

Tuberculosis:

a re-emerging disease in animals and humans

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Summary

Tuberculosis continues to be an important disease both in humans and animals. It causes morbidity, mortality and economic loss worldwide. The occurrence of *Mycobacterium bovis* disease in humans, domesticated and wild animals confirms the relevance of this zoonosis. *M. bovis* in humans continues to be reported in industrialised countries and in immigrants from regions of the world where tuberculosis in cattle is endemic. The real incidence of *M. bovis* in humans in developing countries continues to be roughly underestimated due to the scarcity of appropriate laboratory facilities to isolate and to differentiate *M. bovis* strains. In Latin America, less than 1% of tuberculosis cases are reported as being due to *M. bovis*. However, the economic relevance that meat and dairy industries play in these countries stimulates the promotion of bovine tuberculosis eradication programmes. Human-to-human airborne transmission of *M. bovis* does occur and it may be important where human immunodeficiency virus (HIV) infection in humans is prevalent, *M. bovis* infection in cattle is enzootic and pasteurisation of dairy products is not routinely practised. Eradication of *M. bovis* in cattle and pasteurisation of dairy products are the cornerstones of prevention of human disease.

Measures should be developed to identify and control *M. bovis* infection in wild animals as these may be important reservoirs of infection for domesticated food-producing animals. There is a need for medical and veterinary professionals to cooperate on disease outbreaks. The information presented herein strongly supports the 'One World/One Health/One Medicine' concept.

Keywords

Health, *Mycobacterium*, *Mycobacterium bovis*, *Mycobacterium tuberculosis*, One Health, Public health, Tuberculosis.

Tubercolosi: una malattia riemergente nell'uomo e negli animali

Riassunto

La tubercolosi (TB) continua ad essere una malattia importante per uomo e animali. È causa di morbidità, mortalità e di perdite economiche a livello internazionale. L'insorgenza della malattia da Mycobacterium bovis nell'uomo e negli animali domestici e selvatici conferma la rilevanza di questa zoonosi. Il M. bovis nell'uomo continua a manifestarsi nella popolazione dei paesi industrializzati e negli immigrati provenienti da regioni del mondo dove la TB del bestiame ha

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assunto carattere endemico. La reale incidenza del M. bovis nella popolazione dei paesi in via di sviluppo continua a essere colpevolmente sotto-stimata a causa della mancanza di laboratori adatti a isolare il micobatterio e sa differenziarne i ceppi. In America latina, i casi di TB dovuti al M. bovis sono inferiori all'1%, tuttavia, il peso economico che le industrie di carni e latticini hanno in questi paesi incentiva l'introduzione di programmi di eradicazione. La trasmissione aerea del M. bovis da uomo a uomo può avere rilevanza laddove si osserva una prevalenza dell'infezione da virus dell'immunodeficienza umana (HIV). L'infezione da M. bovis nel bestiame è enzootica e la pastorizzazione dei latticini non è una prassi di routine. L'eradicazione di M. bovis nel bestiame e la pastorizzazione dei latticini sono gli elementi cardine della prevenzione della malattia nell'uomo. Occorre mettere a punto programmi di intervento per l'individuazione e il controllo dell'infezione da M. bovis negli animali selvatici, in quanto possono essere importanti riserve di infezione per gli animali domestici domestici allevati a scopi alimentare. I professionisti medici e veterinari devono unire le forze per prevenire le epidemie. Le informazioni riportate depongono fortemente a favore del concetto "Un solo mondo/Una sola salute/Una sola medicina".

Parole chiave

Mycobacterium, Mycobacterium bovis, Mycobacterium tuberculosis, Salute pubblica, Tubercolosi, Una sola salute.

Introduction

Tuberculosis is an important disease in humans and animals worldwide. The tubercle bacillus infects over 2 billion people or one third of the world's population and it is estimated that 1.5 to 2 million people die from tuberculosis each year (254). A total of 95% of cases occur in people in developing countries. Worldwide, tuberculosis is one of the leading causes of deaths caused by infectious disease.

Significant progress has been made towards the elimination of tuberculosis caused by *Mycobacterium tuberculosis* complex from humans in industrialised countries (71). However, in many countries where financial resources are insufficient to support tuberculosis programmes, only limited

progress has been made towards the control of the disease. The development of multi-drug-resistant and extensively drug-resistant (MDR and XDR) strains has complicated the development of efficacious regimens for the treatment of tuberculosis in humans and has significantly increased the cost associated with the use of multiple drug therapies (284).

Moreover, the susceptibility of human immunodeficiency virus (HIV)-infected individuals to *M. tuberculosis* complex is of major concern to public health officials in developing countries where acquired immune deficiency syndrome (AIDS) is rampant (254). *M. bovis* accounts for only a small percentage of the cases of tuberculosis reported in humans; however, it is a pathogen of significant economic importance in wild and domestic animals around the globe, especially in countries where little information is available on the incidence of *M. bovis* infection in humans (44, 52, 57, 71, 196, 211, 249, 261, 282, 285).

The genus *Mycobacterium* includes several species that cause disease in humans and other animals. The *M. tuberculosis* complex (Fig. 1) includes the following:

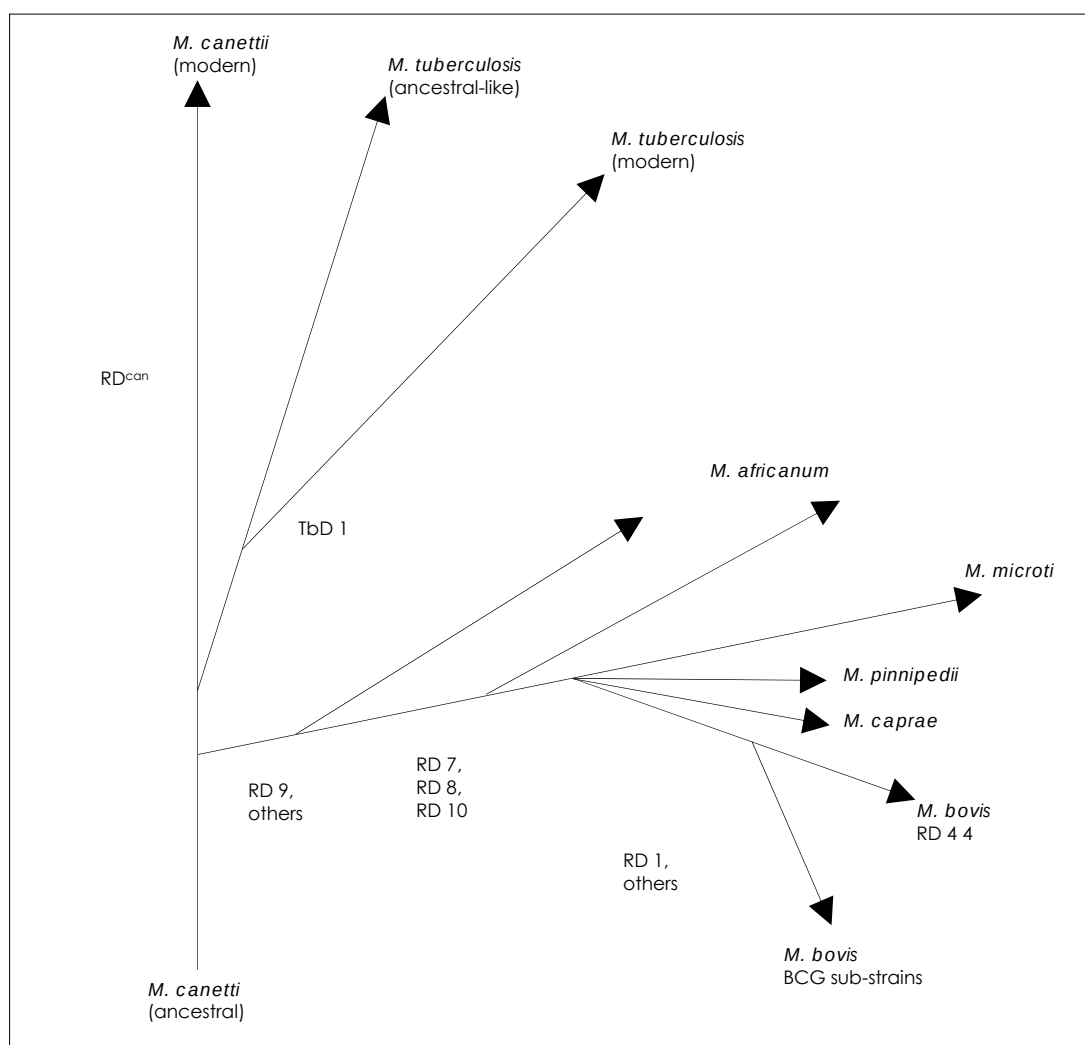
- *M. tuberculosis*
- *M. canettii*
- *M. africanum*
- *M. bovis*
- *M. pinnipedii*
- *M. caprae*
- *M. microti*.

The analysis of the *M. bovis* genome challenged the epidemiological hypothesis that *M. tuberculosis* is a human-adapted variety of *M. bovis* that was acquired from cattle. The irreversible loss of DNA material uncovered by the *M. bovis* genome sequencing project and the systematic analysis of polymorphisms in a large number of strains suggest quite a different scenario (18, 41). This analysis indicates that *M. canettii* is probably the ancestral species of the *M. tuberculosis* complex. Successive DNA deletions, starting by the loss of region RD 9 (RD = regions of difference), originated in *M. africanum*, *M. microti* and *M. bovis*. Moreover, *M. bovis* bacillus Calmette-Guérin (BCG) experienced

further deletions during *in vitro* adaptation and the loss of region RD 1 has been implicated as the region associated with virulence attenuation. In this view, modern *M. tuberculosis* strains originated later from ancestral *M. canettii* by loss of *M. tuberculosis*-specific deletion 1 locus (TbD 1).

Tubercle bacilli were identified more than 120 years ago (130). However, definitive information on the pathogenesis of the

M. tuberculosis complex is not available (41). The tubercle bacillus enters the macrophage by binding to cell surface molecules of the phagocyte. Ingestion of the tubercle bacillus by the phagocytes into the phagosome or intracytoplasmic vacuole protects the organism from the bactericidal components of serum. Following ingestion of the bacillus, lysosomes fuse with the phagosome to form



RD^{can} regions of difference *canettii*
TbD 1 *Mycobacterium tuberculosis*-specific deletion 1 locus
RD regions of difference

Figure 1

Proposed evolution of *Mycobacterium tuberculosis* complex from a common ancestral *M. canettii* strain. During evolution, the ancestral progenitor underwent various deletions (e.g. loss of RD^{can}, RD 9, RD 4, RD 1 giving origin to the micro-organisms of the *M. tuberculosis* complex).

The scheme is based on Brosch *et al.* (18).

Note the two lineages for *M. africanum* with the more ancestral-like lineage originating prior to the loss of RD 7, RD 8 and RD 10.

This scheme is compatible with bovine tuberculosis arising from human tuberculosis.

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phagolysosomes and it is there that the phagocytes attempt to destroy the bacillus (244). However, virulent bacilli have the ability to escape destruction. Virulent mycobacteria survive inside a mononuclear phagocyte by inhibiting phagosome fusion with pre-formed lysosomes, thereby limiting acidification. It has been suggested that pathogenicity of *M. tuberculosis* complex is a multifactorial phenomenon requiring the participation of the cumulative effect of several lipid complexes, such as lipoarabinomannan and phosphatidyl inositol mannoside present in the cell walls of the bacilli (183, 247). Protective immunity against mycobacterial infections is dependent on the activation of a cell-mediated immune response. Inflammatory cytokines, i.e. interleukin 1 (IL-1), IL-2 and tumor necrosis factor- α (TNF- α), produced by mononuclear cells sensitised by mycobacterial antigens recruit natural killer T cells, CD4 T cells, CD8 T cells and gamma delta T cells (213). These cells each produce cytokines that recruit additional cells to the site of infection resulting in the formation of granulomas and containment of infection (183). Granuloma formation is an attempt of the host to localise the disease process. However, in the cases in which the host response is unable to destroy the bacillus due to conditions that compromise immune function resulting in low CD4+T cell counts, such as old age, stress or HIV reactivation may occur, resulting in the release of bacilli and transmission of infection. For details on the current state of knowledge on granuloma formation, readers are referred to D.G. Russell (213).

The susceptibility of different host species varies for *M. tuberculosis* complex depending on the route of exposure, dose of organisms and virulence of the strain (86, 244). Humans, non-human primates and guinea-pigs are very susceptible to *M. tuberculosis* (235). Cattle, rabbits, and cats are susceptible to *M. bovis* and are quite resistant to *M. tuberculosis*. Wild hoofed stock are generally susceptible to *M. bovis* but few reports are available on the isolation of *M. tuberculosis* (85, 142, 218, 234). Swine and dogs are susceptible to both *M. bovis* and to *M. tuberculosis* (235, 236, 241).

For the purpose of presentation and understanding, the information will be divided into three parts. The first part deals with tuberculosis in wild animals, the second provides information on tuberculosis in domesticated animals and the third part presents information on tuberculosis in humans.

Tuberculosis in wild animals

There has been an increased interest in tuberculosis in wild and captive wild animals following outbreaks of the disease in animals in zoos, primate centres, animal colonies and game parks (85, 142, 227, 234, 239, 256, 283). The importance of these occurrences of tuberculosis is emphasised by the difficulty of replacing some rare and endangered species, by the economic losses and by the public health hazard (8, 16, 51, 77, 182, 193, 194, 210, 261, 266, 270, 286).

In the United States and in several other countries, *M. bovis* infection in wild animals has interfered with the eradication of tuberculosis in cattle (37, 38, 45, 62, 96, 98, 123, 131, 133, 157, 188, 195, 198, 204). In several areas where bovine tuberculosis has been eradicated, it was observed that *M. bovis* infection in cervids was responsible for infection in cattle (122, 123). In Michigan, *M. bovis* outbreaks were traced to several cattle herds in 2006 and 2007 resulting in herd depopulation. This resulted in the loss of valuable blood lines and the loss of income related to the export of breeding animals and semen. Estimates indicate that the overall cost of the *M. bovis* outbreaks in deer and cattle in Michigan exceeded US\$100 million (M.J. Gilsdorf, personal communication). Efforts to control and eradicate tuberculosis in wild animals is hampered by a lack of validated *in vivo* and *in vitro* diagnostic tests of suitable sensitivity and specificity and procedures for handling and restraining animals under field conditions to conduct tuberculin skin tests and/or collect specimens for laboratory examination to confirm a diagnosis (241, 248). Therefore, measures need to be developed to identify and eradicate *M. bovis* infection in wild animals.

