIATED HUMAN TB DUE TO *M. BOVIS*

TB and other mycobacterial infections are major opportunistic infections in HIV/AIDS-infected individuals,[48] while HIV/AIDS is a major predisposing factor for TB, including reactivation of disease. The current spreading pandemic of HIV/AIDS infection in developing countries, especially where bovine TB is prevalent in domestic and wild animals, poses an additional serious public heath threat.[7,10,33,36]

DIAGNOSIS

TB can be diagnosed clinically, but usually only in the later stages of the disease. The tuberculin skin test (TST) is universally recognised and is generally used for preliminary diagnosis in bovine TB control programmes. However, in countries with low disease prevalence or disease free status, meat inspection is used for diagnosis and surveillance. Other tests, such as an antibody enzyme-linked immunoassay (ELISA) and the gamma-interferon assay, have been used as supplementary tests in eradication and control. Assays for bovine interferon-gamma have recently been applied to indicate infection.[49] Confirmation of infection, however, often relies on isolation and identification of *M. bovis*.

Clinical examination and necropsy

Many cattle with bovine TB are clinically normal. Some cows with extensive miliary tuberculous lesions also appear clinically normal, but progressive emaciation unassociated with other signs should arouse suspicion of TB. Capricious appetite and fluctuating temperature are also commonly associated with the disease. Pulmonary involvement is eventually characterised by chronic cough, together with dyspnoea and other signs of low-grade pneumonia. Affected animals are docile and sluggish, but the eyes remain bright and alert.[11] A report from Great Britain on the pathological examinations of cattle over 6 months old stated that the primary complex in TB is in the lungs and their associated lymph nodes.[50] More recent studies reported by Neill et al.[51] and Pollock and Neill[25] indicate that the precise processes by which cattle become infected by *M. bovis* may not be completely characterised or understood.

Microscopy

M. bovis can be demonstrated microscopically on direct smears from clinical samples, and on prepared tissue materials. Tissue smears from affected organs stained by the Ziehl-Neelsen (ZN) method can be used to demonstrate the presence of acid-fast mycobacteria, red bacilli on a pale blue background (methylene blue staining), and appear on a green background if re-stained with malachite green. Examination of a haematoxylin-eosin-stained section of lesion for each case that is positive on smear is valuable. This technique is cheap, practical and a useful preliminary diagnostic step in developing countries where other laboratory facilities are not available; furthermore, identification in clinical samples and pathological specimens can be performed rapidly.[30]

Culture on artificial media

Specimens are taken from lesioned lymph nodes and parenchymatous organs such as lungs, liver, spleen, etc. In animals with positive intradermal skin tests but showing no gross pathological lesions, samples from the retropharyngeal, bronchial, mediastinal and mesenteric lymph nodes are collected routinely for culture examination; the supramammary and mandibular glands and liver are sometimes included. Specimens are ground in silver sand, macerated or homogenised in a stomacher and decontaminated. Following centrifugation the sediment is often inoculated onto a set of solid media slopes consisting of egg-based media such as LJ, Coletsos base and Stonebrink's media. Slopes of these media containing pyruvate are inoculated, usually in duplicate, and an agar-based medium, such as Middlebrook 7H10 or 7H11, is also often used. Stonebrink's medium containing sodium pyruvate without glycerol is possibly the best medium. Colonies of *M. bovis* are expected to appear after 3–5 weeks of incubation at 37°C.[52]

A radiometric culture method such as the semi-automated BACTEC 460 system employing a liquid supplemented Middlebrook medium can also be used. Radio-labelled carbon dioxide ($^{14}CO_2$), obtained following metabolism of ^{14}C-labelled palmitic acid incorporated into the culture medium, is measured in an ion chamber system as an indicator of bacterial growth. ZN-stained smears from suspect culture growth are examined by direct light microscopy. Suspected mycobacterial cultures are inoculated into liquid or solid media and incubated for at least 8 weeks at 37°C in air with or without increased CO_2. Identification of presumptively identified mycobacterial isolates is commonly carried out by determining cultural and biochemical properties. The radiometric method provides results significantly more rapidly than traditional culture, but is often sensitive to fungal contamination, particularly when certain animal specimens are used, and will grow atypical mycobacteria or non-mycobacterial organisms not restricted by the antibiotics incorporated.[52] The disadvantages of this method are that the BACTEC method is more expensive, requires an instrument to read the culture vials, and involves handling of radioisotopes. It should be noted that acid-fastness is not restricted to the mycobacteria; other acid-fast organisms include *Corynebacterium* spp., *Nocardia* spp., and *Rhodococus* spp. Colony of MTC organisms in primary cultures can be observed after 4–6 weeks on solid media, and after 13–15 days in radiometric and automated culture systems.[53] The drawback of using radioisotopes has been overcome in a newer system of the Bactec 'MGIT' 960 culture system, which employs a fluorometric detection system. This system is considerably more expensive to buy, however, and has not yet proved consistently effective for culturing *M. bovis* from clinical specimens. Although older, other techniques are still in use[30] such as: 1) bacillary morphology and growth rates; 2) enhancement by glycerol of growth of *M. tuberculosis* isolates and suppression of *M. bovis* isolates; 3) niacin synthesis by *M. tuberculosis* but not by *M. bovis* isolates; 4) enhancement by pyruvate for growth of *M. bovis;* 5) oxygen preference—*M. bovis* is microaerophilic, whereas *M. tuberculosis* is aerobic; 6) growth characteristics—both *M. bovis* and *M. tuberculosis* fail to grow at 25°C and 42°C and hence are strict mesophiles; 7) colony pigmentation—neither *M. bovis* nor *M. tuberculosis* colonies are pigmented; 8) nitrate is reduced by *M. tuberculosis* but not by *M. bovis* isolates; 9) sensitivity to drugs—resistance to pyrazinamide of *M. bovis* isolates and sensitivity of *M. tuberculosis* isolates; 10) susceptibility to thiophene-2-carboxylic acid hydrazide by *M. bovis* but not by *M. tuberculosis* isolates.

Research is ongoing for alternative methods that are rapid, cheap and easy to perform. Recent studies using the mycobacterial antigen (MPB64) revealed discrimination of MTC members from mycobacteria other than tuberculosis (MOTT),[54] while Hasegawa et al. reported using lateral flow immunochromatographic assay (ICA) to detect MPB64 with anti-MPB64 monoclonal antibody, with 100% specificity and sensitivity in differentiating MTC bacteria from MOTT.[55] Other modern laboratory methods can be applied to discriminate mycobacterial isolates on the basis of the genomic characteristics of the organism. However, these methods are expensive for most developing countries with limited financial and technical resources.

