**Report of the Advisory Committee on Dangerous Pathogens, Mycobacterium bovis Working Group**

**Introduction**

Bovine tuberculosis (bTB\(^1\)), caused by *Mycobacterium bovis* (see Appendix 1), poses a significant and growing animal health and economic problem in the UK. Although historically an important zoonosis, and still so in many parts of the world, a combination of pasteurisation of milk (raw milk is the main source of human infection (de la Rua-Domenech, 2006)) and regular testing of cattle, and culling of those found to test positive on herd screening, has reduced the zoonotic risk enormously, such that bTB is no longer seen as a major zoonotic problem in the UK. However, the number of ‘reactor’ cattle (i.e. cattle testing positive for bTB) slaughtered each year has risen in recent years from 5,200 in 2001 to more than 30,000 per year (National Statistics, 2014). Furthermore, although the number of human cases diagnosed in the UK each year is small, the death in 2013 from bTB of a person who had worked in an abattoir raised the issue of a potentially growing risk of occupational infection. In November 2013, this issue was brought before ACDP, who agreed to set up a Working Group with the purpose of assessing the risks of *M. bovis* exposure and transmission to those who work in abattoirs and similar facilities handling cattle known or suspected to be infected with *M. bovis*. The Working Group was tasked with producing a report for ACDP that would provide an evidence base for future ACDP guidance on risk and risk management options in abattoirs dealing with cattle to be applied in this particular occupational setting. The Terms of Reference for the Working Group can be found in Appendix 2, and a list of members is in Appendix 3.

**Summary of the evidence**

As well as reviewing both the scientific and ‘grey’ literature, the Working Group heard expert evidence and opinion from a range of organisations and individuals. We are grateful to these organisations and individuals for sharing their time and experience, and especially for their openness with the Working Group. They are listed in Appendix 4.

Rather than summarising the evidence and views of each expert, the following attempts to review all the evidence and opinions brought to the Working Group in order to highlight what is known and agreed, what is contested and what remains unknown, and to make some suggestions as to future guidance. This review is focussed by the remit given by ACDP to the Working Group, namely occupational bTB in the UK, particularly in the abattoir setting.

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\(^1\) To ensure clarity, bovine tuberculosis (bTB) will be used throughout to describe the infection and disease caused by *Mycobacterium bovis* in any host (e.g. human bTB and bTB in cattle).
The bovine TB epidemic in animals in the UK

There are many reviews and reports that discuss the epidemiology of bTB in the UK, the increasing incidence of both herd outbreaks and individual cases, and the geographic expansion of the epidemic in Great Britain northwards from southwest England and South Wales (Abernethy et al., 2013, Pfeiffer, 2013). This report will focus only on those aspects of the epidemic and the disease in cattle that might affect zoonotic risk, particularly occupational risk in abattoirs (but see 'zoonotic risk and risk management').

Between 2009 and 2013, over 11.1 million cattle were slaughtered in GB, an annual throughput of more than 2 million cattle across a total of 313 abattoirs (2.14 million in 2013) (Animal Health and Veterinary Laboratories Agency, 2014a). Of these abattoirs, eight in England and Wales are currently contracted by the Department for Environment, Food and Rural Affairs (Defra) to undertake the slaughter of *M. bovis* reactor cattle, i.e. those removed from farms on the basis of a positive tuberculin skin test, γ-interferon test, or other diagnostic test. There has been a marked increase in the number of such reactor cattle since 2001: numbers peaked in 2012 at approximately 37,000 and have fallen slightly since (32,000 in 2013) (National Statistics, 2014). Cases of bTB can, however, also be detected at non-reactor abattoirs. These 'slaughterhouse cases' occur relatively infrequently (1,153 cases in 2013 in GB (Animal and Plant Health Agency, personal communication) and are distributed unevenly across the non-reactor abattoirs, reflecting the distribution of bTB in cattle (see Maps 2 and 3, Appendix 5).

Samples (such as lymph nodes or obvious lesions) are taken from both reactor and slaughterhouse cases representative of farm-level outbreaks, and are sent to the Animal and Plant Health Agency (APHA) for culture: around 65-70% of these test positive for *M. bovis*³. However, the main purpose for sending these samples for culture is for genotyping rather than to demonstrate or confirm infection with *M. bovis*.