Moreover, elimination of tuberculosis from wild animals has been hampered by the lack of suitable regulations to limit the sale and transportation of infected or exposed animals. The problem is further magnified by the inability to detect tuberculous animals early in the course of disease because clinical signs are often not present until progressive pulmonary disease develops. Available diagnostic tests (i.e. tuberculin skin tests, gamma interferon assays or the enzyme-linked immunosorbent assay [ELISA]) should be interpreted with caution because the criteria for conducting these tests have not been systematically evaluated in many species. However, positive tuberculin reactions usually indicate infection or previous exposure to mycobacteria. It should be emphasised that negative results are of questionable significance in instances especially when animals have been exposed to *M. bovis*. The time period for development of delayed hypersensitivity varies for different species and for individual animals in a population in which tuberculosis has been diagnosed.

Distribution

Some early reports of tuberculosis describe the disease seen in wild animals in zoos or animal parks (85, 218, 239, 271). In view of the reduction of tuberculosis in domestic animals and humans, one might expect a decrease in the disease in zoo animals. However, there is evidence to indicate that foci of infection may persist in some wild animal populations for long periods of time (42, 85, 90, 133, 135, 204, 233, 256, 272). Moreover, no regulations exist in many countries which require tuberculosis outbreaks in wild or captive wild animals to be reported to regulatory or public health officials. In many instances, necropsies are not conducted. In addition, there is often a reluctance to publicise the occurrence of tuberculosis in these animals because of public relations and loss of revenue (286).

Tuberculosis has not been commonly reported in wild animals, except where they have been exposed to domestic animals or to humans that have the disease (136, 155, 156, 159). The majority of these are considered spill-over cases, where infection is present but not

sustainable in the wildlife population (45, 64, 123, 157). *M. bovis*-infected cattle are considered the most probable reservoir hosts (123). When tuberculosis in cattle in the same geographic areas was eliminated or reduced, comparable declines in the prevalence in wildlife were also seen (39, 101, 149, 186). However, there are now several species of wildlife that are recognised as reservoirs of *M. bovis*. The major wildlife reservoirs for *M. bovis* are ungulates, including African buffalo (*Syncerus caffer*) (12, 65, 126, 133, 157) wood bison (*Bison bison athabasca*) (131, 177) and North American bison (*Bison bison*) (73, 96, 131, 229).

Several species of cervids susceptible to *M. bovis* (16, 37, 64, 68, 72, 80, 101, 118, 179, 202, 203, 204, 217, 220, 256, 263, 265), such as white-tailed deer (*Odocoileus virginianus*) (11, 88, 121, 135, 180, 188, 219), mule deer (*Odocoileus hemionus*) (210) and lechwe (*Kobus lechwe*) (33, 91, 275) are recognised as reservoirs. Red deer/elk/wapiti (*Cervus elaphus*) subspecies (131, 177, 197, 198, 228, 233, 243, 262, 263) are also reservoirs for *M. bovis* in some instances.

European badgers (*Meles meles*) are a reservoir species in the United Kingdom (10, 36, 38, 39, 136, 152, 174). Brushtail possums (*Trichosurus vulpecula*) are a recognised reservoir of *M. bovis* in New Zealand (42, 43, 63). Feral swine (*Suis scrofa*) and wild boar (*Sus scrofa*) were once considered to be spill-over hosts for *M. bovis* (6, 45, 69, 73, 149, 197, 223); however, mounting evidence in Europe indicates that wild boars have become reservoirs of *M. bovis* for other wildlife and domestic animal species (99, 176, 191, 262, 263).

Other wildlife species have been reported with *M. bovis* infection that have a potential to be reservoirs of *M. bovis*; but have not been implicated as reservoirs of the disease. Wild cloven-hoofed animals with *M. bovis* infection include free-ranging axis deer (*Axis axis*) in Hawaii (72, 217), greater kudu (*Tragelaphus strepsiceros*) (128, 157), llama (*Lama glama*) (9, 258), fallow deer (*Dama dama*) (6, 16, 58, 281), roe deer (*Capreolus capreolus*) (58, 105), muntjac (*Muntiacus* spp.) (58), giraffe (*Giraffa camelopardalis*) (84, 197, 218), ferret (*Mustela furo*) (45, 64), feral water buffalo (*Bubalis bubalis*) (45, 108), Arabian oryx (*Oryx leucoryx*)

(81), Sika deer (*Cervus nippon*) (58, 68, 167), tapir (*Tapirus terrestris*) (195), mule deer (210), European wild goat (*Capra aegagrus*) (195), impala (*Aepyceros melampus*) (157), sitatunga (*Tragelaphus spekii*) (195), Bactrian camel (*Camelus ferus*) (21, 195), large spotted genet (*Genetta tigrina*) (157), wildebeest (*Connochaetes* spp.), lesser kudu (*Tragelaphus imberbis*) and topi (*Damaliscus korrigum*) (34), yak (*Bos grunniens*) (218) and eland (*Taurotragus oryx*) (157, 195).

Carnivores and scavengers can acquire *M. bovis* under natural conditions through the consumption of infected carcasses. These species include the fennec fox (*Fennecus zerda*) (111), tiger (*Panthera tigris*) (145, 218), coyote (*Canis latrans*) (19, 180, 181), wolf (*Canis lupus*) (22), fox (*Vulpes vulpes*) (19, 58, 136), lion (*Panthera leo*) (126, 157, 171, 218), cheetah (*Acinonyx jubatus*) (126, 157), leopard (*Panthera pardus*) (157, 218), snow leopard (*Uncia uncia*) (109), weasel (*Mustela* spp.) (45), Iberian lynx (*Lynx pardinus*) (6, 17), bobcat (*Felis rufus*) (19, 180), hyena (*Crocuta* spp.) (155, 157), raccoon (*Procyon lotor*), black bear (*Ursus americanus*) and opossums (*Didelphis virginiana*) (19, 136, 198), mustelids (*Mustela* spp.) (8, 61, 144, 206, 207), stoats (*Mustela erminea*) (58, 205), bush pig (*Potamochoerus porcus*) (157) and warthogs (*Phacochoerus africanus*) (157).

Other wildlife species affected by *M. bovis* include moles (*Scalopus aquaticus*) (58), voles (*Clethrionomys* spp., *Microtus* spp.) (58, 90, 152), rats (*Rattus* spp.) (58), hedgehogs (*Erinaceus europaeus*) (58, 143), rabbits (*Oryctolagus cuniculus cuniculus*) (45, 95, 127), European badgers (58, 157), white rhinoceros (*Ceratotherium simum*) (51, 227), hares (*Lepus europaeus*) (6), primates including a Siamang gibbon (*Symphalangus syndactylus*), Mayotte lemur (*Lemur fulvus mayottensis*), lion tailed macaque (*Macaca silenus*) and Patas monkey (*Erythrocebus patas*) (271), Colobus monkeys (*Colobus guereza caudatus*) (227), baboons (*Papio* spp.) (127, 216, 232, 238, 253), otters (*Lutra lutra*) (58) and Arctic marine mammals (257).

Tuberculosis caused by *M. pennipidii*, a variant of *M. bovis* has been reported in fur seals (*Arctocephalus* spp. and *Arctocephalus forsteri*) and sea lions (*Neophoca cinerea*) (48, 276). The

organism has been reported to cause disease in cattle, gorillas, tapirs and llamas (47). Also, *M. pennipidii* has been isolated from a trainer of seals in New Zealand (251). *Mycobacterium caprae* has been isolated from a dromedary camel (*Camelus dromedarius*) and North American bison (193). The lesions observed closely resembled *M. bovis* infection. *M. africanum* has been isolated from a monkey (252) and *M. microti* has been isolated from wild voles (*Microtus agrestis*) and wood mice (*Apodemus sylvaticus*) (58, 267).

M. bovis has been isolated from captive and wild non-human primates in several countries (34, 127, 157, 216, 227, 232, 234, 238, 271, 273, 283). Tuberculous humans have been the source of *M. tuberculosis* infection in Asian elephants (*Elephas maximus*) (154, 159). In addition, *M. tuberculosis* has caused tuberculosis in non-human primates (89, 104, 234, 239). *M. tuberculosis* has been isolated from captive wild animals (black rhinoceros [*Diceros bicornis*], oryx, addax [*Addax nasomaculatus*] and Asian elephant and Rocky Mountain goats [*Oreamnos americanus*]) originating in nine states in widespread areas of the United States (182, 239). Several other species of the genus *Mycobacterium* have been isolated from wild animals maintained in captivity (195, 234, 237, 241). The widespread occurrence of outbreaks of mycobacterial infections is of concern to public health officials and to veterinarians responsible for the health care of wild animals in zoos, animal parks and primate colonies.

Aetiology

Most clinically significant mycobacteria are slow growing organisms that usually appear on culture media in 3 to 6 weeks (241). Microscopically, the mycobacteria usually appear as slender rods that are 0.3 to 0.6 µm in diameter and 1.5 to 3 µm in length (130). The morphology and staining characteristics of pathogenic mycobacteria are similar to those of saprophytic mycobacteria. An important staining characteristic of mycobacteria is their resistance to acid decolourising agents. Several staining procedures are available for demonstrating acid-fast bacilli (AFB), but the Ziehl-Neelsen or Kinyoun techniques with carbol fuchsin are most widely used.

Tubercle bacilli grow aerobically on *in vitro* cultivation. The optimal temperature for growth of mammalian strains is 37°C. To minimise growth of rapidly growing bacteria or fungi on culture media, sodium hydroxide is usually used to process ground tissue suspensions for inoculation of solid culture media (69, 241). Pyruvate, when added to culture medium, enhances the growth of *M. bovis*, whereas glycerol inhibits the growth of *M. bovis* (110, 125, 249). Liquid culture techniques (i.e. BACTEC) provide for more rapid detection (112). Identification of mycobacterial isolates is accomplished by use of appropriate biochemical, drug susceptibility and supplemental tests or by molecular techniques (106, 260). Polymerase chain reaction (PCR) conducted on formalin-fixed tissues and/or cultures enable rapid identification of the *M. tuberculosis* complex (162). Restriction fragment length polymorphism (RFLP) and spoligotyping are useful in strain differentiation of *M. tuberculosis* and of *M. bovis* (46, 106). More recently, multilocus variable number tandem repeat analysis (MLVA) has been described; this technique, when used alone or in combination with other genomic analyses, may lead to a greater differentiation of *M. bovis* strains (3, 151, 224, 231). Genotyping techniques have been useful in conducting epidemiological investigations to obtain information on the source(s) of infection (62, 99, 106, 120, 123, 138, 161, 191, 192, 260).

Transmission and resistance

In non-human primates, *M. tuberculosis* complex can produce extensive disease involving the parenchyma of the lungs as well as extrapulmonary tissues (127, 232, 237). When animals with advanced disease cough, the organism may be transmitted by aerosol or droplets of exudate containing the bacilli. Animals may also be infected by ingestion of feed and water contaminated with urine, faecal material or exudates from diseased animals that contain tubercle bacilli. Fomites, such as thermometers, are a definite source of spread, as are cages, masks and containers used for food and water.

In addition, elephant, rhinoceros and oryx are examples of animals that appear to be susceptible to *M. tuberculosis* (142, 159). Oryx may develop extensive pulmonary lesions as well as lesions of the uterus and mammary gland. Therefore, in the young, congenital transmission, or transmission by drinking milk from an animal with tuberculous mastitis, may be a factor. Tuberculous humans may also be a source of infection for both susceptible hoofed animals and non-human primates (154, 156, 159). Therefore, measures should be taken to protect these animals from humans infected with *M. tuberculosis*.

Crowding appears to be important in the transmission and spread of tuberculosis in wild animals as in humans. The disease is most common among animals kept in close contact in exhibit pens or barns, or where supplemental feeding is practised (122, 163, 165).

Signs

It is important to emphasise that clinical signs are only rarely apparent in wild animals. Clinical signs of tuberculosis, when present in wild mammals, are often variable. The extensiveness of disease is related to the virulence of the organisms, the route of infection, the stage of infection and several host-related factors. Wild animals with pulmonary tuberculosis may exhibit a cough and show some evidence of dyspnoea. When visceral lesions are present, an enlarged spleen and/or liver may be palpated on physical examination. Regional lymph nodes may be enlarged in advanced cases and, in some instances, may rupture and drain to the surface. Emaciation may result from inappetence in chronic cases. The hair coat may appear rough and occasionally alopecia is apparent. In acute cases resulting from massive exposure to *M. tuberculosis* complex, the disease may spread rapidly and no obvious signs develop prior to death.

Tubercles are often found in bronchial, mediastinal and portal lymph nodes. Other tissues that may be affected include lungs, liver, spleen and the surfaces of body cavities. When tuberculous lesions are located in the

parenchyma of lungs, a productive broncho-pneumonia may be present. Dyspnoea, emaciation and a roughened hair coat are apparent in animals with advanced disease. In some cases where generalised tuberculosis is present, lesions have been reported in the genital tract. A mucopurulent discharge from the vagina may persist for prolonged periods of time. The occurrence of tuberculous mastitis in an oryx emphasised the significance of a thorough physical examination supported by adequate laboratory examination (142).

The obvious difficulty with observing and handling animals in the wild or farmed herds, or in those in confined colonies, is that animals with progressive tuberculosis remain in these populations when no signs of tuberculosis are present. It is essential to conduct suitable diagnostic procedures, including delayed type hypersensitivity tests and *in vitro* tests while animals are held in isolation prior to introduction into a herd or exhibit.

Gross pathology

Wild mammals found to be tuberculous at necropsy after natural death are often without prior suspicion of tuberculosis. Gross lesions may be extensive involving entire organs of one or both body cavities; however, the anatomical sites of lesions, the extent of pathological involvement and the consistency of nodular formations with some caseous necrosis are often present before unthriftiness is often apparent (245).

Tuberculous lesions observed at necropsy usually have an appearance of yellowish caseous necrotic areas in nodules of firm white to light grey fibrous tissue. Tubercles may not appear discrete in instances where lesions become diffuse with adjacent tissues. Often other bacteria and agents are present within tuberculous lesions which may affect gross appearance. Some lesions observed in lymph nodes of the head, particularly the medial retropharyngeal nodes or thoracic cavity of cervids, may have a purulent consistency, whereas others may be partially dry (181, 188, 243, 245).