Molecular diagnostics

Culture is still internationally considered the gold standard for detection of mycobacteria; however, the intensity of labour required and the possible presence of viable non-cultivable mycobacteria in some clinical specimens requires more appropriate methods. Molecular diagnostic methods are in principle attractive replacements for traditional procedures, but such tests

should have demonstrable, sustained improvement in sensitivity, specificity and reproducibility. They may be convenient and less costly when used for high throughput, but the availability of appropriate resources and trained staff is important and must be considered, particularly in developing countries. There is often a tendency to underestimate the difficulties inherent in introducing and applying these technologies to pathogens such as mycobacteria.

In mycobacteriology, for obvious reasons, molecular technologies have been applied primarily to enhance detection and typing of *M. tuberculosis*. More recently, however, through international collaborative efforts, attention has been directed at *M. bovis* as a significant animal pathogen with zoonotic potential. The intracellular nature and impermeability of mycobacterial cell walls, together with the presence of polymerase chain reaction (PCR) inhibitors in clinical specimens, limit the efficiency of PCR detection. This can be problematic and impact on the potential uses of PCR detection with certain clinical specimens and in particular with specimens taken from tuberculous cattle, where low numbers of bacilli are common. Although amplification-based detection kits are commercially available, a recent review indicates that detection of *M. bovis* in animals has focused primarily on 'in-house' amplification-based systems.[56]

Molecular biology techniques have now provided a means to detect and differentiate *M. bovis* isolates. Such differentiation should enable systematic epidemiological surveys to be conducted and, potentially, the origins of infection to be traced. Fingerprinting of *M. bovis* has been extremely effective using the restriction fragment analysis technique referred to as REA.[57] However, widespread adoption of the method has been curtailed by issues such as potential cost and difficulties in technology transfer. A modified method of pulse field gel electrophoresis (PFGE) for *M. bovis* has had little uptake generally, as most methods now have advanced to exploiting the repetitive DNA sequences that occur in the mycobacterial genomes. Two types of repetitive DNA are known in bacterial genomes—dispersed repeats and tandem repeats. Dispersed repeats include mobile genetic elements, including insertion sequences (ISs) and prophages.[58] Most of these ISs are located in the same genome position in members of the MTC; however, there are significant differences between species that are useful for diagnostics. The insertion element IS*6110*, which belongs to the IS3 family, is the most abundant and best-characterised. The IS*6110* copy number varies from 0 to 25 in MTC bacteria, whereas in *M. bovis* isolates the IS*6110* copy number is low, often occurring singly, especially in isolates from cattle.[59] Interestingly, and usefully exploitable, the IS*6110* copy number is often higher in *M. bovis* isolates from more 'exotic' animal species.[57]

Finding repetitive DNA in the genomes of MTC bacteria enabled an array of typing or fingerprinting techniques to be developed based on DNA amplification or Southern blotting, or a combination of the two.[60] DNA polymorphism in MTC is found in *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis* BCG, *M. bovis* subsp. caprae, *M. microti* and *M. canettii*.[61] Strain typing of *M. tuberculosis* isolates by IS*6110*-RFLP (restriction fragment length polymorphism) is now internationally accepted and is used to monitor the efficacy of human TB control programmes and to detect point source outbreaks.[62] However, for *M. bovis* isolates from cattle, because the IS*6110* copy number is low, additional probes are often required in RFLP typing to improve discrimination.[59] It is widely accepted that the RFLP techniques are highly discriminating, but they are also cumbersome and inconvenient to use. Other methods have therefore been developed, such as the spacer-oligotyping (spoligotyping) technique, which employs PCR amplification of the DNA sequence of the highly polymorphic direct repeat (DR) locus and reverse-cross blot hybridisation to detect the presence or absence of spacer DNA sequences, mostly from *M. tuberculosis*. The polymorphism is carried by these spacers, which are variable in length (35–41 bp). Spoligotyping is easily executed and produces a simple digital pattern, but it is less discriminating than REA or RFLP.[63] Further ex-amination of *M. tuberculosis* and *M. bovis* isolates has revealed additional spacer sequences that may further improve discrimination.[64] Spoligotyping is possibly the most widely used typing method for differentiation of isolates belonging to the MTC; because of its simplicity it is becoming a favoured method for typing *M. bovis* isolates. The method has been employed for simultaneous diagnosis and typing of mycobacterial isolates.[63,65]

The availability of the complete genome sequences for *M. tuberculosis* and *M. bovis*, together with advances in bioinformatics, has revealed novel information about DNA repeat sequences that has been employed to develop new typing methods for discriminating isolates.[58] One example is the variable number of tandem repeat (VNTR) typing method,[66] which through amplification of PCR products has exploited the VNTR loci comprising repeat units of varying size and copy number.[67,68] This method has the advantage of having the potential for automation and is hence useful for high throughput. There is little doubt that further advances will bring new genomic information, but these will have little immediate value for widespread adoption in developing countries.

Molecular technologies are currently rarely employed in developing countries, and where they are available, priority is often given to human cases. In Guinea-Bissau, Kallenius et al. successfully differentiated *M. bovis* and *M. tuberculosis* from other mycobacteria by combining biochemical tests with IS*6110* RFLP and spoligotyping.[22] A recent study in Ethiopia showed the importance of molecular techniques in

differentiating between *M. tuberculosis*, *M. africanum* and *M. bovis*, which were found to cause tuberculous lymphadenitis in humans.[69] Genotypic characterisation of mycobacterial isolates previously identified as *M. africanum*, and isolates from TB patients in Africa (Burkina Faso, Ivory Coast, Senegal, Mauritania, Benin, Burundi, Rwanda, Cameroon, Central African Republic and Madagascar) and France, was performed on the basis of IS*6110* RFLP analysis, IS*1081* RFLP analysis, spoligotyping, VNTR typing, and the polymorphism of the *oxyR*, *pncA* and *mtp40* loci. The results obtained showed that the majority of *M. africanum* isolates were characterised by a specific spoligotyping pattern that was intermediate between those of *M. tuberculosis* and *M. bovis*.[62]

PREVALENCE OF *M. BOVIS* IN SUB-SAHARAN AFRICA

In sub-Saharan Africa, where nearly 2 million TB cases occur each year, it is unknown what role cattle-derived *M. bovis* plays in the epidemic of TB.[70] However, there is substantive evidence of significant transmission of *M. bovis* in pastoral communities with close human-to-livestock contact.[71] In Sahelian countries, for example, there are large communities in which no livestock screening for bovine TB is conducted and people are exposed to direct contact with animals and consume unpasteurised milk and milk products. Due to a lack of capability to isolate and differentiate the organism from TB cases, cases due to *M. bovis* are possibly underreported, and may represent a significant threat to the community.[7]