Cattle are tested for *M. bovis* infection according to the risk of infection in the area in which they are kept. In high risk areas, testing may occur annually or even more often, whereas in low risk areas testing may occur only every four years (or less frequently in some herds in Scotland). Reactor cattle are those that test positive, most commonly in the Single Intradermal Comparative Tuberculin Test (SICTT): the test compares the immune response to *M. bovis* and *M. avium*. Having a comparative skin test improves its specificity (to around 99.5%) but decreases its sensitivity (to around 80%) (de la Rua-Domenech et al., 2006, Karolemeas et al., 2012). Furthermore, as in other species, including humans, the skin test can fail to detect infection in individual cattle with late-stage TB. Hence, slaughterhouse cases (detected by

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² For the purposes of this report, the term ‘non-reactor abattoir’ refers to those abattoirs that are not contracted to handle reactor cattle, and the term ‘reactor abattoir’ refers to those abattoirs that are contracted to handle reactor cattle.

³ Such animals are then designated ‘confirmed.’ It is important in terms of control in cattle not to equate lack of microbiological confirmation with either lack of infection or a 'false positive' test, since various studies show the skin test to be more sensitive than culture of *M. bovis*. 

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having lesions) can arise through infrequent testing of cattle in low risk areas, but also through test insensitivity and/or late-stage disease. Because the aim of routine skin testing is to remove infected cattle as soon as possible, ideally before they become infectious, of more relevance to assessing risk to humans from reactor cattle may be the proportion of reactors confirmed microbiologically or with visible tuberculous lesions (around 50% in 2010-2013 (Animal Health and Veterinary Laboratories Agency, 2014b)).

There is an evidence-based consensus that the main route of transmission of *M. bovis* amongst cattle is through respiratory aerosols. However, other sources of infection are possible, including contaminated food and pasture (either contaminated by cattle or by wildlife, in particular badgers) (Phillips et al., 2003). Perhaps key to this issue is that infection in cattle is more common in densely-housed animals with poor ventilation, as might be expected of a respiratory infection spread by aerosol.

Studies of the pathogenesis of bTB in experimentally-infected cattle, combined with abattoir surveys of naturally infected cattle, have shown that following experimental infection, cattle may shed *M. bovis* in upper respiratory tract secretions during the very early stages of infection. However, not all studies detect such shedding, suggesting that it is transient, may be an artefact of abnormal doses or routes of infection and/or involve only small numbers of bacteria. Otherwise, infection tends to be limited to the lungs and associated lymph nodes until late in infection when generalised infection can occur. This late-stage generalised disease can include infection of the udder and shedding of *M. bovis* into milk. Widespread, systemic disease, while common in some countries with few controls, is rare in the UK owing to the regular testing of cattle for infection, at individual and herd levels, and the removal of the majority of infected individuals during the early stages of infection. This, combined with pasteurisation, is why milk is no longer seen as an important source of human infection in the UK, although occasional cases have been reported in farmers drinking unpasteurised milk from their own, infected, cattle.

Systemic disease can involve spread via the blood. The Working Group spent some time discussing whether or not bacteraemia, and the aerosols generated during slaughter, might create a risk of human infection. No evidence for such transmission could be found and, because of the stage of infection at which the overwhelming majority of infected cattle are slaughtered, the Working Group concluded that while the possibility of aerosolised bacteria in blood could not be ruled out completely, the likelihood of this as a source of human infection was negligible. Similarly, urine is unlikely to be a vehicle for transmission because renal lesions are rare in bovine tuberculosis (Phillips et al., 2003). Faeces, however, provides a theoretically larger, although still small, risk as, although enteric infection in cattle is rare (it is much more common in, for example, camelids), faeces could be contaminated as a result of secondary ingestion of saliva or sputum containing *M. bovis*.

Other species can also become infected with *M. bovis*. However, with the exception of badgers, the rates of infection in other species are very low
compared to those in cattle. Between 2002 and 2012, 153 cases were reported in pigs, 114 in sheep and goats and 34 in farmed deer (Defra, personal communication), making the abattoir-related risk of infection from these species very small compared to that from cattle. Perhaps of greater public health risk is infection from companion and smallholder animals. There were 222 cases in alpacas and llamas and 147 in domestic cats reported between 2002 and 2012 (Defra, personal communication): both these species present risks of infection to their owners, who have closer and more prolonged contact with these animals than do farmers or abattoir workers. Indeed, there have been several recent cases of zoonotic transmission from both camelids and cats.

**Epidemiology of M. bovis in people in the UK**

Before animal disease controls were mandated in the 1950s annual deaths from tuberculosis (TB) acquired zoonotically in the UK numbered around 2,500 people (Ministry of Agriculture Fisheries and Food, 1965), representing approximately 6% of deaths due to all forms of TB (Hardie and Watson, 1992). There has been a substantial and sustained decline in the incidence of *M. bovis* in people following control measures including wide-scale pasteurisation of milk, regular, compulsory tuberculin skin testing of cattle herds and compulsory slaughter of reactor cattle (de la Rua-Domenech, 2006).