Microscopic pathology

A tubercle is described as a granulomatous lesion, characteristically composed of a caseous, necrotic centre bordered by a zone of epithelioid cells, some of which may have formed multinucleated giant cells, an accumulation of lymphocytes, a few granulocytes and an encapsulation of fibrous connective tissue of varying thickness (241). There is considerable variation in the apparent ability of some wild mammals to form tubercles associated with connective tissue and for epithelioid cells to form multinucleated giant cells.

Tuberculous lesions from camelids and wild bovines often contain a central area of caseation with some mineralisation surrounded by a zone of epithelioid cells and lymphocytes, and may be encapsulated by a thick zone of fibrous connective tissue.

Lesions examined from oryx, kudu, nilgai (*Boselaphus tragocamelus*) and sable antelope (*Hippotragus niger*) closely resemble those in other bovidae. Nodular areas of caseation and epithelioid cells exhibit an apparent lack of connective tissue involvement.

Tuberculous lesions from baboons and several species of monkeys usually demonstrate microscopic similarities with those in other wild mammals. Tubercles from non-human primates infected with *M. bovis* do not have significant histological differences to differentiate them from lesions caused by *M. tuberculosis*.

Diagnosis

Clinical signs are of limited value in establishing a presumptive diagnosis of tuberculosis in wild animals. Although lesions may involve several organs in the thoracic and abdominal cavities, the animal may not show evidence of disease. Emaciation, dyspnoea and a roughened hair coat may be apparent in some advanced cases; however, these may not be present until a few days or weeks before death.

Ante-mortem diagnostic tools used in livestock have been applied to several wildlife species. Skin testing has been used widely in captive

and free-ranging cloven-hoofed animals (100, 227, 228, 231, 242, 243, 245); but the sensitivity and specificity of these tests in wildlife often vary from those seen in domestic cattle and raise concerns regarding their use in tuberculosis control programmes in some species (265).

In some non-human primates the tuberculin skin test remains negative for several weeks following exposure. Despite certain limitations, the tuberculin test is considered the most practical procedure for the diagnosis of tuberculosis in non-human primates (89). Some *in vitro* cell-mediated immune assays have been developed, such as gamma interferon assay. These assays may be used as supplemental tests; however, the efficacy of these tests remains to be determined in many species. Factors that may influence tuberculin skin responsiveness in monkeys include inhibition of the reaction by isoniazid (INH) therapy, desensitisation following repeated tuberculin skin testing and infection with certain viruses, such as rubeola (94, 200).

Radiological examinations have proved to be of value in identifying lesions in tuberculous baboons (238). Advanced cases in non-human primates with lung granulomas caused by *M. bovis* and *M. tuberculosis* appear as nodular masses. Similar findings have been reported in kudus from which *M. bovis* was isolated on necropsy.

Adequate dosage is an important factor in performing tuberculin tests in non-human primates. Tuberculins prepared for human use are not suitable because the doses are too low. Products used in non-human primates must be standardised in the species to be tested. Several tuberculins prepared by different methods are in use for testing exotic animals. In the past, Koch's Old Tuberculin (KOT) originally was prepared from a heated concentrate of glycerol-veal broth in which tubercle bacilli were grown. The bacilli were killed by flowing steam and removed; the filtrate was evaporated to 10% of its original volume. Later, a tuberculin for veterinary use referred to as 'old tuberculin' (OT) was produced by the United States Department of Agriculture (USDA) on a synthetic medium

free of animal protein. Three strains of *M. tuberculosis* were used. The skin test agent was produced in the same general manner as KOT, except that it was evaporated to only 20% of its original volume and reconstituted to 40% using phosphate buffered saline containing 0.5% phenol and glycerol.

Refined tuberculins referred to as purified protein derivatives (PPDs) have been prepared by the precipitation of culture filtrates on which *M. bovis* was grown using ammonium sulphate or trichloroacetic acid (103). An advantage of PPDs is that they may be standardised on the basis of their protein nitrogen content. Moreover, these products can be biologically balanced by tests in guinea-pigs and/or cattle for use in comparative tests.

Tuberculin skin tests in exotic cloven-hoofed animals are most often conducted in the cervical region; in camelids, the tests are conducted in the axillary region just behind the front leg. In non-human primates, one tenth of a ml (5 000 tuberculin units [TU]) of USDA-PPD is usually injected into an upper eyelid and the site is observed for induration at 24, 48 and 72 h. Tests using biologically balanced *M. avium* PPD and *M. bovis* PPD tuberculin injected at separate sites on the abdomen lateral to the linea alba may enable the differentiation of sensitisation due to *M. tuberculosis* complex from that due to *M. avium* complex and other acid-fast organisms.

In vitro cellular techniques, such as the lymphocyte proliferation assays (i.e. gamma interferon assay) using specific mycobacterial antigens, appear to be of some value. Test results are available within one week; this would enable the rapid detection and removal of infected animals from the population, a very important aspect in the control and elimination of the disease. Lymphocyte stimulation tests have an advantage in that the animals are handled only once, compared to twice for tuberculin skin tests; furthermore, the immune status of the animals is not altered by repeated skin testing.

Serological tests have been developed to detect mycobacterial antibodies in wild animals with disease (23, 34, 240, 241). These tests have not

permitted definitive diagnoses of tuberculosis in certain animals, mainly because of non-specific reactions and false-negative reactions (159). Studies on sera from monkeys infected with *M. bovis* indicate the usefulness of ELISA for the diagnosis of tuberculosis in monkeys and other wild species with advanced disease (34, 146, 240). However, tests to detect antibodies are of little or no value in the identification of animals in the early stages of infection (248). The potential use of multiple antigens in detecting infections in the sera of wild animals has been reported; however further investigations are needed to improve the sensitivity for the tests to be of practical value in the elimination of disease from populations in which tuberculosis has been diagnosed (146, 147, 159).

Histopathological examinations of tissues collected at necropsy or on biopsy are useful to establish a diagnosis of tuberculosis. Lesions may vary for different animals; however, typical lesions are usually characterised by the presence of epithelioid cells and multinucleated giant cells. Caseation necrosis and mineralisation may be present. Molecular techniques have been developed for the detection of *M. tuberculosis*, *M. bovis* and *M. avium* complex in formalin-fixed tissue specimens (162).

Slaughter surveillance is useful in some animals (i.e. cervids) to identify gross lesions typical of tuberculosis (265, 281). Tissues should be collected for culture or PCR whenever possible because this is the only way to confirm a diagnosis of tuberculosis. Specimens for mycobacteriological examinations should be submitted to a laboratory either in a saturated solution of sodium borate or frozen. Since many of the pathogenic strains of mycobacteria grow slowly, cultures should be incubated for 8 to 10 weeks at 37°C. PCR and RFLP are currently available in most reference diagnostic laboratories (106).

Prevention and control

In instances where the reservoir of tuberculosis for wildlife is domestic cattle, control and eradication of the disease in cattle has reduced spill-over into susceptible wildlife species (35,

100, 186). Research on vaccines for potential use in wildlife is ongoing. Problems exist regarding the efficacy of vaccines to prevent disease. Moreover, guidelines have not been developed for effective vaccine delivery systems for wildlife (49, 114).

Control of tuberculosis in wildlife populations is extremely difficult when there is a wildlife reservoir of disease; efforts have been directed at reducing levels of disease in the wildlife species, reducing wildlife population numbers and keeping infected animals from potential hosts (64, 177, 181). Cases in point include *M. bovis* in badgers in the United Kingdom, brushtail possums in New Zealand elk, white-tailed and mule deer in the United States, bison in Canada and African buffalo in South Africa.

Sporadic cases of tuberculosis in free-ranging white-tailed deer and mule deer have been reported (11, 135, 195) but these cases were isolated incidents and attributed to spill-over from infected cattle herds. It has been hypothesised that the initial infection of white-tailed deer with *M. bovis* in Michigan occurred in the 1930s, when severe winters drove deer onto cattle farms with high levels of tuberculosis (165, 180). The area currently affected by bovine tuberculosis has been concentrated in this region: the north-eastern lower peninsula of Michigan (165, 180, 219).

Until the current disease outbreak in 1994, traditional paradigms assumed that the normal behaviour of white-tailed deer, a non-herding species, would not provide sufficient opportunities for disease transmission to develop endemic tuberculosis (11, 195). However, the seasonal aggregation of deer in 'yarding areas' in the winter for shelter and feed, the practice of large-scale supplemental winter feeding of deer by residents and contact between infected does and their fawns have provided sufficient opportunity for disease transmission (163, 180, 219).

After recognition of the presence of endemic bovine tuberculosis in Michigan, several control measures have been implemented to control and eradicate the disease in wildlife. Active surveillance of wildlife and livestock,

and bans on supplemental feeding of deer, combined with measures to reduce the size of the deer herds in the tuberculosis-affected area were initiated and the prevalence of tuberculosis in wild deer has declined from a peak over 2% in the late 1990s to 0.3% in 2007; (158). Spill-over from infected deer from 1996 to 2003 has been seen in coyotes, raccoons, black bears, elk, bobcats, red foxes and opossums (19, 158, 180).

Wild animals acquired for zoos, animal parks or primate colonies should come from sources known to be free of disease. Quarantine should be imposed on imported animals; the period of quarantine should be a minimum of 60 days and preferably 120 days. When tuberculosis is diagnosed in an animal colony or holding facility, it is necessary to thoroughly clean and decontaminate the facilities using a substituted cresylic such as 1-Environ at 7 to 19 day intervals.

Treatment

Anti-tuberculosis drugs used in humans have been used to treat disease in elephants, monkeys, oryx, addax, giraffe and great apes in captivity. The dosage and period of time for which animals are maintained on drug(s) appear to be variable to obtain and maintain suitable blood levels to inhibit tubercle bacilli (84, 89, 159, 268, 273). Two or more anti-tuberculosis drugs (i.e. INH), rifampin or ethambutol) should be used simultaneously when treating animals that have signs of disease (234, 273). *In vitro* drug susceptibility tests should be conducted on cultures prior to initiation of long-term therapy; these tests should be repeated on recent isolates at periodic intervals if animals fail to respond to therapy. Assistance should be obtained from a reference laboratory that can conduct tests to determine drug susceptibility profiles.

There is evidence that INH is immuno-suppressive; therefore, the use of the tuberculin test to detect tuberculous animals in a colony receiving INH therapy may be of limited value (94). To obtain a valid test, it is recommended that INH therapy be discontinued 30 days prior to tuberculin skin testing of animals. The risk that infected

animals will develop fulminating disease is a reason often presented for failure to discontinue drug therapy prior to conducting delayed-type tuberculin skin tests.

INH is an inexpensive drug used to treat elephant, non-human primates, giraffe, camels and some other wild animals (23, 85, 87, 89, 159, 234, 273). The INH dosage in elephant or monkeys ranges from 7.5 to 20 mg/kg per day for 3 months to 1 year; however, a lower dose is recommended in new world monkeys. The drug may be added to food or fruit juice or dissolved in water. The water intake may vary considerably from one day to another under various environmental conditions; therefore, the addition of INH to food ensures more uniform ingestion. In elephant, INH can be placed in the rectum in animals that refuse to accept the drug by oral administration. (G. West, personal communication). For cloven-hoofed animals, such as kudu, oryx, llama and rhinoceros, INH should be administered at the rate of 5-10 mg/kg per day for 1 year, and may be reduced during the second year of therapy (234). Peracute deaths have been reported in giraffe treated with INH (84). Ethambutol and rifampin have been used in treating Arabian oryx (J. Haagsma, personal communication). Para-aminosalicylic acid (PAS) and streptomycin are other drugs which have been used to treat animals infected with *M. tuberculosis* complex.

Tuberculosis in domestic animals

Cattle

Distribution of disease

Tuberculosis due to *M. bovis* is found in cattle throughout the world. The disease was probably introduced into the Americas in colonial times by imported cattle. Tuberculosis was prevalent in cattle in Europe, with areas showing as many as 50% of the animals infected. Where eradication programmes have been conducted, bovine tuberculosis has decreased considerably and it has been eliminated in some Scandinavian and European countries; however, in some

developing countries of Africa, Asia and South America, the occurrence of tuberculosis in cattle remains extremely high. Outbreaks continued to occur in 2007 and 2008 in widespread geographic areas of the United States (California, Minnesota, New Mexico, Colorado, Oklahoma and Michigan) (Michael Carter, personal communication).

Once considered to be a rarity, wildlife reservoirs of *M. bovis* infection are being recognised as serious impediments to the control of tuberculosis in cattle in areas where control and eradication programmes were able to virtually eliminate the disease from livestock (96, 123). In the United Kingdom, New Zealand, Canada, South Africa and the United States, wildlife reservoirs of *M. bovis* (European badgers, brushtail possums, elk, bison, African buffalo and white-tailed deer, respectively) have interfered with the eradication of tuberculosis in cattle (123). While it has been suggested that cattle were originally the most likely source of *M. bovis* for the wildlife hosts, each of these host species was capable of sustaining *M. bovis* once the disease became established in the free-ranging wildlife population (133, 164, 172, 181, 186, 204). Genomic analysis demonstrating that cattle share *M. bovis* with wildlife (6, 46, 99, 106, 151) and levels of bovine tuberculosis in cattle have been linked to the prevalence of the disease in wildlife (39, 74, 101, 149, 181).

The presence of wildlife reservoirs of tuberculosis creates a serious barrier to the eradication of the disease in livestock (96, 98, 131, 133, 204). Disease control in wildlife is difficult, and outbreaks of tuberculosis in cattle due to exposure to wildlife are very costly. In the United States, estimates of the overall cost of *M. bovis* outbreaks in farmed and wild deer and cattle in the State of Michigan was in excess of US\$100 million (96). In Canada, the estimated cost of management strategies of bovine tuberculosis in the Riding Mountain area is US\$1.5 million annually (131). Current approaches to control this aspect of tuberculosis include increasing biosecurity measures to separate infected wildlife from cattle, feed, water, and cattle housing, reducing the size of the wildlife reservoir

population and reducing/controlling levels of tuberculosis in wildlife (101, 173, 214). Measures need to be developed to identify and eradicate *M. bovis* infection in wild animals since these animals are an important source of infection for domestic food-producing animals.