In Nigeria, Idigbe et al. found *M. bovis* in 4% of patients with lower respiratory tract symptoms.[72] Hoffner et al. reported isolation and biochemical characterisation of *M. tuberculosis* and *M. bovis* in humans in Guinea-Bissau.[73] Vekemans et al. have retrospectively analysed the TB registers of Bobo Dioulasso, Burkina Faso, which correlated prevalence of cattle-related TB in ethnic groups.[74] In Burundi, Rigouts et al. isolated *M. bovis* in 38% of clinically suspected bovines.[75] DNA fingerprinting revealed 4–8 copies of IS*6110* for all *M. bovis* isolates with some degree of polymorphism. In Madagascar, a proportion of *M. bovis* (1.25%) was observed among sputum smear-positive patients and among extra-pulmonary TB patients (1.30%).[76] Jiwa et al., referring to the high morbidity and mortality due to HIV/AIDS in the Kagera area of Tanzania, suggested that the presence of bovine TB in cattle necessitates further investigation into the role of animal-derived bovine TB in human health.[77] Kazwala et al. emphasised that non-tuberculosis complex mycobacteria are a danger to human health in countries such as Tanzania, where the number of people with impaired immunity due to HIV/AIDS infection is growing.[42] Ledru et al. suggest that most TB cases in African HIV/AIDS patients are due to exogenous re-infection rather than to reactivation of endogenous *M. tuberculosis*.[78] Considering the association of HIV/AIDS with TB in humans, similar risk may occur in individuals exposed to infection with *M. bovis*.[17]

Livestock

M. bovis is endemic in Uganda.[79] Vekemans et al. reported purified protein derivative (PPD) TST of cattle, with 13% positive reactions and isolation of mycobacteria in 26% of 60 retailed milk samples collected in markets in Burkina Faso.[74] Jiwa et al. report a 0.2% prevalence of bovine TB in the Lake Victoria area of Tanzania.[77]

Kazwala et al. isolated mycobacterial species from the raw milk of pastoral cattle in the Southern Highlands of Tanzania.[42] Although the number of *M. bovis*-positive samples was low, the habit of pooling milk may still pose a public health danger to milk consumers. There is very little information, mostly in the form of unpublished reports, on the prevalence of *M. bovis* in livestock in Chad, where Schelling et al. found a 17% prevalence of bovine TB using the PPD tuberculin test.[80] Quantification of *M. bovis* in livestock is thus clearly important. Chad, where 949 cases of human TB were reported for a total population of nearly 7 million in 1996, is suspected to have an incidence of TB ranging from 100 to 250 per 100 000 population. In this situation an incidence rate of *M. bovis*-derived TB of 4–25/100 000 can be expected, depending on levels of exposure to infected livestock and milk.[41]

Although bovine TB in cattle is widespread in Africa, some member states fail to report the annual prevalence and incidence of the disease to the OIE, while others tend to report the disease sporadically, at intervals of several years (Table).

Sub-Saharan Africa covers large expanses of arid territory, where dairy cattle production is limited due to inadequate forage; in these areas goats are commonly kept for milk and meat production, and are locally known as 'poor man's cows' due to their adaptability to the high temperatures and efficient conversion of inedible roughage to edible animal protein. Contact between goats with cattle at pasture and in huts at night is common, and may expose them to infection with *M. bovis*. Similarly, in Spain, Garcia-Marine has reported isolating *M. bovis* from goats housed with cattle at night to protect them from wild carnivores.[81]

Game animals

In those countries where bovine TB has been eliminated, wild and feral tuberculous animals constitute a serious risk of re-infection for domestic animals. Woodford found *M. bovis* in warthog (*Phacochoerus aethiopicus*) and buffalo (*S. caffer-sparrman*) in the Ruwenzori National Park in Uganda.[82] On follow-up

Table Bovine tuberculosis in cattle in 43 African countries, 1992–2001*

Country	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
Algeria	+	+	+	+	+	+	+	+	+	+
Angola	+	+	+	+	+	+	+	NR	+	NR
Botswana	+	NR	000	1993	NR	NR	NR	NR	NR	NR
Burkina Faso	+	+	NR	+	+	+	+	+	NR	NR
Cameroon	+	+	NR	NR	?	+	+	+	+	+
Cape Verde	NR	+	NR	NR	?	?	NR	1987	NR	NR
Central African Republic	?	?	+	+	+	NR	NR	NR	NR	NR
Chad	+	+	+	+	. . .	+	+	NR	NR	NR
Comoros	?	?	NR	NR	NR	NR	000	NR	NR	NR
Ivory Coast	+	+	+	+	+	+	+	+	+	+
Democratic Republic of Congo	NR	NR	NR	NR	NR	+	NR	NR	NR	NR
Egypt	+	+	+	+	+	+	+	+	+	+
Eritrea	+	+	+	NR	+	+	+	+	+	+
Ethiopia	+	+	+	+	+	. . .	. . .	NR	NR	+
Gabon	NR	NR	NR	+	NR	NR	NR	NR	NR	NR
Gambia	NR	NR	NR	NR	. . .	NR	NR	NR	NR	NR
Ghana	+	+	+	+	+?	+	+	NR	+	+
Guinea	. . .	NR	. . .	. . .	. . .	NR	NR	NR	NR	NR
Kenya	000	NR	NR	NR	+	+	+	+	+	+
Lesotho	. . .	NR	NR	NR	NR	NR	NR	NR	+	NR
Libya	+	?	+	NR	NR	+	+	+	+	+?
Madagascar	+	+	+	+	+	+	+	+	NR	NR
Malawi	+	+	NR	+	+	+	+	+	NR	+
Mali	+	NR	NR	NR	+	+	+	+	+	NR
Mauritius	+	+	NR	NR	+	NR	NR	NR	NR	NR
Morocco	+	+	+	. . .	+	+	+	+	+	NR
Mozambique	+	+	+	NR	1995	+	+	+	NR	NR
Namibia	1984	1984	+	+	+	1995	1995	1995	1995	1995
Niger	+	+	+	+	NR	+	+	NR	NR	+
Nigeria	+	+	+	NR	+	NR	NR	NR	NR	+
Reunion (FR)	+	NR	NR	NR	+	+	+	+	+	+
Sao Tome & Principe	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Senegal	+	NR	NR	NR	. . .	. . .	. . .	NR	NR	NR
Seychelles	NR	NR	NR	NR	NR	NR	NR	NR	000	NR
South Africa	+	+	+	+	+	+	+	+	+	+
Sudan	+	NR	NR	NR	1992	1992	1992	1992	1992	1992
Swaziland	NR	+	NR	+	+?	+	+	NR	+	+
Tanzania	+	NR	NR	+	NR	+	+	+	+?	+
Togo	+	NR	NR	+	NR	+	NR	NR	+	+
Tunisia	+	+	+	+	+	+	+	+	+	+
Uganda	+	+	+	+	+	+	+	+	+	+
Zambia	+	NR	. . .	+	. . .	+	+	NR	+	NR
Zimbabwe	NR	NR	NR	1990	1990	1990	1990	1990	1996	1996