In 2013, 29 human *M. bovis* TB cases were notified in the UK (Appendix 6), and the annual number of cases has been very low for more than a decade. Although exposure data are collected on culture-confirmed cases of *M. bovis*, determining risk factors for individual cases is complicated because of the long latent period of TB, which leads to inaccurate recall by patients, so identifying the likely source of infection is often impossible. Latency is poorly understood but is generally thought to reflect a balance between the host's immune response and immune evasion by the bacteria. Immunosuppression resulting from other diseases, poor nutrition, age or medical treatments can significantly increase the risk of reactivation and the rate of progression to clinical disease.

A further means of identifying the sources of human infection may be genotyping isolates of *M. bovis* and comparing them with cattle isolates. While isolates from bTB in cattle are routinely genotyped by spoligotyping, those from humans are usually typed only by VNTR (see Appendix 1). This makes comparison of human and bovine isolates in the UK difficult. VNTR typing of just under half of the human isolates has revealed some clustering, confirmed several cases of human-to-human transmission and, very recently, provided evidence of transmission between cats and their owners. Clearly there is an urgent need for more work in this area.

People aged ≥65 years account for the largest proportion of *M. bovis* cases (Appendix 7), the majority having been born in the UK. In people aged 15 to 44 years the majority of *M. bovis* cases were born outside the UK. The proportion of *M. bovis* cases in children aged ≤14 years continues to be very low. These data suggest strongly that cases of *M. bovis* TB in people in the UK are most likely to be due to reactivation of latent infection acquired before
widespread pasteurisation of milk and implementation of compulsory TB control programmes in cattle, or to infection acquired abroad.

Transmission from cattle to people

*M. bovis* can be transmitted to people via three main routes: ingestion, inhalation or direct contact with mucous membranes and skin abrasions (de la Rua-Domenech, 2006). The infectious dose is unknown but has been quoted as being in the order of tens to hundreds of organisms by the respiratory route and millions by the oral route (O’Reilly and Daborn, 1995). Infection via the respiratory route is therefore plausible but requires close contact with a tuberculous animal. Rarely, person-to-person transmission of *M. bovis* can occur, and outbreaks have been reported (Mandal et al., 2011).

Ingestion

Traditionally, prior to the introduction of control measures, consuming unpasteurised milk from infected cows was the main vehicle for *M. bovis* infections in people. *M. bovis* is completely inactivated by pasteurisation. Although selling unpasteurised cow’s milk to the final consumer is still permitted in England, Wales and Northern Ireland under certain conditions, unpasteurised cow’s milk sales represent a very small fraction (estimated 0.01%) of total UK liquid milk sales market. Furthermore, dairy herds supplying unpasteurised milk for sale must be officially TB free (Regulation (EC) 853/2004) and undergo annual tuberculin skin testing. Selling unpasteurised cow’s milk is banned in Scotland and there are no known sales in Northern Ireland (Advisory Committee on the Microbiological Safety of Food, 2011). In 2011 the Advisory Committee on the Microbiological Safety of Food (ACMSF) formally assessed the risk to consumers from consumption of unpasteurised milk and milk products and considered the risk of infection to be very low (Advisory Committee on the Microbiological Safety of Food, 2011).

In theory, transmission could also occur via consumption of meat/meat products, although there is no evidence that this has happened either in the UK or, from the published literature, elsewhere. In June 2013 the European Food Safety Authority published a scientific report on meat inspection which concluded that the risk of *M. bovis* infection in people from eating meat was negligible as it was considered not to be a meat-borne pathogen (European Food Safety Authority, 2013).

Inhalation

Transmission via aerosolised bacilli excreted from the respiratory tract of diseased cattle is the most efficient mode of transmission and the infectious dose is considered to be much lower than that for the oral route (Collins, 1983). There is, therefore, a potential risk for people who handle animals (or carcases) infected with *M. bovis* and bTB is a recognised occupational zoonosis (Advisory Committee on Dangerous Pathogens, 2003, de la Rua-Domenech, 2006, Public Health England, 2014). Cases of *M. bovis* infection have been described in abattoir workers and in others with occupational contact with livestock, including reactor cattle (Robinson et al 1998; Gibson et al 2004; Smith et al 2004; Mandal et al 2011). It is also interesting to note that a geographical overlap of cases of human *M. bovis* infection with the
distribution of bovine tuberculosis in UK cattle has been observed (Mandal et al. 2011).

**Direct Contact**

Traumatic inoculation of bovine TB into the skin during manipulation of carcases or direct contact with infected animals can produce localised skin, tendon, mucosal or lymph node lesions. This mode of transmission is now extremely rare in the UK, with only one case documented in the mid-2000s in a veterinary surgeon treating an infected alpaca before the diagnosis of TB had been made (Twomey et al., 2010).