M. tuberculosis is only rarely isolated from cattle. Lesions generally are limited to lymph nodes of the head. Cattle exposed to tuberculous caretakers often respond to tuberculin skin tests. Therefore, the importance of hypersensitivity due to *M. tuberculosis* infection in cattle should be considered only in herds where no gross lesion responses are routinely reported.

Tuberculosis in cattle has decreased where control and eradication programmes have been based on tuberculin testing and slaughter of reactor animals. In the United States in 1916, 0.53% of the cattle examined on post-mortem were condemned for tuberculosis whereas in 1978 less than 0.0016% were condemned for this disease. During a five-year period from 1972 to 1977, *M. bovis* was isolated from cattle originating in 32 states and Puerto Rico (241). In 2007 and 2008, *M. bovis* was identified in 14 herds in 6 states. (Michael Carter, personal communication). These occurrences emphasise the need to maintain efforts to identify foci early in order to minimise the spread of disease to other herds through the sale and movement of *M. bovis*-infected cattle.

Aetiology and transmission

M. bovis, a slowly growing non-chromogenic acid-fast bacterium, is the agent most often responsible for bovine tuberculosis (237). However, *M. caprae* has been isolated from cattle in several countries in central Europe (195). Among cattle, the tubercle bacillus is transmitted through the air or by contaminated feed and water. It has been shown that mycobacteria can be isolated from the nasal secretions of cattle reacting to tuberculin. Pulmonary exudates of the cow are usually swallowed; consequently, the organisms pass with the faeces to contaminate the ground and feed. Congenital transmission of tuberculosis has been recorded. Genital lesions have been reported; therefore, the importance of spread by this route should be

considered in a herd where infection persists. By comparison, the different serovars of *M. avium* complex isolated from lesions in slaughter animals, with few exceptions, caused only localised lesions in the mesenteric lymph nodes and other tissues.

Signs and pathology

The signs of tuberculosis in cattle usually vary with the distribution of tubercles in the body (241). Clinical evidence of disease may not become apparent in chronic cases until the terminal stages of disease. In cases where progressive pulmonary disease exists, the animals may show signs of dyspnoea with an associated cough. The pathogenesis and observation of gross lesions in cattle are similar to those reported previously in this chapter for wild animals. Microscopic examination of tubercles in the lungs usually reveals central areas of necrosis and mineralisation, with borders of epithelioid cells and well-defined capsules composed of fibrous connective tissue. Multinucleated giant cells are commonly observed.

Diagnosis

Since clinical evidence of disease is usually lacking in tuberculous cattle, a presumptive diagnosis is often made on the basis of an animal's response to tuberculin injected into the skin of the caudal fold (caudal-fold tuberculin [CFT] test). The tuberculin skin test has been referred to as the master key to bovine tuberculosis control and eradication programmes in many countries of the world (175, 266). Much has been written about the sensitivity of different test sites and the various tuberculins available for use in cattle (124, 184, 185, 186). PPD tuberculins have been produced for veterinary use from the culture filtrate of *M. bovis* (103). These products, when used with biologically balanced *M. avium* PPD in a comparative cervical skin test (CCT), provide useful information for the differentiation of skin responses caused by *M. bovis* from those caused by other mycobacteria (248).

Gamma interferon assays have been developed for use in detection of *M. bovis* infection in cattle (264, 274). The specificity of this test has

been improved by the use of specific mycobacterial antigens (i.e. ESAT-6) in cattle (199). These assays have been evaluated as a supplemental test in some countries. Studies in cattle experimentally infected with *M. bovis* suggest that skin testing affects gamma interferon and antibody responses (187). Information is needed in cattle naturally infected with *M. bovis* under field conditions in different regions of a country.

Serological tests have been developed for detecting bovine tuberculosis; but these tests have not been widely used because they fail to have adequate sensitivity to detect animals in the early stages of disease and because of problems associated with non-specific reactions (248). The lack of sensitivity of ELISA and other serological tests is important because a large percentage of the *M. bovis*-infected cattle in a herd are often not in advanced stages of disease. However, ELISA may be of some limited value in slaughter surveillance to detect cattle with advanced disease.

To confirm a diagnosis of tuberculosis, it is necessary to isolate and identify the aetiological agent using bacteriological procedures and/or molecular techniques (106, 241, 248).

Vaccination and treatment

Vaccination with BCG has been attempted in cattle in Africa and other countries with no success (175, 285). Vaccination with BCG has not come into widespread use as BCG often provides only partial protection in cattle (20). Some evidence indicates BCG plus ESAT-6:CFP10 confers some protection in cattle that have been experimentally exposed to *M. bovis*; however, information is not provided on the time interval at which animals were necropsied following challenge (153). At the present time, no practical or effective approaches have been developed for vaccinating animals against *M. bovis*. It is important to emphasise that some vaccinated animals develop disease and are contagious; these animals may remain in the herd and serve as sources of infection for other animals. BCG vaccination of cattle has been abandoned because of the low protective effect and

because BCG vaccination interferes with the tuberculin testing used in eradication programmes (277).

More recently, there has been an increased interest in DNA vaccination against tuberculosis (32, 114). With the availability of the genome sequence of *M. bovis*, it may now be possible to evaluate protective DNA sequences (92). Moreover, DNA vaccines may not elicit delayed hypersensitivity responses; therefore, they would have the advantage of not interfering with the tuberculin skin test or the gamma interferon assay. It should be emphasised that evaluation of new vaccines must be conducted in naturally occurring outbreaks of *M. bovis* under field conditions (186).

Some anti-tuberculosis drugs have been evaluated in treatment of tuberculous cattle. However, no systematic studies have been made to ascertain the efficiency of these agents in eliminating *M. bovis* or its effect on tuberculin responsiveness. Therefore, the use of INH or other drugs is not recommended.

Swine

M. tuberculosis, *M. bovis* and *M. avium* ssp. *avium* and *M. avium* ssp. *hominisuis* have been isolated from swine with tuberculous lesions (236). Outbreaks of *M. tuberculosis* have been associated with the feeding of uncooked garbage from hospitals or residences housing human cases. Regulations in many developed countries require that garbage be cooked before being fed to animals. Enforcement of these regulations has contributed to the reduction of disease caused by *M. tuberculosis*. Since tuberculous individuals can spread disease directly to pigs by sputum or body excretions, they should not be allowed to care for swine. The lesions in swine infected with *M. tuberculosis* are often associated with lymph nodes of the gastrointestinal tracts. Caseous lesions are most commonly found in the mesenteric or submaxillary lymph nodes; however, microscopic lesions have been observed in the portal and thoracic nodes and in the parenchyma of the lungs. The lesions, characterised by the presence of epithelioid cells, with occasional giant cells, cannot be

differentiated consistently, on either gross or microscopic examination from those caused by *M. avium*.

Swine are quite susceptible to *M. bovis* (235). Progressive lesions are usually observed in the lungs; well-defined tubercles may be present in the liver, spleen and lymph nodes in the thoracic and abdominal cavities. Microscopically, granulomas may contain caseated centres with some mineralisation. Giant cells and epithelioid cells are usually present in lesions in the lungs. Although AFB can be demonstrated on appropriately stained sections, it should be emphasised that only a few tubercle bacilli may be observed. Outbreaks of infection are usually found on premises where tuberculosis has been diagnosed in cattle. Yards or buildings contaminated with faecal material that contains viable organisms may serve as a source of infection. Milk from infected cows has also been cited as a possible source of tubercle bacilli. Animals are usually infected by ingestion of the organism. There is little evidence for pig-to-pig transmission. Tuberculin tests conducted by injection of 5 000 TU of PPD in the skin of the dorsal surface of the ear or vulva are useful for diagnosis.

Horses

Horses can be infected with *M. bovis*, *M. avium* and *M. tuberculosis*; however, information from experimental inoculations suggests that horses are relatively resistant (85). The incidence of disease in horses is very low in those countries with national programmes to eradicate tuberculosis in cattle. The course of disease in horses appears to be chronic, with the first signs being loss of body condition, despite a normal appetite. Lesions are often present in the liver and mesenteric lymph nodes. Tubercles are seldom seen in the spleen and kidneys; however, the spleen may be enlarged to several times the normal size. Lung lesions were usually present in *M. bovis* infections. Skeletal lesions have been reported.

Microscopically, the granulomas in lymph nodes and lungs are characterised by accumulations of epithelioid cells and

multinucleated giant cells. Mineralisation is rarely observed; however, necrosis may be present.

Skin tests conducted in an eyelid or in the cervical region reveal that some horses without disease react to tuberculin. There is no definitive information on the causes of non-specific tuberculin sensitivity in the horse. The diagnosis of tuberculosis in horses should be based on isolation and identification of the organism.

Goats

Information on the occurrence of tuberculosis in goats indicates they are susceptible to *M. bovis*, but quite resistant to *M. tuberculosis* (235, 237). *M. tuberculosis* ssp. *caprae* has been isolated from goats in Spain (5). In natural and experimental infections with *M. bovis*, lesions are usually present in the lungs and associated lymph nodes. Tubercles may be present in the liver and spleen. Histologically, the lesions are very similar to those observed in cattle. Well-defined granulomas are observed and characterised by the presence of epithelioid cells and numerous giant cells. AFB are usually present; however, the number of organisms varies greatly depending on the animal.

The diagnosis of tuberculosis in goats is usually made at necropsy. The tuberculin skin test appears to be a reliable method for detecting animals infected with *M. bovis*. *M. bovis* PPD (5 000 TU) can be injected into the skin in the cervical region. The test sites should be observed for induration and swelling at 48 h and 72 h post-injection. An increase in skin thickness of 4 mm or more is considered a positive reaction. A confirmative diagnosis of *M. bovis* requires a positive culture from lesions, because *M. avium* ssp. *avium* and ssp. *paratuberculosis* cause disease in this species.

Sheep

Tuberculosis is rare in sheep. *M. bovis* may cause lesions similar to those observed in cattle. Evidence for generalisation of disease includes the presence of lesions in lungs, bronchial and mediastinal lymph nodes, as well as spleen and kidneys (195). The lesions

are often large and calcified. *M. avium* also produces pulmonary lesions in sheep. The tuberculin skin test is of value in the diagnosis of tuberculosis in sheep (195, 261). It should be emphasised that both *M. avium* and *M. bovis* PPD injected at separate sites should be used because of the susceptibility of sheep to *M. avium* ssp. *avium*.

Dogs

It is generally believed that the susceptibility of dogs to *M. bovis* and *M. tuberculosis* is similar to that of humans (78, 237). The occurrence of disease is usually related to exposure to a tuberculous patient or to *M. bovis* infected cattle (225). In naturally occurring cases, lesions are most often found in the lungs, liver and kidneys; however, tubercles may also be observed in the pleura and peritoneum (85, 93). The lesions are invariably exudative in type and appear grey in colour. The absence of calcification has been considered characteristic of tuberculosis in dogs; however, exceptions do occur.

Microscopic examination of the lungs may reveal coalescing lesions with central areas of caseation. Numerous leukocytes and macrophages are present; however, multinucleated giant cells are not usually observed.

Tuberculin skin tests have been applied but the efficacy of these tests is unknown. Intradermal tests can be made on an eyelid, in the cervical region or in the medial aspect of a rear leg. *M. bovis* PPD and *M. avium* PPD (5 000 TU) can be used; the injection sites should be examined at 48 h for the presence of induration, erythema and necrosis. The diagnosis of tuberculosis in dogs is usually based on mycobacteriological examination.

Cats

Cats appear to be very resistant to *M. tuberculosis* (85, 235). The incidence of tuberculosis in cats is related to the presence of *M. bovis* infection in cattle and other species (85, 116). Ingestion of contaminated materials, including milk and offal from infected cattle or wildlife, has been incriminated as the most common source of infection for cats (53, 59, 60, 120, 225). Therefore, the primary site of

infection is considered to be the alimentary tract. Transmission of *M. bovis* through injection (bites or open wound contamination) has also been documented (53, 64, 116, 205). Feral cats have been reported as spill-over hosts for *M. bovis* (45, 53, 169, 205) and other pathogenic mycobacteria (15, 102), but pose little likelihood of serving as a reservoir of disease for other species.

Tuberculin skin tests have been described and evaluated for detecting infected cats. These can be of value in epidemiological investigations on bovine tuberculosis (120). Since cats infected with *M. bovis* often develop pulmonary lesions, they may serve as a possible source of infection for cattle and other domestic animals.

Tuberculosis in humans

The sections on tuberculosis diagnosis and treatment and prevention and control measures are based on current practices used in the United States. They may also be relevant to other low-incidence, industrialised countries; however, some details of management are likely to vary. There are likely to be substantial differences in practices from those of high-burden countries. It is recommended that readers also review their national tuberculosis programme and relevant World Health Organization (WHO) guidelines.

Aetiology and transmission

In humans, tuberculosis is a pulmonary and systemic disease caused by *M. tuberculosis* complex species, predominantly *M. tuberculosis*. It is spread from person to person by airborne transmission of droplet nuclei 1–5 µm in diameter. Several factors determine the probability of transmission, as follows:

- infectiousness of the source patients – positive sputum smear for AFB or cavities on chest radiograph being strongly associated with infectiousness
- host susceptibility of contacts
- duration of exposure
- the environment in which exposure takes place – small, poorly ventilated space providing the highest risk (26).

Even among household contacts of tuberculosis patients, the risk of infection is relatively low and is generally reported to be 30% or less (79). In addition, animal and human studies have demonstrated that tuberculosis transmission may dramatically decrease within days to weeks of instituting effective treatment.

Epidemiology

In the United States, there had been a steady 5% to 6% annual decline in the case rate of tuberculosis until 1984 (115). Between 1985 and 1992, however, the annual incidence of tuberculosis increased by 20% (115). This increase was concentrated in young, urban, racial and ethnic minority populations. Tuberculosis was also found to be prevalent among the homeless, illicit drug users and inmates of correctional facilities. In many of these groups, the rise in tuberculosis was linked to high rates of HIV infection. A second trend emerged with increased immigration to the United States of people from countries where tuberculosis is prevalent. Before 1986, foreign-born inhabitants accounted for 22% of tuberculosis cases.