* Data were extracted from the following sources: FAO-OIE-WHO Animal Health Yearbooks, Rome, Italy: OIE, 1992–1997, and OIE World Animal Health in 1997. Parts 1 and 2, Paris, France: OIE, 1998–2001.
+ = low sporadic occurrence; NR = disease not reported; 000 = never reported; (1987) = year of last occurrence; ? = suspected but not confirmed; . . . = no information available; +? = serological evidence and/or isolation of causative agent, no clinical disease.

of an outbreak of *M. bovis* in a population of feral baboons in Kenya, Sapolsky and Else concluded that the source of infection was animals feeding on village abattoir offal of *M. bovis*-infected cows.[83]

Bovine TB is now a particularly serious problem in South Africa's Kruger National Park, where bovine TB was diagnosed for the first time in an African buffalo in 1996.[84] In the same year, Keet et al. reported bovine TB in a cheetah, two lions and a baboon (*Papio ursinus*) from the park.[12] It is assumed that they contracted the disease directly or indirectly from tuberculous buffaloes. Tuberculous granulomatous lesions in the lungs were extensive and constituted the predominant changes in all three animal species. African buffalo form an integral part of the ecosystem, serving as a source of infection for predators and the environment, and they are one of the preferential prey animals for lions in Kruger Park.[85] Weak, young, old and debilitated animals are more vulnerable to predation by lions and other large predators.

Transmission of the infection to herds of wildebeest was confirmed for the first time in 1998. The continuing geographical spread of the disease to animal species such as kudu (*Tragelaphus strepsiceros*), baboons (*Papio* sp.), lions (*Panthera leo*), cheetah (*Acinonyx jubatus*) and leopards (*P. pardus*) living in the parks and consequently to free-ranging species that may act as maintenance hosts of the infection is a matter of serious concern.[86] From a conservation point of view, bovine TB therefore potentially poses a serious threat to endangered species.

CONTROL AND PREVENTION

As Cousins has suggested,[87] there are various reasons for attempting to eradicate bovine TB: 1) the risk of infection to the human population; 2) loss in productivity due to infected animals; and 3) animal market restrictions set by countries with advanced eradication programmes. The priority they are given will vary depending on factors specific to the country in question. In Africa, the economic losses associated with livestock contracting bovine TB has either never been studied or has not been examined sufficiently. However, data can be obtained from other countries where bovine TB is prevalent. In Argentina, for example, the annual loss due to bovine TB is approximately US$63 million.[2] Based on these estimates, it can be inferred that bovine TB may pose a serious economic risk to Africa. Moreover, preliminary reports submitted by member states to the OIE indicate that the disease occurs almost everywhere on the African continent, with inevitable economic and public health implications (Table).

In industrialised countries, control and eradication of bovine TB has been successfully carried out by regular testing and removal of infected animals under mandatory national bovine TB programmes. Such programmes have been successful in many European Union member states and in seven central European countries between 1953 and 1980.[15] In developing countries, however, bovine TB remains a major animal health problem, mainly because these countries cannot shoulder the financial burden required to implement a control programme and compensate for slaughtered animals. Limited access to education, poor information networks and lack of disease surveillance are other factors that limit the implementation of any such programme.

Vaccination

BCG, an attenuated strain produced by continuous subculture of a wild-type *M. bovis* isolate from cattle, has played a crucial role in controlling human TB, particularly in children. However, its use for bovine TB is less effective. Using BCG vaccination to control bovine TB is an option that has been considered in European countries, North America and some African countries. However, because of its limited effectiveness, the Joint WHO/FAO Expert Committee on Zoonoses stated in its second report that 'The committee is of the opinion that vaccination has no place in the eradication of bovine TB in cattle'.[88] In a report by Waddington and Ellwood, attempts to protect cattle against bovine TB by BCG vaccination had no success.[89]

Buddle et al. found an absence of protective immunity in BCG-vaccinated cattle, possibly linked to immune responses developed to environmental mycobacteria, although it might have been expected that exposure to the shared antigens of environmental mycobacteria would provide acquired protection from bovine TB.[90] A study conducted by Corner et al. on the use of vaccine against bovine TB in brushtail possums (*T. vulpecula*) revealed 69% efficacy of BCG vaccine.[91] Skinner et al. recently examined the efficacy of vaccination with BCG alone and a DNA prime-BCG boost regimen in cattle challenged with virulent *M. bovis*. The prime-boost regimen significantly enhanced protection in six parameters compared to significant enhancement of protection in only two parameters for BCG alone.[92] This was demonstrated by fewer animals with severe lung lesions, fewer lymph nodes with lesions per animal, a smaller proportion of animals with lesions, lower mean lung and lymph node lesion scores and less *M. bovis* isolated from retropharyngeal and thoracic lymph nodes compared to non-vaccinated challenge animals.[91]

A vaccination strategy employing BCG would obviously necessitate developing differential diagnostic assays to distinguish vaccinates from non-vaccinates, as BCG vaccination causes sensitivity to tuberculin, the PPD routinely used in skin testing tuberculous cattle. Because of this and the varying efficacy of BCG in cattle, recent research capitalising on advances in immunology and molecular biology has focused on alternatives to BCG, including novel attenuated *M. bovis* strains, sub-unit vaccines and recombinant DNA vaccines.[93] The recently available *M. bovis* genome sequence should have a significant impact on new generation vaccine candidates.[94]

Eradication of bovine TB using compulsory test and slaughter strategies has proven difficult even in industrialised countries, where cattle movement can usually be controlled. In some of these countries, where natural reservoirs of the disease in wild animals pose a serious risk of transmission to domestic livestock, the problems have been exacerbated, and eradication programmes remain unsuccessful. In most African countries, controlling free movement of animals within a country is difficult and movement between countries cannot be regulated, primarily due to a lack of border controls. In addition, spread of bovine TB amongst wildlife in game parks in Africa is increasingly being recognised as a serious problem, with consequences for domestic animals. African countries may therefore find advantages in vaccinating susceptible animals. Daborn and Grange have suggested strategic vaccination of susceptible domestic animals in endemic areas as a feasible option for Africa, where control of bovine TB is a much more acceptable and practical measure and eradication is not the objective.[9] Skinner et al. reported that vaccination could potentially be used to control bovine TB in countries where wildlife reservoirs exist and in those that cannot afford conventional control procedures.[95] Development and production of an effective vaccine with appropriate methods and strategies for delivery could therefore contribute to

bovine TB control in Africa. This is obviously a task best undertaken at a global level and applied locally in appropriate scenarios.

CONCLUSIONS

Although other infectious diseases that are more fatal for animals are prevalent in Africa, bovine TB is a significant zoonotic human pathogen that aggravates the 'triple trouble' of HIV/AIDS and TB infection and malnutrition. International market requirements in trading of animals and their products seek demonstrable high health status of food animals. In the long run, therefore, developing countries such as those in Africa need to control and eventually eradicate bovine TB, as agriculture remains the backbone of many of these nations' economies.

Numerous actions could be taken to assist bovine TB control in both animals and humans. In general, information about zoonotic diseases and their potential impact on human health should be disseminated appropriately in developing countries. The following remedial measures are suggested for bovine TB:

- While pasteurisation of milk is essential to render milk free of *M. bovis* for human consumption, this option is not applicable in rural African communities due to lack of infrastructures and traditional use of curdled milk; this custom should be eradicated by educating the public to boil milk before consumption.
- As inspection of abattoir meat is limited in urban areas for the same reasons as milk pasteurisation, thoroughly cooking meat would reduce human TB due to *M. bovis* and other food-borne infectious diseases.
- Economic and technical assistance by industrialised countries is essential to promote control of TB in general and of bovine TB in particular. The decision makers of all African nations, particularly heads of government and departments of culture, health, education and agriculture, can play a role by creating the infrastructure necessary to achieve this goal.
- In the context of global eradication of TB, elimination of bovine TB in domestic and wild animals could be considered as a long-term objective for developing countries such as in Africa; nevertheless, control of the disease is now essential. The development and use of new and effective vaccines to susceptible animals are therefore a priority.

For those who have the capacity to perform identification of both *M. tuberculosis* and *M. bovis* isolates, the contributions to human infection from animal sources need to be verified, and the emergence of multidrug-resistant strains needs to be monitored. This will provide substantial information for the national and international scientific community to assess the character of new strains. Despite the high cost of general, widespread application in developing countries, molecular typing may be necessary and could enhance epidemiological surveillance of the disease and comparison of strains in different regions of the world.

Although it may be considered as a local or regional issue, bovine TB in the developing world should be seen in the context of the devastating effect of the HIV/AIDS pandemic associated with mycobacterial infections. The majority of these nations' populations live in resource-poor settings where nutritional depletion results in impairment of the host immune function and failure of anti-tuberculosis treatment and vaccination. The international community must therefore respond rapidly to these problems to curb any additional contribution to the growing global TB pandemic, with its consequent disastrous effect on humans.

Acknowledgements

The work of Dr Jakob Zinsstag, Switzerland, was supported by NCCR North-South IP4. This work was supported by grant N° QD1191, from the National Agency for Agricultural Research of the Ministry of Agriculture, Czech Republic.

References

1 Anon. Framework for effective tuberculosis control. Geneva, Switzerland: World Health Organization, 1994: 179 pp.
2 Cosivi O, Grange J M, Daborn C J, et al. Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. Emerg Inf Dis 1998; 4: 59–70.
3 Thoen C O, Steele J H, eds. *Mycobacterium bovis* infection in animals and humans. Arnes, IA: Iowa State University Press, 1995; 355 pp.
4 Anon. Zoonotic tuberculosis (*Mycobacterium bovis*): a memorandum from WHO (with participation of FAO). Bull World Health Organ 1994; 72: 851–857.
5 Murray C J, Styblo K, Rouillon A. Tuberculosis in developing countries: burden, intervention and cost. Bull Int Union Tuberc Lung Dis 1990; 65: 6–24.
6 Zumla A, Squire S B, Chintu C, Grange J M. The tuberculosis pandemic: implications for health in the tropics. Trans Royal Soc Trop Med Hyg 1999; 93: 113–117.
7 Daborn C J, Grange J M, Kazwala R R. The bovine tuberculosis cycle—an African perspective. J Appl Bacteriol 1996; 81: S27–S32.
8 Grange J M. The future and the challenge. Mycobacteria and human disease. 2nd ed. London, UK: Arnold, 1996; 2: 7–10.
9 Daborn C J, Grange J M. HIV/AIDS and its implications for the control of animal tuberculosis. Br Vet J 1993; 149: 405–417.
10 Pavlik I, Machackova M, Ayele W Y, et al. Incidence of bovine tuberculosis in wild and domestic animals other than cattle in six Central European countries during 1990–1999. Vet Med Czech 2002; 47: 122–131.
11 Blood D C, Radostis O M, eds. Disease caused by mycobacteria—IV. In: Veterinary Medicine. 7th ed. London, UK: Bailliere Tindall, 1989; 7: 710–740.
12 Keet D F, Kriek N P J, Penrith M L, Michel A, Huchzermeyer H. Tuberculosis in buffaloes (*Syncerus caffer*) in the Kruger National Park: spread of the disease to other species. Onderstepoort J Vet 1996; 63: 239–244.
13 Collins C H, Grange J M. The bovine tubercle bacillus. J Appl Bacteriol 1983; 55: 13–29.