By 1997, these numbers had increased to 39% and in 2006 they reached 57% (30). Extensive national, state, and local control efforts have led to a new decline of the annual incidence of tuberculosis since 1992, falling 48% between 1992 and 2006 with 13 779 tuberculosis cases reported in 2006 (case rate of 4.6 per 100 000 persons) (30). Despite this welcome decline, the associations of this disease with conditions such as HIV infection, homelessness, drug use, foreign birth and racial/ethnic minority status remain. For example, the tuberculosis case rate in 2006 for foreign-born persons was 9.6 times greater than the rate for United States-born persons and for blacks was 8.4 times greater than for whites (30). Of perhaps even greater concern, the decrease in overall case rates has slowed in recent years, from 7.8% per year from 1993 to 2000 to 3.8% per year from 2000 to 2006 (31).

Drug resistance

Multidrug-resistant (MDR) tuberculosis is defined as tuberculosis caused by organisms

resistant to at least INH and rifampin, the two drugs that have been most effective for tuberculosis treatment. MDR tuberculosis is difficult to treat and generally requires a minimum of 18 to 24 months of therapy with second-line medications (less effective and more toxic); surgery is also indicated in some patients (50). The greatest risk factor for the presence of MDR-tuberculosis is a history of prior treatment for tuberculosis (97, 140).

Since 1993, the proportion of patients with primary MDR tuberculosis decreased from 2.5% to approximately 1.0% (30). The number of MDR tuberculosis cases in 2006 was 111, and only 20 of those cases were in United States-born people (30). Both in the United States-born and foreign-born patients, decreases have been seen in the percentage of cases with primary MDR tuberculosis, although the decline in the United States-born group has been greater. Since 1999, the percentage of United States-born persons with tuberculosis who have MDR tuberculosis has remained at approximately 0.6%. However, of the total number of reported primary MDR tuberculosis cases, the proportion occurring in foreign-born persons increased from 26% (105 of 410) in 1993 to 82% (91 of 111) in 2006 (30).

Extensively drug-resistant (XDR) tuberculosis is defined as tuberculosis caused by organisms that are MDR and also resistant to any fluoroquinolone and at least one second-line injectable drug (amikacin, kanamycin, capreomycin). XDR tuberculosis is very difficult to treat (the cure rate may be as low as 30-40%). In the United States, a total of 48 cases of XDR tuberculosis were reported between 1993 and 2006.

Pathogenesis

Tuberculosis infections occur when susceptible people inhale droplet nuclei containing tubercle bacilli and the droplet nuclei reach the alveoli of the lungs. The tubercle bacilli that reach the alveoli are ingested by alveolar macrophages and the majority of these bacilli are destroyed or inhibited. A small number multiply intracellularly and are released when the macrophages die. If alive, these bacilli may spread through the bloodstream to more

distant tissues and organs, including areas in which tuberculosis disease is most likely to develop: the apexes of the lungs, the kidneys, the brain and bones and through the lymphatic system to regional lymph nodes. This process of dissemination primes the immune system for systemic responses.

On account of the primed immune system, extracellular bacilli attract macrophages and other immunologically active cells. The immune response kills most of the bacilli and the remaining bacilli are confined through the formation of granulomas. At this point, latent tuberculosis infection (LTBI) has been established, which may be detected by using the Mantoux tuberculin skin test or interferon gamma release assays. Within weeks after infection, the immune system is usually able to halt the multiplication of the tubercle bacilli, preventing further progression.

In some people, the tubercle bacilli overcome the defences of the immune system and begin to multiply, resulting in the progression from LTBI to tuberculosis disease. This process may occur soon after or many years after infection. Unless treated, approximately 3-5% of persons who have been infected with *M. tuberculosis* will develop tuberculosis disease in the first two years after infection and another 2-5% will develop disease sometime later in life. Thus, approximately 5-10% of people with normal immune systems who are infected with *M. tuberculosis* will develop tuberculosis disease at some point in their lives. Immuno-compromised persons have a much higher risk of progression from infection to disease. For example, HIV-infected persons not receiving antiretroviral therapy have an 8% annual risk of progression (222).

Detection of tuberculosis

The tuberculin skin test (TST) is a major tool for detecting tuberculosis infection. The TST (Mantoux method) is performed by the intracutaneous injection of 5 TU of PPD. The extent of induration is measured 48 h to 72 h later. The interpretation of the TST is based on an individual's epidemiological risk factors for tuberculosis infection and progression to disease. The 2000 American Thoracic Society

(ATS)/Centers for Disease Control and Prevention (CDC) guidelines for interpretation of TST results are as follows:

- 5 mm induration is considered positive for:
 - individuals with HIV infection or other comparable immunosuppression (equivalent to receiving 15 mg or greater of prednisone for one month or more)
 - close contacts with an active tuberculosis patient
 - patients with a chest radiograph suggestive of prior tuberculosis (e.g. fibro-nodular) disease (also termed 'inactive disease')
- 10 mm induration is considered positive for:
 - recent immigrants (within the last five years) from high-incidence countries
 - injection drug users
 - residents and employees of high-risk congregate facilities, such as nursing homes, homeless shelters, or prisons
 - mycobacterial laboratory personnel
 - persons with underlying medical conditions, such as diabetes, silicosis, end-stage renal disease, certain malignancies and low body weight (loss of at least 10% of ideal body weight)
 - children younger than four years of age and infants, children, or adolescents exposed to adults at high risk
- 15 mm induration is considered positive for all others (1).

A positive TST result is considered to indicate the presence of infection with *M. tuberculosis*. In the United States, it is recommended that persons who test positive receive treatment for LTBI to prevent progression to disease. Thus, an intent to test for LTBI should indicate an intent to treat for LTBI when found. Consequently, testing should be reserved for persons at high risk for LTBI or at high risk to progress to disease, based on their epidemiological profile.

In a number of situations, the TST is neither sensitive nor specific for tuberculosis infection. A positive TST result is a manifestation of type IV delayed hypersensitivity. Certain biological conditions, such as viral illnesses including HIV infection, malignancies and

other debilitating illness (including advanced active tuberculosis) and certain medications, will suppress the type IV response and T-lymphocyte function. In addition, proper application of the TST requires careful attention to technique and interpretation. Tuberculin skin testing should be performed by well-trained and experienced operators. False-positive test findings can occur for a number of reasons, including cross-reactions caused by non-tuberculous (atypical) mycobacterial infection.

The QuantiFERON®-TB Gold (QFT-G, Cellestis Limited, Carnegie, Victoria, Australia) assay is an *in vitro* test that detects the release of interferon gamma (IFN- γ) from lymphocytes of sensitised persons when their fresh heparinised whole blood is incubated with peptide mixtures simulating two *M. tuberculosis* proteins called ESAT-6 and CFP-10 (27). A newer version of the test, QFT-G In Tube, includes a third antigen, TB 7.7. QFT-G and QFT-G In Tube have been approved by the United States Food and Drug Administration (FDA) for use as an *in vitro* diagnostic aid in diagnosing *M. tuberculosis* infection, including both LTBI and tuberculosis disease.

Current data indicate that ESAT-6, CFP-10 and TB 7.7 are secreted by all *M. tuberculosis* and pathogenic *M. bovis* strains, but are absent from all BCG vaccine strains (4). These proteins are also absent from commonly encountered non-tuberculous mycobacteria, with the exception of *M. kansasii*, *M. szulgai* and *M. marinum* (4). Thus, QFT-G offers the possibility of detecting *M. tuberculosis* infection with greater specificity than has been possible previously with tests that used tuberculin PPD as the tuberculosis antigen (e.g. TST).

Although the performance of QFT-G has not been sufficiently evaluated in selected populations of interest (e.g. HIV-infected), available data indicate that QFT-G is as sensitive as TST for detection of tuberculosis disease and more specific than TST for detection of LTBI (27, 170). CDC guidelines for QFT-G recommend that QFT-G can be used instead of TST in all circumstances in which TST is currently used (27). This includes initial

and periodic screening of individuals at risk for tuberculosis infection and screening of exposed persons in contact investigations. However, because there are insufficient data regarding the performance of QFT-G in certain clinical situations, the guidelines recommend following a negative QFT-G with additional testing (e.g. TST) or monitoring in such situations. Examples of clinical scenarios in which additional testing or monitoring should be considered include patients with severe immunosuppression (e.g. HIV infection) who have had recent exposure to a patient with tuberculosis and patients about to undergo treatment with potent tumour necrosis alpha antagonists.

QFT-G represents one type of IFN- γ release assay. The other type of assay, called Elispot, enumerates individual lymphocytes producing IFN- γ after peripheral blood mononuclear cells are incubated with similar antigens (76). A commercial Elispot test (T-SPOT, Oxford Immunotech, Oxford) for the diagnosis of tuberculosis infection was recently approved by the FDA for use in the United States.

Treatment of latent tuberculosis

The principal preventive tool in the United States has been treatment of LTBI with INH. In the 1950s, when INH became available as an inexpensive, bactericidal and relatively non-toxic drug for the treatment of tuberculosis disease, controlled trials were instituted to determine its efficacy for the treatment of LTBI. In more than 70 000 patients, the United States Public Health Service (USPHS) and others consistently demonstrated a 60% to 70% case reduction rate attributable to INH therapy (79). Follow-up for as long as 15 years confirmed the long-term protection INH provides against progression to disease.

Concern regarding toxicity, especially hepatotoxicity, and the need for adherence to a prolonged course of therapy have limited the effectiveness of INH therapy as a public health intervention. Older studies have revealed that INH-associated liver injury occurred in about 1% of patients and deaths secondary to INH-induced liver injury were reported (132). More recently, however, public health clinics in

Seattle and San Diego reported incidences of hepatotoxicity of 0.1% and 0.3%, respectively, among over 14 000 patients treated (139, 178). There were no deaths reported and only one hospitalisation. Despite the low incidence of liver injury, completion rates for six months of therapy were below 65% in both reports.

Both the ATS and CDC recommend that people with LTBI receive treatment (1). Clinical monitoring, on a monthly basis at minimum, is recommended for all patients receiving INH. Routine transaminase monitoring should be reserved for individuals at particular risk for hepatotoxicity, including those who are pregnant or in the immediate post-partum period (first three months) or those with HIV infection, a history of liver disease, a history of excess alcohol use, or other risks for liver disease. The preferred duration of INH therapy is nine months for all groups of patients, including those with HIV infection, those with a chest radiograph suggestive of prior tuberculosis disease (inactive tuberculosis) and children (1). Six months of treatment is considered an acceptable alternative for immunocompetent adults without evidence of prior tuberculosis on chest radiograph, but this shorter duration is felt to be less effective based on existing data.

For contacts exposed to INH-resistant, rifampin-susceptible tuberculosis patients, rifampin (four months duration) can be used (1). It is important to note that rifampin and a closely related medication, rifabutin, interact with protease inhibitors and non-nucleoside reverse transcriptase inhibitors that are used to treat HIV. Consultation with an expert familiar with both HIV and tuberculosis treatment is recommended in this situation. For MDR tuberculosis exposures, some have suggested the use of pyrazinamide and ethambutol, or pyrazinamide in combination with a fluoroquinolone (50). However, data on the efficacy of these regimens are not available.

Clinical presentation and diagnosis of tuberculosis

Tuberculosis disease has a wide array of clinical manifestations, both pulmonary and

extrapulmonary. Inhaled droplet nuclei of *M. tuberculosis* initially lodge in the middle or lower lung zones where regional ventilation is greatest, resulting in a local inflammatory reaction with spread to regional lymph nodes and subsequent haematogenous dissemination. Distant organs, especially the kidneys, bones, central nervous system, as well as the lung apices, are seeded, but overt clinical disease of these areas does not usually ensue. A low-grade fever and symptoms of an upper respiratory illness may also be present. The chest radiograph may show a small area of pneumonitis and often hilar and paratracheal lymphadenopathy. Prominent hilar adenopathy is frequent in children; it is found less commonly in adults (134).

This initial infection, termed primary tuberculosis, resolves spontaneously in most individuals. Healed lesions appear on chest radiograph as calcified parenchymal nodules and are often associated with calcified hilar lymph nodes. In a small percentage of individuals, the initial infection progresses and can manifest as follows:

- rupture of subpleural infectious foci into the pleural space, resulting in tuberculous pleuritis
- extensive caseous pneumonia
- enlargement of tuberculous lymph nodes, causing bronchial obstruction (collapse-consolidation lesions)
- rupture of a tuberculous focus into a bronchus, leading to extensive endo-bronchial spread throughout one or both lungs
- rupture of a tuberculous focus into a pulmonary blood vessel with haematogenous spread leading to acute disseminated disease (148).

Tuberculosis can reactivate months to years after containment of the primary infection. The factors causing reactivation of lesions are poorly understood. Certain conditions increase the likelihood of progression of LTBI to disease, including malnutrition, alcoholism, poorly controlled diabetes mellitus, silicosis, immunosuppression (by disease processes or drugs), the post-partum period, gastrectomy, chronic haemodialysis and jejunoileal bypass

surgery (1). In most patients, however, no predisposing factor can be identified.

Radiographically, reactivation or post-primary pulmonary tuberculosis usually presents as an infiltrate in the apical and posterior segments of the upper lobes (148). Patients can be entirely asymptomatic or have non-specific symptoms of chronic respiratory infection (e.g. fever, weight loss, productive cough and haemoptysis). Chest radiographs may reveal somewhat nondescript fibronodular or fluffy alveolar-filling process in the upper lung fields, but frequently show cavity formation, fibrosis with volume loss, or both. New haematogenous dissemination and extra-pulmonary disease may follow pulmonary reactivation.

The definitive diagnosis of pulmonary tuberculosis depends on obtaining a positive culture from infected secretions or tissues. If cultures are negative or obtaining a culture is not possible, presumptive diagnosis can be made from clinical inference and therapeutic trials. The TST provides information as to whether tuberculous infection is present, but does not distinguish between disease and LTBI. False-negative TST results are common in immunosuppressed patients (advanced tuberculosis itself being sufficiently immunosuppressive). A typical chest radiograph is helpful, but non-specific: a variety of non-tuberculous processes can have a similar appearance.

Spontaneous or aerosol-induced sputum sampling (at least three specimens) is the method of choice for bacteriological assessment. Initially, the specimens are stained by the Ziehl-Neelsen or fluorescent techniques for AFB. Specimens should be cultured for mycobacteria. Cultures are essential for the following reasons:

- smears alone will miss up to 50% of active tuberculosis cases
- mycobacteria other than *M. tuberculosis* can produce positive smears
- cultures are necessary for drug susceptibility testing.