14 Yates M D, Pozniak A, Grange J M. Isolation of mycobacteria from patients seropositive for the human-immunodeficiency virus (HIV) in South East England—1984–1992. Thorax 1993; 48: 990–995.
15 Pavlik I, Ayele W Y, Parmova I, et al. Incidence of bovine tuberculosis in cattle in seven Central European countries during the years 1990–1999. Vet Med Czech 2002; 47: 45–51.
16 Lee K, Mills A. Strengthening governance for global health research—the countries that most need health research should decide what should be funded. Br Med J 2000; 321: 775–776.
17 ÓReilly L M, Daborn C J. The epidemiology of *Mycobacterium bovis* infections in animals and man—a review. Tubercle Lung Dis 1995; 76: 1–46.
18 Rastogi N, Legrand E, Sola C. The mycobacteria: an introduction to nomenclature and pathogenesis. Rev Sci Tech Off Int Epiz 2001; 20: 21–54.
19 Behr M A, Small P M. A historical and molecular phylogeny of BCG strains. Vaccine 1999; 17: 915–922.
20 van Soolingen D, Hoogenboezem T, De Haas P E W, et al. A novel pathogenic taxon of the *Mycobacterium bovis* complex, Canetti: characterization of an exceptional isolate from Africa. Int J Syst Bacteriol 1997; 47: 1236–1245.
21 Aranaz A, Cousins D, Mateous A, Dominguez L. Elevation of *Mycobacterium tuberculosis* subsp. *caprae* Aranaz et al. 1999 to species rank as *Mycobacterium caprae* comb. nov., sp. nov. Int J Syst Evol Microbiol 2003; 53: 1785–1789.
22 Kallenius G, Koivula T, Ghebremichael S, et al. Evolution and clonal traits of *Mycobacterium bovis* complex in Guinea-Bissau. J Clin Microbiol 1999; 37: 3872–3878.
23 Brosch R, Gordon S V, Marmiesse M, et al. A new evolutionary scenario for the *Mycobacterium bovis* complex. Proc Nat Acad Sci USA 2002; 99: 3684–3689.
24 Wray C. Survival and spread of pathogenic bacteria of veterinary importance within the environment. Vet Bull 1975; 45: 543–550.
25 Pollock J M, Neill S D. *Mycobacterium bovis* infection and tuberculosis in cattle. Vet J 2002; 163: 115–127.
26 Neill S D, Pollock J M, Bryson D B, Hanna J. Pathogenesis of *Mycobacterium bovis* infection in cattle. Vet Microbiol 1994; 40: 41–52.
27 Whipple D L, Bolin C A, Miller J M. Distribution of lesions in cattle infected with *Mycobacterium bovis*. J Vet Diagn Invest 1996; 8: 351–354.
28 Mcllroy S G, Neill S D, McCracken R M. Pulmonary-lesions and *Mycobacterium bovis* excretion from the respiratory-tract of tuberculin reacting cattle. Vet Rec 1986; 118: 718–721.
29 Neill S D, O'Brien J J, Hanna J. A mathematical model for *Mycobacterium bovis* excretion from tuberculous cattle. Vet Microbiol 1991; 28: 103–109.
30 Pritchard D G. A century of bovine tuberculosis 1888–1988—conquest and controversy. J Comp Pathol 1988; 99: 357–399.
31 Thoen C O, Himes E M. Pathogenesis of *Mycobacterium bovis* infection. Progr Vet Microbiol Immunol 1986; 2: 198–214.
32 Francis J. Bovine tuberculosis including a contrast with human tuberculosis. London, UK: Staples Press Ltd, 1947: 220 pp.
33 Cosivi O, Meslin F X, Daborn C J, Grange J M. Epidemiology of *Mycobacterium bovis* infection in animals and humans, with particular reference to Africa. Rev Sci Tech Off Int Epiz 1995; 14: 733–746.
34 Torning K. Bovine tuberculosis. Dis Chest 1965; 50: 241–246.
35 Sjögren I, Hillerdal O. Bovine tuberculosis in man—reinfection or endogenous exacerbation. Scand J Respir Dis 1978; 59: 167–170.
36 Grange J M, Yates M D. Zoonotic aspects of *Mycobacterium bovis* infection. Vet Microbiol 1994; 40: 137–151.
37 van Soolingen D, De Haas P E W, Haagsma J et al. Use of various genetic markers in differentiation of *Mycobacterium bovis* strains from animals and humans and for studying epidemiology of bovine tuberculosis. J Clin Microbiol 1994; 32: 2425–2433.
38 van Soolingen D. Molecular epidemiology of tuberculosis and other mycobacterial infections: main methodologies and achievements. J Intern Med 2001; 249: 1–26.
39 Guerrero A, Cobo J, Fortun J et al. Nosocomial transmission of *Mycobacterium bovis* resistant to 11 drugs in people with advanced HIV-1 infection. Lancet 1997; 350: 1738–1742.
40 Edginton M E, Sekatane C S, Goldstein S J. Patients' beliefs: do they affect tuberculosis control? A study in a rural district of South Africa. Int J Tuberc Lung Dis 2002; 6: 1075–1082.
41 Annon. Global tuberculosis control. Geneva, Switzerland; World Health Organization, 1997: 1–221.
42 Kazwala R R, Daborn C J, Kusiluka L J, Jiwa S F, Sharp J M, Kambarage D M. Isolation of *Mycobacterium* species from raw milk of pastoral cattle of Southern Highlands of Tanzania. Trop Anim Health Prod 1998; 30: 233–239.
43 Pavlik I, Ayele W Y, Parmova I, Melicharek I et al. *Mycobacterium bovis* in animal and human populations in six Central European countries during 1990–1999. Vet Med-Czech 2002; 48: 83–89.
44 Liss G M, Wong L, Kittle D C, et al. Occupational exposure to *Mycobacterium bovis* infection in deer and elk in Ontario. Can J Public Health 1994; 85: 326–329.
45 Macallan D C. Malnutrition in tuberculosis. Diagn Microbiol Infect Dis 1999; 34: 153–157.
46 Krieger J, Higgins D L. Housing and health: time again for public health action. Am J Public Health 2002; 92: 757–768.
47 Triggle D J. Medicine in the 21st century or pills, politics, potions, and profits: where is the public policy? Drug Dev Res 2003; 59: 269–291.
48 Raviglione M C, Snider D E, Kochi A. Global epidemiology of tuberculosis—morbidity and mortality of a worldwide epidemic. JAMA 1995; 273: 220–226.
49 Neill S D, Cassidy J, Hanna J, et al. Detection of *Mycobacterium bovis* infection in skin test-negative cattle with an assay for bovine interferon-gamma. Vet Rec 1994; 135: 134–135.
50 Stamp J T. Bovine pulmonary tuberculosis. J Comp Pathol 1948; 58: 9–23.
51 Neill S D, Bryson D G, Pollock J M. Pathogenesis of tuberculosis in cattle. Tuberculosis 2001; 81: 79–86.
52 Kubica G P. Clinical microbiology. In: Kubica G P, Wayne L G, eds. The mycobacteria, a source book, Part A. New York, NY: Marcel Dekker, 1984: 680 pp.
53 Eisenach K D. Molecular diagnostics. In: Rutledge C, Dale J, eds. *Mycobacterium*—molecular biology and virulence. Oxford, UK: Blackwell Science, 1998: 161–179.
54 Abe C, Hirano K, Tomiyama T. Simple and rapid identification of the *Mycobacterium bovis* complex by immunochromatographic assay using anti-MPB64 monoclonal antibodies. J Clin Microbiol 1999; 37: 3693–3697.
55 Hasegawa N, Miura T, Ishii K, et al. New simple and rapid test for culture confirmation of *Mycobacterium bovis* complex: a multicenter study. J Clin Microbiol 2002; 40: 908–912.
56 Hughes D. Exploiting genomics, genetics and chemistry to combat antibiotic resistance. Nature Rev Gen 2003; 4: 432–441.
57 Collins D M. DNA typing of *Mycobacterium bovis* strains from the Castlepoint area of the Wairarapa. N Z Vet J 1999; 47: 207–209.
58 Gordon S V, Heym B, Parkhill J, Barrell B, Cole S T. New insertion sequences and a novel repeated sequence in the genome of *Mycobacterium bovis* H37Rv. Microbiol 1999; 145: 881–892.
59 Skuce R A, Brittain D, Hughes M S, Neill S D. Differentiation of *Mycobacterium bovis* isolates from animals by DNA typing. J Clin Microbiol 1996; 34: 2469–2474.
60 Tanaka M M, Small P M, Salamon H, Feldman M W. The dynamics of repeated elements: applications to the epidemiology of tuberculosis. Proc Nat Acad Sci USA 2000; 97: 3532–3537.

61 Kremer K, van Soolingen D, Frothingham R, et al. Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium bovis* complex strains: interlaboratory study of discriminatory power and reproducibility. J Clin Microbiol 1999; 37: 2607–2618.

62 Viana-Niero C, Gutierrez C, Sola C, et al. Genetic diversity of *Mycobacterium africanum* clinical isolates based on IS*6110*-restriction fragment length polymorphism analysis, spoligotyping, and variable number of tandem DNA repeats. J Clin Microbiol 2001; 39: 57–65.

63 Roring S, Brittain D, Bunschoten A E, et al. Spacer oligotyping of *Mycobacterium bovis* isolates compared to typing by restriction fragment length polymorphism using PGRS, DR and IS*6110* probes. Vet Microbiol 1998; 61: 111–120.

64 van Embden J D A, van Gorkom T, Kremer K, Jansen R, van der Zeijst B A M, Schouls L M. Genetic variation and evolutionary origin of the direct repeat locus of *Mycobacterium bovis* complex bacteria. J Bacteriol 2000; 182: 2393–2401.

65 Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of *Mycobacterium bovis* for diagnosis and epidemiology. J Clin Microbiol 1997; 35: 907–914.

66 Magdalena J, Vachee A, Supply P, Locht C. Identification of a new DNA region specific for members of *Mycobacterium bovis* complex. J Clin Microbiol 1998; 36: 937–943.

67 Skuce R A, McCorry T P, McCarroll J F, et al. Discrimination of *Mycobacterium bovis* complex bacteria using novel VNTR-PCR targets. Microbiology 2002; 148: 519–528.

68 Smittipat N, Palittapongarnpim P. Identification of possible loci of variable number of tandem repeats in *Mycobacterium bovis*. Tubercle Lung Dis 2000; 80: 69–74.

69 Kidane D, Olobo J O, Habte A, et al. Identification of the causative organism of tuberculous lymphadenitis in Ethiopia by PCR. J Clin Microbiol 2002; 40: 4230–4234.

70 Daborn C J. Bovine tuberculosis in the tropics—a call to arms. In: Tacher G, Leteneur L, eds. Proceedings of the VIIth International Conference of the Institute of Tropical Veterinary Medicine. Yamoussoukro, Côte d'Ivoire: Institute of Tropical Veterinary Medicine, 1992: 359–368.

71 Mposhy M, Binemo-Madi C, Mudakikwa B. Incidence of bovine tuberculosis on the health of the populations of North-Kivu (ZAIRE). Rev Elev Med Vet Pay 1983; 36: 15–18.

72 Idigbe E O, Anyiwo C E, Onwujekwe D I. Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. J Trop Med Hyg 1986; 89: 143–148.

73 Hoffner S E, Hjelm U, Kallenius G. Susceptibility of *Mycobacterium malmoense* to antibacterial drugs and drug-combinations. Antimicrob Agents Chemother 1993; 37: 1285–1288.

74 Vekemans M, Cartoux M, Diagbouga S, et al. Potential source of human exposure to *Mycobacterium bovis* in Burkina Faso, in the context of the HIV epidemic. Clin Microbiol Infect 1999; 5: 617–621.

75 Rigouts L, Maregeya B, Traore H, Collart J P, Fissette K, Portaels F. Use of DNA restriction fragment typing in the differentiation of *Mycobacterium bovis* complex isolates from animals and humans in Burundi. Tubercle Lung Dis 1996; 77: 264–268.

76 Rasolofo-Razanamparany V, Menard D, Rasolonavalona T, et al. Prevalence of *Mycobacterium bovis* in human pulmonary and extra-pulmonary tuberculosis in Madagascar. Int J Tuberc Lung Dis 1999; 3: 632–634.

77 Jiwa S F H, Kazwala R R, Aboud A A O, Kalaye W J. Bovine tuberculosis in the Lake Victoria Zone of Tanzania and its possible consequences for human health in the HIV/AIDS era. Vet Res Commun 1997; 21: 533–539.

78 Ledru E, Ledru S, Zoubga A. Granuloma formation and tuberculosis transmission in HIV- infected patients. Immunol Today 1999; 20: 336–337.

79 Office International des Epizooties. World Animal Health. Part I. Paris, France: OIE, 1992: 263–264.

80 Schelling E, Diguimbaye C, Daoud S. Zoonoses in nomadic populations of Chad—preliminary results obtained in humans and livestock. In: Salman M D, Paul S, Ruch-Gallie R, eds. Proceedings of the 9th Conference of the International Society of Veterinary Epidemiology and Economics. Breckenridge, CO: International Society of Veterinary Epidemiology and Economics, 6–11 August 2000, [abstract No. 396].

81 Garcia-Marine F. Report of meetings of the IUATLD scientific committee on tuberculosis in animals. Paris, October 15–16 1992. Paris, France: IUATLD Newsletter March, 1993: 14–16.

82 Woodford M H. Tuberculosis in wildlife in the Ruwenzori National Park Uganda. 1. Trop Anim Health Prod 1982; 14: 81–88.