The newest laboratory tools for the diagnosis of tuberculosis are nucleic acid amplification

(NAA) tests performed on direct specimens (i.e. without the need for growing cultures) (24). Two commercially available NAA tests have been approved by the United States FDA for use on AFB smear-positive respiratory specimens, namely: the PCR and transcription-mediated amplification. NAA tests can be performed in several hours. The combination of a positive AFB smear and positive NAA test is essentially diagnostic of active tuberculosis. Negative NAA tests in the face of positive AFB smear suggests that patients have infection with non-tuberculous mycobacteria. NAA tests are also approximately 25-30% more sensitive than the AFB smear and can be of use when smears are negative and the clinical suspicion for tuberculosis remains moderate or high. Transcription-mediated amplification has also been approved by the FDA for use on AFB smear-negative specimens. There is less experience with the use of NAA tests for non-respiratory specimens. Several studies suggest they can be useful for the diagnosis of extrapulmonary tuberculosis, especially for meningitis. The finding of a positive NAA test does not obviate the need for cultures, as NAA tests do not give any information about drug susceptibilities.

Extrapulmonary tuberculosis can occur with or without concurrent active pulmonary tuberculosis. Most frequently, the pathogenesis is that of recrudescence of a previously quiescent haematogenous lesion. However, upper airway and laryngeal disease, lymphatic tuberculosis and pleural or pericardial tuberculosis commonly arise by extension from contiguous structures. Gastrointestinal tuberculosis can follow ingestion of expectorated infectious sputum or ingestion of unpasteurised dairy products from cattle infected with *M. bovis* (a rare cause of human tuberculosis).

Tuberculosis in HIV-infected patients often presents differently from that observed in immunocompetent patients (107). Atypical features of tuberculosis found in HIV-infected patients include the following:

- higher frequency of negative TSTs (61% vs 10%)

- higher frequency of extrapulmonary sites (60% vs 28%)
- higher frequency of diffuse or miliary infiltrates (60% vs 32%)
- higher frequency of hilar adenopathy (20% vs <5%)
- higher frequency of normal chest radiographs with pulmonary involvement (15% versus <1%)
- lower frequency of focal infiltrates (35% vs 68%)
- lower frequency of cavities (18% vs 67%).

Atypical clinical and radiographic features of tuberculosis in HIV patients are more likely to be seen in those patients with lower CD4 counts, especially below 200 (117). Patients infected with HIV are much more likely to develop rapidly progressive, sometimes fatal disease.

Treatment of tuberculosis

Drugs used to treat tuberculosis can be divided into first-line and second-line agents. The first-line (i.e. most effective and least toxic) drugs consist of INH, rifampin, pyrazinamide and ethambutol (2). INH and rifampin are very effective bactericidal drugs. The principal adverse reactions of INH include hepatitis and neuritis. The major side-effects of rifampin are hepatotoxicity and hypersensitivity reactions (226). Some evidence suggests that the combination of rifampin and INH can be associated with a greater incidence of liver injury than with either drug alone. Hypersensitivity reactions, including a flu-like syndrome, thrombo-cytopaenia and, rarely, acute renal failure, have been reported, usually occurring with intermittent rifampin therapy. Rifampin increases hepatic metabolism of some drugs, causing important drug interactions. Pyrazinamide is used for the first two months in many treatment regimens. Its principal side-effects are hepatotoxicity and hyperuricemia; the latter rarely leads to gout or renal failure. Ethambutol is a bacteriostatic agent that has been in general use for over three decades. A dose of 25 mg/kg daily is used for two to three months and then decreased to 15 mg/kg. Retrobulbar optic neuritis has occasionally complicated therapy

with doses in excess of 20 mg/kg for prolonged periods, but is rarely seen when using 15 mg/kg.

Second-line medications are generally reserved for therapy of drug-resistant disease or for patients intolerant of first-line medications. Fluoroquinolones are among the latest additions to the anti-tuberculous armamentarium. They are generally well tolerated and several have good *in vitro* activity against *M. tuberculosis*. Levofloxacin and moxifloxacin are the first choices of medications in this class because they have superior *in vitro* activity compared to other older fluoroquinolones and they also have a good safety profile. Rare gastrointestinal side-effects, such as nausea and bloating, and neurological side effects, including dizziness, insomnia, tremulousness and headaches, may occur with fluoroquinolones. Several aminoglycoside antibiotics are of proven efficacy in the therapy of tuberculosis. Unfortunately, they all require intramuscular or intravenous administration and have a high incidence of serious side-effects.

Streptomycin, the first drug available for tuberculosis therapy, is still used occasionally; however, its value is limited by dose-related renal and eighth cranial nerve toxicities and an increasing incidence of drug resistance. Other aminoglycosides, such as capreomycin, kanamycin and amikacin, have similar toxicities and may be slightly less effective. PAS, ethionamide and cycloserine are oral preparations that are usually used only in MDR tuberculosis. PAS and ethionamide can cause severe gastrointestinal distress, whereas cycloserine is associated with personality changes, depression, frank psychoses and, in high doses, seizures.

For patients with INH- and rifampin-susceptible pulmonary tuberculosis, standard treatment is divided into an initial phase of two months (8 weeks) followed by a continuation phase of four months (18 weeks) (2). Given the relatively high rate of INH resistance in adults, their initial-phase treatment should consist of INH, rifampin, pyrazinamide and ethambutol for two months. For children, ethambutol is usually not needed

unless there is particular concern for INH resistance or the child has a clinical pattern usually seen in adults (i.e. upper lobe infiltration, cavity formation). Once susceptibility results become available, ethambutol may be discontinued (or omitted if drug susceptibility results are known prior to beginning treatment) if the organism is susceptible to INH and rifampin.

If pyrazinamide cannot be included in the initial phase of treatment, or if the isolate is resistant to pyrazinamide alone, the initial phase should consist of INH, rifampin and ethambutol (at least until drug susceptibility results are known) (see above) administered daily for two months. Situations where pyrazinamide may be withheld include severe liver disease, gout and pregnancy. The initial phase may be given daily for eight weeks, daily for two weeks and then twice weekly for six weeks, or three times weekly for eight weeks. Twice weekly therapy is never recommended for HIV-infected patients with CD4 counts below 100 in the initial or continuation phase of tuberculosis treatment (see below) (29).

The standard continuation phase therapy for tuberculosis susceptible to INH and rifampin consists of INH and rifampin (2). Treatment may be given daily, twice weekly (except in HIV-infected patients and when the CD4 count is below 100), or three times weekly. For HIV-seronegative patients with non-cavitary pulmonary tuberculosis and negative sputum-smear results at the completion of two months of treatment, an alternative continuation phase treatment is INH and rifapentine (a long-acting analogue of rifampin) administered once weekly. The duration of the continuation phase is four months (six months total treatment) for most patients with drug-susceptible tuberculosis. However, patients that did not have pyrazinamide included in their initial phase should have their continuation phase extended to seven months (nine months total treatment). In addition, patients with positive sputum cultures after two months of therapy and cavities on chest radiograph are more likely to fail treatment or relapse. Therefore, such patients should also

have their continuation phase extended to seven months as should patients being treated with once weekly INH and rifapentine with positive sputum cultures after two months of treatment regardless of chest radiographic findings.

Since the 1990s, the use of directly observed therapy (DOT) by local health departments has become a major tool in tuberculosis control. With DOT, some or all doses of medication are taken in the presence of a nurse or other ancillary healthcare worker. This can be done by having the patient come to the clinic or by sending an outreach worker to the patient's home. Use of DOT minimises the risk of treatment failure and acquired drug resistance due to non-adherence.

To monitor response to therapy, sputum cultures should be collected every month until cultures are negative for two consecutive months. Since over 90% of patients have negative sputum cultures after three months of treatment, any individual with a positive sputum culture at this point should be carefully evaluated to try to identify the aetiology of this delayed response to therapy. A patient with a positive sputum culture after four months of treatment is considered a treatment failure. Possible reasons for treatment failure include non-adherence and medication malabsorption. In addition to addressing the reasons for treatment failure, repeat susceptibility testing should be performed to assess for acquired drug resistance. If the treatment regimen is going to be modified, at least three new drugs to which the patient's organism would be expected to be susceptible should be added. A cardinal rule of tuberculosis therapy is that a single drug should never be added to a failing regimen.

Patients infected with organisms that are resistant to INH, rifampin, or both, require modifications in their drug regimens (2, 50). For tuberculosis that is INH-resistant (but rifampin-susceptible), the recommended daily regimen is rifampin, ethambutol and pyrazinamide for six months. A fluoroquinolone may be added to strengthen this regimen in patients with extensive disease. For tuberculosis resistant to rifampin (but INH-

susceptible), the recommended therapy is INH, ethambutol and a fluoroquinolone for 12 to 18 months with pyrazinamide added for the first two months. Tuberculosis that is resistant to at least INH and rifampin is termed MDR. Treatment of MDR tuberculosis is often complex and should be performed in consultation with a tuberculosis expert. MDR tuberculosis should be treated with four to six medications to which the organism is susceptible. Therapy should continue for at least 18 to 24 months. Surgical resection of heavily diseased areas of the lungs is sometimes used as an adjunctive therapy for MDR tuberculosis and should be considered in suitable candidates.

Chemotherapy for extrapulmonary tuberculosis does not differ in principle from that for pulmonary tuberculosis (2). The duration of therapy is the same as for pulmonary disease, with the exception of:

- meningitis, for which nine to 12 months of treatment is recommended
- bone and joint disease, for which some experts recommend extending treatment to nine months.

Corticosteroids should be used routinely in the treatment of central nervous system tuberculosis, including meningitis and pericarditis, but are not recommended as an adjunct for treatment of other forms of tuberculosis.

Tuberculosis treatment of HIV-infected patients is similar to that of HIV-negative patients, although there are several differences (29). On account of the complex drug interactions between many antiretroviral medications and rifamycins, it is strongly recommended that tuberculosis treatment of patients on such drugs be carried out in consultation with a clinical HIV expert. The combination of once weekly INH and rifapentine should never be used in HIV-infected individuals as this has resulted in high rates of treatment failure and relapse associated with acquired rifampin resistance. Biweekly therapy should not be used in HIV-infected patients with CD4 counts below 100 for the same reason. Currently, the combination of efavirenz-based antiretroviral therapy

(efavirenz plus two nucleosides) and rifampin-based tuberculosis treatment, at their standard doses is the preferred treatment for HIV-related tuberculosis. For patients unable to take non-nucleoside reverse transcriptase inhibitor-based antiretroviral therapy, the combination of rifabutin (as a substitute for rifampin) with protease inhibitor-based antiretroviral therapy is the preferred form of treatment.

Temporary exacerbation of tuberculosis symptoms and lesions can occur in patients with HIV who are taking antiretroviral therapy. This phenomenon, known as the immune reconstitution inflammatory syndrome or the paradoxical reaction, has been attributed to recovery of the delayed hypersensitivity response in these patients and increased exposure to tuberculosis antigens following the initiation of bactericidal anti-tuberculous therapy. In general, modifications of tuberculosis therapy and antiretroviral therapy are not necessary, and a short course of corticosteroids may ameliorate symptoms associated with this reaction if it is severe.

Adverse drug reactions can occur at any time during treatment. Often difficult to diagnose, they can be confused with manifestations of tuberculosis or other concurrent illnesses. Sometimes drug reactions are relatively mild so that stopping therapy is not warranted. Specific reactions can be handled by discontinuing the suspect drug. Often drugs have overlapping toxicity and reactions are non-specific (e.g. fever, rash, jaundice). In such cases, all drugs should be stopped for a brief period (e.g. one week) and then reintroduced singly, the least likely offender first. Some clinicians re-initiate drugs at low doses, whereas others resume full-dose therapy. If a reaction appears a second time, then another drug may need to be substituted. (One drug can be added to a successful regimen as long as the entire regimen is adequate.)

All patients receiving tuberculosis treatment should be counselled and tested for HIV infection. Patients with risk factors for hepatitis B or C should be tested for these viruses. Baseline serum transaminase, bilirubin, alkaline phosphatase, creatinine

levels and a platelet count should be measured for all adults. Visual acuity and colour testing should be performed on all patients receiving ethambutol. Monitoring recommendations for individual second-line drugs can be found in the ATS/CDC/Infectious Diseases Society of America 2003 Treatment of Tuberculosis statement (2).

Surgery is rarely necessary except in selected cases of MDR tuberculosis and for complications of tuberculosis such as:

- emergency treatment of massive haemoptysis
- therapy of bronchopleural fistulas
- drainage of true (purulent) tuberculous emphysemas
- for relief of mechanical problems in skeletal tuberculosis, such as spinal stabilisation procedures in selected individuals with Pott's disease.

Human tuberculosis in the United States caused by *Mycobacterium bovis*

While human *M. bovis* disease is rare in the United States overall, it appears to be more common in certain geographic regions of the country. Approximately 1% of human tuberculosis in the United States is caused by *M. bovis*, but from 1994-2000, 7% of culture-positive tuberculosis patients in San Diego had disease caused by *M. bovis* (113, 138). A large majority (90%) of these patients were Hispanic persons born in the United States or Mexico, and most had extrapulmonary disease. A study of paediatric tuberculosis in the San Diego region revealed that for many children infected with tuberculosis the only risk factor was ingestion of unpasteurised dairy products from Mexico (13). These findings have implicated dairy products from Mexican cattle herds as the major source of *M. bovis* infection for patients in San Diego. Ultimately, eradication of human *M. bovis* disease in this setting will require eradication of the organisms in the infected dairy herds by culling. In the short term, more extensive efforts at educating the public about the dangers of consuming raw dairy products are needed.

Compared to transmission from cattle to humans, the role of human-to-human airborne transmission in the spread of *M. bovis* is controversial. The predominant view has been that human-to-human transmission is a rare event and is only likely to occur in populations that are particularly susceptible to tuberculosis (e.g. HIV-infected persons) (14, 107, 208). Prior reports of clusters of cases with social and molecular epidemiological links and evidence from contact investigations of patients with pulmonary *M. bovis* have suggested that human-to-human transmission does occur, even in non-immunosuppressed persons. A recent report by Evans *et al.* is the best documented instance of multiple events of likely human-to-human transmission (75). Based on these findings, it seems prudent from a public health standpoint, to treat pulmonary *M. bovis* patients in the same manner as pulmonary *M. tuberculosis* patients.