83 Sapolsky R M, Else J G. Bovine tuberculosis in a wild baboon population—epidemiologic aspects. J Med Primatol 1987; 16: 229–235.

84 Bengis R G, Kriek N P J, Keet D F, Raath J P, DeVos V, Huchzermeyer H F A K. An outbreak of bovine tuberculosis in a free-living African buffalo (*Syncerus caffer-Sparrman*) population in the Kruger National Park: a preliminary report. Onderstepoort J Vet Res 1996; 63: 15–18.

85 Morris P J, Thoen C O, Legendre A M. Pulmonary tuberculosis in an African lion (*Panthera leo*). J Zoo Wildl Med 1996; 27: 392–396.

86 Zieger U, Pandey G S, Kriek N P J, Cauldwell A E. Tuberculosis in kafue lechwe (*Kobus leche kafuensis*) and in a bushbuck (*Tragelaphus scriptus*) on a game ranch in Central Province, Zambia. J South Afric Vet Assoc 1998; 69: 98–101.

87 Cousins D V. *Mycobacterium bovis* infection and control in domestic livestock. Rev Sci Tech Off Int Epiz 2001; 20: 71–85.

88 World Health Organization Technical Report Series. Joint WHO/FAO Expert Committee on Zoonoses, Second Report. Geneva, Switzerland: WHO, 1959: 169 pp.

89 Waddington F G, Ellwood D C. An experiment to challenge the resistance to tuberculosis in BCG-vaccinated cattle in Malawi. Br Vet J 1972; 128: 541–552.

90 Buddle B M, Keen D, Thomson A, et al. Protection of cattle from bovine tuberculosis by vaccination with BCG by the respiratory or subcutaneous route, but not by vaccination with killed *Mycobacterium vaccae*. Res Vet Sci 1995; 59: 10–16.

91 Corner L A L, Norton S, Buddle B M, Morris R S. The efficacy of bacille Calmette-Guerin vaccine in wild brushtail possums (*Trichosurus vulpecula*). Res Vet Sci 2002; 73: 145–152.

92 Skinner M A, Buddle B M, Wedlock D N, et al. A DNA prime *Mycobactetium bovis* BCG boost vaccination strategy for cattle induces protection against bovine tuberculosis. Infect Immun 2003; 71: 4901–4907.

93 Ginsberg A M. What's new in tuberculosis vaccines? Bull World Health Organ 2002; 80: 483–488.

94 Garnier T, Eiglmeier K, Camus J C, et al. The complete genome sequence of *Mycobacterium bovis*. Proc Natl Acad Sci USA 2003; 100: 7877–7882.

95 Skinner M A, Wedlock D N, Buddle B M. Vaccination of animals against *Mycobacterium bovis*. Rev Sci Tech OIE 2001; 20: 112–132.

RÉSUMÉ

La tuberculose bovine est une affection caractérisée par le développement progressif de lésions granulomateuses spécifiques ou de tubercules dans les tissus pulmonaires, dans les ganglions lymphatiques ou dans d'autres organes. L'agent causal de la maladie est *Mycobacterium bovis*. Les espèces bovines, y compris les bisons et les buffles, sont sensibles à cette affection, mais presque tous les animaux à sang chaud peuvent être atteints. La

sensibilité à l'égard de la maladie n'est pas égale dans toutes les espèces ; certains sont des hôtes-réservoir (terminaux) et d'autres des hôtes de persistance. En Afrique, la maladie affecte principalement le bétail ; toutefois, l'infection d'autres animaux de ferme ou domestiques, tels que les moutons, les chèvres, les cochons, les chiens et les chats, n'est pas rare. Les ruminants et les carnivores sauvages sont également atteints et sont les réservoirs naturels de l'agent infectieux dans le gibier. L'homme est lui aussi sensible à la maladie, les groupes à risques les plus élevés étant les individus atteints d'une infection concomitante par le VIH/SIDA. En Afrique, on admet que la tuberculose humaine est généralement due à *M. tuberculosis* ; toutefois, une proportion inconnue des cas est due à *M. bovis*. Cette infection chez les humains est sous-déclarée par suite de l'incapacité d'un grand nombre de laboratoires à faire la distinction entre *M. bovis* et *M. tuberculosis*. Aucun des rapports nationaux soumis à l'OIE et à l'OMS par les Etats membres des pays d'Afrique ne mentionne l'importance de *M. bovis* dans les cas de tuberculose humaine. La consommation de lait non pasteurisé et de viande insuffisamment cuite ainsi que des contacts étroits avec les animaux infectés représentent pour les humains les principales sources d'infection. Cette revue tente d'examiner l'impact de la tuberculose bovine sur la santé des animaux et des humains.

RESUMEN

La tuberculosis bovina es una enfermedad caracterizada por un desarrollo progresivo de lesiones granulomatosas específicas o tubérculos en el tejido pulmonar, en los ganglios linfáticos o en otros órganos. *Mycobacterium bovis* es el agente causal de esta enfermedad. Las especies bovinas, incluyendo los bisontes y los búfalos, son susceptibles a esta enfermedad, pero casi todos los animales de sangre caliente pueden ser afectados por ella. Todas las especies no son sensibles de la misma manera ; algunas son huéspedes reservorio (terminales) y otras huéspedes de mantención. En África la enfermedad afecta primariamente al ganado ; sin embargo, no es infrecuente la infección de otros animales de granja o domésticos, tales como las ovejas, las cabras, los cerdos, los perros y los gatos. Los rumiantes salvajes y los carnívoros también son afectados y son reservorios naturales del agente infeccioso en la selva. El hombre también es susceptible a la enfermedad, los grupos de más alto riesgo siendo los individuos con infección concomitante VIH/SIDA. En África, se sabe que la tuberculosis humana es ampliamente causada por *M. tuberculosis* ; sin embargo, una proporción desconocida de casos es debida a *M. bovis*. Esta infección en los humanos es subdeclarada, debido a las limitaciones diagnósticas de muchos laboratorios para distinguir *M. bovis* de *M. tuberculosis*. Ninguno de los informes nacionales sometidos a la OIE y a la OMS por los estados miembros de los países africanos hace mención de la importancia de *M. bovis* en los casos de tuberculosis humana. El consumo de leche no pasteurizada y de carne incorrectamente tratada por el calor y el contacto con los animales infectados representan las principales fuentes de infección para los humanos. Esta revisión intenta examinar el impacto de la tuberculosis bovina sobre la salud de los animales y de los humanos.