Respiratory precautions, as determined by local health department policies and procedures, should be instituted for all patients with potentially contagious *M. bovis*. Directly observed therapy is the preferred method of treatment for tuberculosis patients, regardless of whether the infecting organism is *M. tuberculosis* or *M. bovis*. Contact investigation should be conducted in the same manner for *M. bovis* and *M. tuberculosis* patients, and priority of the contact investigation should be assigned based on patient characteristics, such as sputum AFB smear results or cavity on chest radiograph, not the species of tuberculosis infecting the patients. Finally, it is important that all tuberculosis patients be offered HIV counselling and testing.

While controlled clinical trials have not been performed to determine the efficacy of treatment for *M. bovis* disease in humans, programmatic data suggest that treatment outcomes are similar to those found for treatment of *M. tuberculosis* disease when standard regimens, based on drug-susceptibility testing, are used (e.g. nine months of INH and rifampin for *M. bovis* that is susceptible to both drugs) (141). As no diagnostic test exists that can distinguish *M. tuberculosis* from *M. bovis* when the

infection is latent, the relative efficacy of LTBI treatment for *M. bovis* cannot be evaluated. Nevertheless, it seems reasonable to treat persons with LTBI where *M. bovis* is the suspected cause, especially since *M. tuberculosis* infection cannot be excluded as a possibility (77).

Tuberculosis in Latin America and the Caribbean

Tuberculosis continues to be a major cause of illness and death worldwide. According to the WHO, there were an estimated 9.2 million new cases of tuberculosis in 2006 (139 per 100 000 population), 8% of them HIV+ and more than 5% MDR. Distribution of tuberculosis shows enormous differences according to the region of the world. Most of the 22 high-burden countries that collectively account for 80% of tuberculosis cases are situated in Africa and one (Brazil) in the American region. The number of estimated tuberculosis cases in the Americas was 330 724 in 2006 (37/100 000), representing only 3.6% of the total worldwide. Of these, 4.9% corresponded to the United States, Canada, Cuba and Jamaica, where incidence rates were 4, 5, 9 and 7/100 000, respectively. On the other hand, the highest incidence rates in the region were found in Haiti, Bolivia and Peru, that attained, respectively, 299, 198 and 162 per 100 000 (Table I). However, tuberculosis morbidity globally decreased in the region between 1990 and 2006 from 65 to 37 per 100 000 population (280).

While a majority of the tuberculosis cases are due to *M. tuberculosis*, the real incidence of *M. bovis* in humans continues to be roughly underestimated or even ignored for most of the countries in the Latin American and the Caribbean region.

There is a great heterogeneity in terms of geographic surface, human and cattle population, as well as in the development of cattle breeding and dairy industry among countries of this region (83, 280). Two of the main meat exporting countries in the world, Brazil and Argentina, are situated in South America. Their cattle populations are respectively 189 and 51 million head (Table II).

Table I

Estimated burden of tuberculosis and percentage of positive human immunodeficiency virus cases in thirteen countries of Latin America and the Caribbean, 2006

Country	No. cases (tuberculosis incidence per 100 000, all forms)	No. of smear-positive cases (tuberculosis incidence per 100 000)	Human immunodeficiency virus prevalence in incident cases (%)
Argentina	15 231 (39)	6 787 (17)	4.0
Bolivia	18 562 (198)	8 344 (89)	0.5
Brazil	93 933 (50)	59 371 (31)	13.0
Chile	2 417 (15)	1 085 (7)	1.1
Colombia	20 522 (45)	9 192 (20)	2.1
Cuba	1 018 (9)	458 (4)	0.3
Dominican Republic	8 534 (89)	3 812 (40)	3.3
Ecuador	16 958 (128)	7 612 (58)	1.1
Mexico	22 473 (21)	10 087 (10)	1.1
Panama	1 463 (45)	638 (19)	14.0
Peru	44 815 (162)	20 076 (73)	2.0
Uruguay	910 (27)	397 (12)	14.0
Venezuela	11 271 (41)	5 005 (18)	5.8

Source: de Kantor *et al.* (56)

The economic relevance that meat and dairy industries play stimulated the political decision from national authorities, to promoting bovine tuberculosis (BTB) control programmes in order to eradicate BTB, in addition to other cattle diseases that limit the economic benefits obtained by exporting meat and dairy products. In a similar way, sanitary restrictions applied by the United States to the entrance of cattle destined for public

consumption imported from Mexico, due to the detected tuberculosis infection in these cattle, supported the decision of controlling and eradicating BTB from beef cattle in this country (66).

Globally, about 70% of cattle bred in Latin America are held in areas with relatively high prevalence of BTB infection and only 17% in areas virtually free of BTB. In general, official reports on the epidemiological situation of

Table II

Total bovine population in thirteen Latin American and Caribbean countries, estimated percentages of bovine tuberculosis infection in dairy cattle, and confirmed *Mycobacterium bovis* isolates from humans, 2000-2006

Country	Bovine population (millions)	Percentage of PPD reactors in dairy cattle	<i>Mycobacterium bovis</i> isolates from humans
Argentina	51.0	≥1.0	Yes
Bolivia	7.1	≥1.0	No
Brazil	189.0	0.5-1.0	Yes
Chile	2.8	0.5-1.0	No
Colombia	25.0	0.1-1.0	No
Cuba	2.4	<0.1	No
Dominican Republic	1.9	0.1-1.0	No
Ecuador	3.6	≥1.0	Yes
Mexico	26.0	0.1-1.0	Yes
Panama	1.2	<0.1	No
Peru	7.5	0.5-1.0	No
Uruguay	11.7	<0.1	No
Venezuela	13.5	<0.1	Yes

PPD purified protein derivative

Source: de Kantor & Ritacco (55), de Kantor *et al.* (56), de Kantor & Torres (57), Food and Agriculture Organization (83), Ritacco *et al.* (212), World Organisation for Animal Health (280)

cattle infection are only limited to certain areas, and do not have national coverage (54, 211). Consequently, information given in this review is based on regional surveys and other field research work, and on notifications made by the countries to the World Organisation for Animal Health (*Office International des Épizooties*: OIE) and Food and Agriculture Organization (FAO) (83, 280).

Even though BTB infection continues to be prevalent in most of these countries and affects dairy cattle in particular, there has been a sustained improvement in control activities over the last decade. Milk pasteurisation, as well as sanitary controls and surveillance in the dairy industry, have limited the risk of infection by the digestive route in the general population. However, at the same time, those who work in close contact with cattle, in breeding and milk producing establishments, or in slaughterhouses, continue to be exposed to acquiring infection by the respiratory route (56).

Currently, most countries in Latin America and the Caribbean have public health laboratory networks, with facilities to perform diagnostic techniques for tuberculosis, from smear examination by Ziehl-Neelsen, to culture and drug susceptibility testing (DST) of the mycobacterial isolates. In their reference laboratories, molecular and phenotypical identification tests are also performed. In the last decade, an outstanding development of molecular methods has taken place in the region, where these techniques were applied in cooperation with tuberculosis control programmes, in public and in animal health, to explore epidemiological issues.

The certainty in the diagnosis of human or bovine tuberculosis is only achieved by isolating the bacillus and identifying the species, on the bases of the corresponding phenotypic and molecular tests. Recently, it has been demonstrated that a mutation in the glycerol-kinase gene of the *M. bovis* genome inactivated this enzyme and, consequently, *M. bovis* was enabled to metabolise glycerol (125). For this reason, *M. bovis* needs pyruvate as a C source to grow, especially on egg containing culture media. Thus, only if

pyruvate is added to these media in place of glycerol, can *M. bovis* be cultured with ease. Löwenstein Jensen and Ogawa are the glycerol containing egg media usually employed by the laboratories in this region, mainly due to their relatively low cost and robustness (190). *M. bovis* rarely grows there and, if it does, it takes more than two months to develop dysgonic colonies that are small and very difficult to detect. For this reason, only in studies where egg media containing pyruvate, or other rich semi-synthetic liquid media systems, are employed for culturing mycobacteria, can reliable information on the relevance of BTB in humans be obtained. Recently, in several national reference laboratories, BACTEC 12B and BBL MGIT 960 liquid media were introduced for the recovery of mycobacteria. Positive identification of *M. bovis* can be performed by genetic tests (i.e. AccuProbe *M. tuberculosis* complex culture identification test kit, Gen-Probe, San Diego, California) and by phenotypic/biochemical reactions for niacin and nitrate which are negative for *M. bovis* (56, 112, 190).

Table I shows that almost half the number of tuberculosis cases diagnosed in the region were confirmed bacteriologically. However, in most of these, confirmation was only made by the microscopic smear examination. This technique, which is rapid, economical and relatively specific, enables the detection of highly infectious pulmonary cases that are the sources of infection in the community which can then be treated until cure. However, as is well known, mycobacterial species cannot be differentiated by acid-fast staining, which means the information on *M. bovis* cases remains unknown. Differentiating *M. bovis* from *M. tuberculosis* is not considered a public health priority in the countries of Latin America and the Caribbean because the current standard treatment with an initial phase consisting of INH, rifampin, pyrazinamide and ethambutol, is quite effective against both infecting agents. Even though bovine tubercle bacilli are inherently resistant to pyrazinamide, they are sterilised by the combined action of the remaining three

drugs, except in case of strains already resistant to these drugs (278).

According to the laboratory standards for tuberculosis control programmes, culture is usually reserved for the diagnosis of extra-pulmonary tuberculosis cases in children and the suspected pulmonary tuberculosis-negative children to microscopy examination. The use of this method is also recommended for HIV co-infected or suspected MDR-tuberculosis, so that the DST can be performed on positive cultures (190). In 2005 and 2006, the total number of tuberculosis microscopy laboratory services (level I, classically 'one microscope lab') in the Americas (excluding the United States and Canada) as reported to the WHO, was 12 554, while in 1908 culture techniques of other laboratories were also applied ('complexity level II'). In only 100 other laboratories (level III, maximum complexity), DST and species identification tests were performed (279). As a comparison, the United States reported laboratory findings from a total of 1 657 laboratories and Canada 10. All of these laboratories have adequate facilities for smear, culture, DST and species identification. Uruguay reported only one laboratory with a similar capacity and complexity of those in United States and Canada.

In the remaining countries, the proportion of level III to the basic level I, was approximately 1:126. These differences clearly explain the difficulties to obtain information on the number of tuberculosis cases caused by *M. bovis* infection. For similar reasons, DST cannot be offered for every new tuberculosis case diagnosed. Tuberculosis chemotherapy regimens applied in these countries are based on epidemiological estimates obtained from periodical countrywide surveys. In these surveys, the prevalence of drug resistance and MDR is investigated on statistically designed samples of the populations of patients (278). Currently, a standard chemotherapy regimen with four drugs applied in the first two months, followed by two drugs in the continuation phase (two months) has been adopted in most of these countries. The DOT strategy minimises the risk of treatment failure

and acquired drug resistance due to non-adherence (278).

Taking into consideration these limitations in the laboratory diagnostic facilities, the proportion of *M. bovis* cases to the total of human tuberculosis reported, can only be determined from specially designed studies in which, during a period (one to two years at least), all specimens submitted to tuberculosis diagnosis in one or more laboratories, are cultured on the appropriate media and the isolated mycobacteria are analysed to differentiate *M. bovis* from *M. tuberculosis* and other *Mycobacterium* species. When this type of information is available, correlation between the prevalence of *M. bovis* infection in humans and its evolution in time in relation to that of the infection in cattle for a particular region or country can be analysed (56).

Tuberculosis at a global level

Tuberculosis has affected humans since prehistoric times. It has been identified in ancient human remains from virtually every region on Earth. The characterisation of the full genome sequence of *M. tuberculosis* (41) has laid the basis for significant advances in the understanding of the organisms. Previous understanding of the origin of tuberculosis postulated that it began as a zoonosis at the time that humans domesticated and lived closely with cattle, although molecular genetic studies (18) have suggested that this may not have been the case, and that human tuberculosis (*M. tuberculosis*) may have predated tuberculosis in animals (*M. bovis*).

The micro-organisms have spread to all groups in the human population and constitute a major threat to human health globally. Over the past decade, over 8 million new cases of tuberculosis are thought to occur each year. Although the disease is relatively rare (affecting less than 1 person in every 1 000 per year), it has a major impact on the economic productivity of the population because it is a chronic disease with a relatively high case fatality rate if left untreated (50% of those affected will die of the disease) and because the target group affected is primarily persons aged between 15 and 49 years of age, the

economically productive segment of the society.

The disease is not uniformly distributed in the global population. While in some countries (the United States, Canada and the Nordic countries), the case rate is less than 5 cases per million in the population, 80% of all cases occur in just 22 countries, primarily in sub-Saharan Africa and Asia. There is a close association between poverty and risk of tuberculosis both in the global distribution and within industrialised countries where the few cases that occur are found in poor, marginalised and vulnerable groups in the population. Indeed, even in industrialised countries, where the case rate is now extremely low, the risk of developing disease among the marginalised and vulnerable groups is almost as high as in poor countries where tuberculosis is much more common (70).

Progress in the global control of tuberculosis has been challenged by several important factors. The first is the close association of tuberculosis with poverty. This is largely explained by a much higher risk of exposure due to poor ventilation and housing, more crowded living conditions associated with higher numbers of contagious patients in the community. While some remain optimistic that substantial progress is being made in the fight against poverty, there are many reasons to believe that the distribution of wealth is less equal today than it was even two decades ago. The trend in inequality in wealth distribution will certainly determine the trend in the tuberculosis burden.

A second major factor that mitigates against success in conquering tuberculosis globally is the close association between tuberculosis and the epidemic of HIV. This immediately increases the number of patients who develop tuberculosis (rising from a lifetime risk of one in ten in those who are immune competent to a risk of that size within a single year among those living with HIV).

This larger number of cases of tuberculosis leads, secondarily, to an increased risk of infecting other members of the society and a subsequent escalation in the number of cases

from this source as well. This has led to a geometric rise in the numbers of patients affected by tuberculosis in many countries in southern Africa, such that the case rates of tuberculosis are now reaching historic highs. This led the WHO in 2005 to declare tuberculosis an emergency within the region. There are early, but not conclusive, indications that the rising impact of HIV on tuberculosis may be reaching a peak, although it can be expected that the numbers of tuberculosis cases will continue to rise annually for the near future, with the result that sub-Saharan Africa will contribute increasingly to the total global burden of disease, with its economic consequences in communities already struggling to survive. The third challenge to global efforts to contain this disease is the emergence of drug resistance. Resistance to tuberculosis medications is present in some of every large population of micro-organisms due to genetic mutations in the organisms.

Such bacilli may infect others and the disease they cause will be resistant to this medicine (primary resistance). If the sequence is repeated, resistance develops to further medication. *M. tuberculosis* (XDR-TB) and XDR-TB more so, is a form of the disease that is very difficult to cure. The WHO estimates that there are just under half a million MDR-TB cases each year in the world (279).

Bovine tuberculosis in several Latin American and Caribbean countries

Argentina

The National Programme of BTB Control and Eradication (*Servicio Nacional de Sanidad y Calidad Agroalimentaria*: SENASA) was launched in 1998. Its main goal is the eradication of BTB in dairy cattle. Its activities are coordinated with the dairy industry, university veterinary schools, technological-agricultural institutions (*Instituto Nacional de Tecnología Agropecuaria*: INTA) and the veterinary associations in the country. By 2006, the number of dairy herds officially certified as being tuberculosis-free by SENASA was 6 739 of the national total of 12 000, with approximately 2 million milk producing cows. Of the 1.8 million bovines in the tuberculosis-

free herds, 90% are dairy cows. These are located in the provinces of Buenos Aires, Entre Ríos, Santa Fe, La Pampa and Córdoba. A total of 6 193 private accredited veterinarians guarantee a good coverage in the control and surveillance services in these provinces. Argentina exports meat and dairy products to 155 countries. Dairy products must show a certified origin from officially tuberculosis-free herds.

Between 1969 and 2006, an annual average of 10 million cattle were slaughtered and submitted to veterinary inspection. The percentage of animals that suffered partial or total condemnation for tuberculosis decreased from 6.7% to 1.0% in this period (57, 255).

Human tuberculosis due to *M. bovis* has been systematically investigated in several reference laboratories. Figures from Buenos Aires and Santa Fe provinces show a tendency to decline in time; this can be related to the improvement in food hygiene, pasteurisation of milk and dairy products, and in the progress obtained in the BTB control activities led by SENASA (54, 57, 67, 189, 211) (Table III).

Bolivia

Bovine tuberculosis is prevalent in dairy cattle. Surveillance activities are being conducted in several regions of the country (Beni, La Paz, Santa Cruz) by the application of intradermal tuberculin tests using PPD and by culling reactor animals (54). In the public health field, there is a well organised tuberculosis laboratory network. Cultures are performed on

glycerol containing egg media. A pyruvate egg medium (Stonebrink) has been introduced for experimental trials, but no *M. bovis* case has been reported (M. Camacho, personal communication, 2006).

Brazil

According to surveys and official reports, the average prevalence of BTB infection in cattle is 1.3%. It is estimated that cattle in 5% of herds are infected, but the percentage could be as high as 15% in large dairy herds. Bovine tuberculosis has been confirmed bacteriologically in cattle and swine samples. The National Programme for BTB Eradication was launched in 2001. A standardised cervical tuberculin test with bovine PPD has been adopted countrywide (166). Between 1996 and 2006, only three human *M. bovis* cases were confirmed from over 7 000 specimens examined by bacteriological methods. According to this information, the current *M. bovis* prevalence among tuberculosis cases would be below 0.05% (56).

Chile

Bovine tuberculosis infection affects 56% of cattle herds located in Regions V and Metropolitan (Santiago), but only 5.1% in Regions VIII to X (150, 212). According to reports to the OIE in 2006, 1.1% of 8 400 bovine animals submitted for PPD tuberculin tests in the Araucaria, Bio Bio and Los Lagos areas were positive (83, 278). *M. bovis* strains have been isolated from cattle condemned in slaughterhouses (Metropolitan area, Santiago)

Table III
Tuberculosis due to *Mycobacterium bovis*
Findings from two reference laboratories in Argentina

Place	Years	HIV (–) or not investigated No. of cases of <i>Mycobacterium bovis</i> (%)	HIV (+) No. of cases of <i>Mycobacterium bovis</i> (%)
Buenos Aires City	1981–1991	1 000 (0.95)	240 (0.83)
	2000–2006	5 551 (0.22)	1 391 (0.58)
Santa Fe Province	1977–2000	3 946 (2.46)	ND*
	2001–2006	5 901 (1.02)	ND*

HIV human immunodeficiency virus

ND not done

* no HIV(+) tuberculosis case diagnosed

Source: de Kantor & Ritacco (54), de Kantor & Ritacco (55), de Kantor *et al.* (56), Di Lonardo & Di Lenardo (67), Ritacco *et al.* (211), Ritacco *et al.* (212)

and submitted for molecular analysis (209). No case of tuberculosis in humans due to *M. bovis* has been reported in the last twelve years.

Colombia

Less than 0.01% of carcasses submitted for veterinary inspection were condemned for BTB; therefore, *M. bovis* infection is not considered important among cattle (54, 83). However, BTB infection in dairy cattle has been detected by tuberculin tests in 1.2% of herds investigated and *M. bovis* has been isolated from bovine specimens. According to periodic reports submitted to the OIE, BTB in cattle continues to be detected in the Cundinamarca and Antioquia provinces (2006-2007). Positive animals are removed from the herd for slaughter (56). Since 1988, each specimen submitted to the national reference laboratory in Bogotá has been inoculated onto two egg media, one containing glycerol and the other pyruvate. *M. bovis* has been isolated (56).

Cuba

The Control and Eradication Programme for BTB started in the early 1960s. The comparative tuberculin test with mammalian and avian PPD was adopted and the positive animals sent to slaughter. In the 1980s, most of the positive animals necropsied did not have macroscopic lesions and *M. bovis* was not isolated by culture from the specimens submitted (190). Surveillance activities are being continued on a regular basis. In 2005, an outbreak was notified to the OIE where one animal was found to be positive out of 1 700 tested (278). To our knowledge, no human case due to *M. bovis* has been reported in the last 20 years.

Ecuador

Recently the presence of *M. bovis* has been confirmed bacteriologically in samples from tuberculin-positive dairy cattle (201). *M. bovis* has been isolated from two children with extrapulmonary tuberculosis; ingestion of the organism was the suspected route of infection.

Mexico

The National Campaign against BTB has concentrated its actions on cattle. It is especially active in the northern states of the

country, from which nearly 1.2 million head are exported to the United States each year. The Animal Health authorities (*Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación*: SAGARPA) reported in 2007 that 24 regions or states of the country achieved the 'in eradication' status, while the other 22 applied strict control measures (221).

Bovine tuberculosis infection persists in dairy herds. It also affects beef cattle destined for domestic consumption (161) from the central and southern states. In a study performed in 2000 in Queretaro, 17% of 1 201 carcasses submitted to veterinary inspection were condemned due to tuberculosis lesions; *M. bovis* was isolated from 79% of the samples with most of the isolates from retropharyngeal and mediastinal lymphatic nodes (160). In 2004, the USDA prohibited the importation of Holstein and cross spayed heifers and steers from Mexico because of the high incidence of BTB in the Holstein breed (259). In 2006, Mexico reported the detection of BTB in 94 herds to the OIE. A test and slaughter policy was implemented (280).

As explained above, in public health laboratories, bacteriological diagnosis of tuberculosis is usually made by sputum smear examination. Presence of BTB in humans had not been confirmed until recently.

In San Diego, California, 7% of the tuberculosis cases reported between 1994 and 2000 were caused by *M. bovis*. Over 90% of these cases were detected in the Mexican immigrant community. About 25% of patients were United States-born Hispanic children and the main site of disease was extra-pulmonary (138).

More recently, *M. bovis* infection was reported in 35 patients in New York City between 2001 and 2004; 90% of the cases were diagnosed in Mexican-born immigrants and children. Outbreaks were associated with the ingestion of unpasteurised dairy products from Mexico. No evidence of human-to-human transmission was found (28). Results of a comprehensive food investigation indicated that fresh cheese brought to New York City from Mexico was a likely source of infection (129). It must be

emphasised that 30% of the 7 million litres of milk annually produced in Mexico is not pasteurised. This represents an unequivocal risk of infection (287).

Panama

Panama attained the advanced stages of BTB eradication in the early 1990s. However, in 1997, BTB was reported in Bocas del Toro, a province located 500 km west of Panama City that can only be reached by air or sea. In a recent survey, 5% of 60 animals reacted to tuberculin skin tests with PPD. *M. bovis* was cultured from samples collected at necropsy (25). There are no reports of cases of *M. bovis* isolated from humans.

Peru

In 2002, in a survey conducted on 220 370 bovine animals in several regions of the country the prevalence of tuberculin reactors was 0.19% (82). However, percentages are significantly higher in dairy herds in the Lima (5.3%) and Arequipa (2.4%) regions (56). In 2004, a total of 139 709 samples were submitted for culture by the Public Health Laboratory Network and 11.74% of these were positive for *M. tuberculosis*. No *M. bovis* case in humans has been confirmed. However, since only glycerol containing egg media was used, the presence of *M. bovis* as a source of human disease can not be ruled out (56).

Uruguay

Regular BTB control activities implemented by the dairy cooperative industry, were introduced in the 1960s. Tuberculin reactors detected were sent directly to special abattoirs. As a result of these early activities, by 1989 only 0.4% of dairy herds had one or more reactor animals. The official campaign of BTB eradication was launched in 1996. In 2007, five outbreaks were reported to the OIE; in all cases, the animals were slaughtered immediately and the carcasses destroyed. At the national tuberculosis reference public health laboratory, appropriate media were used for isolation of *M. bovis*. However, *M. bovis* has not been isolated from human specimens in the last 20 years. There is a good correlation here between the situation in animal and public health (56).

Venezuela

In 2003, a tuberculin testing survey was performed in several regions of the country. Of 707 693 cattle tested, only 0.04% of the animals were reactors in 5.2% of the herds investigated. From 1999 to 2006; 8.55% of nearly 15 000 human samples submitted for culture on egg media containing glycerol and pyruvate were positive for the *M. tuberculosis* complex. One isolate was identified as *M. bovis* (Biomedicine Institute, Caracas) (56).

Human-to-human transmission of *Mycobacterium bovis*

Recently, J.T. Evans *et al.* (75) reported six cases of *M. bovis* in young people in the United Kingdom. Only in the first patient diagnosed (apparently the source case), was there evidence of zoonotic exposure and consumption of unpasteurised milk. Five of the six cases presented smear-positive pulmonary disease at diagnosis and a history of symptoms for several months before commencing treatment. They could have had multiple opportunities to spread the infection by the respiratory route. These data confirm that the transmission of *M. bovis* among humans, from a smear-positive tuberculosis pulmonary patient to a susceptible but not necessarily immunodepressed contact can take place and the infection can evolve to pulmonary disease, following a pattern similar to that observed in the transmission of *M. tuberculosis*.

Three nosocomial outbreaks of MDR-TB caused by *M. bovis* in patients infected with HIV had been reported in Spain in the late 1990s (40). It was shown that AIDS patients with a CD4 cell count below 50/l and exposed to the index patient had an increased risk of developing *M. bovis* disease (14, 215). Cases of tuberculosis caused by the same genetically identified *M. bovis* pattern were later detected in immunocompetent persons. Recently in Argentina two genetically identical *M. bovis* isolates were recovered within a five year interval from two tuberculosis pulmonary patients, a father and daughter, who were not infected by HIV (74).

These reports confirm yet again that *M. bovis* persists in humans and may be responsible for morbidity and mortality (71). Several comments arise from the above information, as follows:

- the human-to-human transmission of *M. bovis* exists and is possible in cases with or without severe immunosuppression
- *M. bovis* strains can mutate to MDR and these mutants can maintain the original pathogenicity for humans
- eventually cattle and other animals may be re-infected with bovine tubercle bacilli including MDR *M. bovis* strains.

Tuberculosis continues to be an important disease both in humans and animals. It causes substantial morbidity, mortality and economic losses worldwide. There is significant variation in terms of how different organisms of the *M. tuberculosis* complex affect specific animals, including humans. However, there are also important intersections between animals and humans with regard to tuberculosis and perhaps the best example is the occurrence of *M. bovis* disease in humans and domesticated and wild animals.

M. bovis persists in humans, causing pulmonary and extrapulmonary disease. Unlike the transmission of *M. bovis* from cattle to humans, the role of human-to-human airborne transmission in the spread of *M. bovis*, although controversial, does occur (246, 249). The predominant view has been that human-to-human transmission is a rare event and is only likely to occur in populations that are particularly susceptible to tuberculosis (e.g. HIV-infected persons) (14). Prior reports of clusters of cases with social and molecular epidemiological links with patients with pulmonary *M. bovis* have suggested that human-to-human transmission does occur, even in non-immunosuppressed persons (75, 137, 138, 230, 249). The report by Evans *et al.* is the best documented instance of multiple events of likely human-to-human transmission (246). This may be important in Africa where HIV infection in humans is prevalent, *M. bovis* infection in cattle is enzootic and pasteurisation of dairy products is not routinely practised (287). In such a setting, disease

introduced into the human population from cattle could spread from human to human. However, it should be noted that in Kruger National Park, South Africa, where tuberculosis is widespread in wild animals *M. bovis* was not found to be an occupational hazard nor was aerosol transmission implicated as a mechanism for human infection (269).

Future perspectives

Investigations are needed to elucidate the relative importance of *M. bovis* on tuberculosis incidence in humans, especially in developing countries (71). Efforts should be concentrated in countries where HIV infection is widespread since these people are more susceptible to *M. bovis*. Eradication of *M. bovis* in cattle and pasteurisation of dairy products are the cornerstones of prevention of human disease (7). Standard public health measures used to manage patients with contagious *M. tuberculosis* should be applied to contagious patients with *M. bovis* to stop person-to-person spread. Finally, measures should be developed to identify and control *M. bovis* infection in wild animals as these animals may be important reservoirs of infection for domesticated food-producing animals.

It is important to emphasise that pathogenic tubercle bacilli have a wide host range; several species of the genus *Mycobacterium* infect humans as well as wild and domestic animals (235). There is therefore a need for medical and veterinary medical professionals to cooperate in disease outbreaks (119, 270). This concept has been promoted previously (168, 175, 250). However, it should be emphasised that this is of increasing importance in tuberculosis control in the 21st century because of the occurrence of drug resistant strains and reports of immunosuppression of host responses resulting in increased susceptibility to tubercle bacilli.

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