**A recent fatal case of bTB**

As the Working Group was set up in part in response to a recent fatal case of bTB in an abattoir worker, the Group spent some time investigating the background to this unfortunate event.

A 53 year-old male abattoir worker from the West Midlands, who had been brought up on, and worked on, cattle farms, died in May 2013 of multiple organ failure resulting from severe pulmonary pneumonia. He had been on immunosuppressive therapy for an inflammatory bowel disease since January 2012. The abattoir in which he worked was one of those contracted by Defra to slaughter reactor cattle. Screening of 49 family members and work contacts using the Interferon-Gamma Release Assay (IGRA) identified four people who tested positive, all of whom had histories of potential exposure both within and beyond the abattoir. One hospital contact also tested IGRA positive: this hospital worker was non-UK born and originally from a high tuberculosis prevalence area.

*M. bovis* was isolated from lung lesions taken post mortem from the abattoir worker and from a sputum sample from one close contact (indicating active pulmonary infection), but not from anyone else. Genotyping demonstrated no differences in VNTR-MIRU profiles between the two isolates (VNTR Profile 45542; MIRU Profile 2222425322; VNTR+Profile 223342241). Furthermore, the abattoir worker’s isolate clustered within spoligotype 25a, the spoligotype (see Appendix 1) consistently found in cattle in the same area (see Map 4, Appendix 5).

Determining the source of any infection is often difficult but in this case made the more so by multiple potential sources of infection: farms, family members and the abattoir. Furthermore, the abattoir worker who died may have had a latent infection that was not recognised prior to commencing the immunosuppressive treatment for inflammatory bowel disease, and that may have reactivated due to the treatment. The Working Group was advised that the published literature suggests it would take around twelve months to reactivate latent tuberculosis following immunosuppression. Thus, exposure and infection with *M. bovis* could have occurred in the past, either whilst growing up and living on a farm, or during the course of his work in the abattoir, and have been subsequently reactivated by his immunosuppressed status. Alternatively, it is also possible that the suppressed immunity of the deceased would have significantly increased his vulnerability to *de novo* *M.*
bovis infection and disease resulting from a more recent exposure in the abattoir.

The Working Group heard that while spoligotype 25a is that most frequently associated with reactor cattle dealt with at the abattoir where the deceased worked, the farm on which he worked, despite routine testing, had no records of bTB infection. This spoligotype has been found in the region since 1992, suggesting long-term geographic persistence, and is found in both cattle and badgers.

The Working Group are of the view that while there is evidence of M. bovis spoligotype 25a in the abattoir and no evidence for infection on the farm on which the deceased most recently worked, it is impossible to determine the time or place of infection. The combined evidence provided by all parties leaves room for doubt as to the precise nature of exposure that led to infection in this case. The Coroner recorded a verdict of work-related death, and while stressing that he could not be sure whether the infection was contracted recently or in the past, in the abattoir or on a farm, he thought it most likely that it was acquired at the abattoir.

Zoonotic risk and risk management
The Working Group’s remit was to focus on the risk of occupationally acquired bTB, in particular the risk of transmission from cattle to humans in abattoirs. However, it is worth noting that other potential sources of human infection also exist. Other livestock species that might both contract bTB and be encountered in an abattoir (sheep and pigs) have been mentioned already but, it was felt, currently pose a negligible risk, certainly compared to reactor cattle. However, should such cases increase in frequency, the risk should be reviewed. Other risks are less occupation-related. While it could be argued that the expanding epidemic of bTB in cattle in the UK might increase the risk of food-borne infection (and not just through milk), other groups have reviewed this and thus far found no grounds for significant concern (Advisory Committee on the Microbiological Safety of Food, 2011).

Risks of infection in bovine abattoirs
In considering the zoonotic risk associated with cattle in abattoirs, the Working Group visited a reactor abattoir and heard a variety of views on which, if any, stages in the process of slaughter have the potential to generate the conditions required for the dissemination of M. bovis into the work environment and the potential to infect those working there. The group also heard two main opinions among stakeholders as to which animals might pose the greatest (if any) risk: reactor cattle in dedicated abattoirs and ‘slaughterhouse cases’ in non-reactor (i.e. the majority of) abattoirs.

The occupational risk in abattoirs handling reactor cattle arises from the large number of reactor, i.e. infected, cattle handled. Most stakeholders involved in abattoirs argued that the low incidence of M. bovis infection in the general population, together with an absence of qualitative and quantitative data to accurately inform risk, led to the conclusion that the risk from M. bovis in abattoirs is very low, and that controls to prevent or reduce exposure are not
needed beyond the basic hygiene measures that are currently in place in any abattoir. However, with the significant rise in the number of reactor cattle slaughtered over the last 10 years and the concentration of reactor slaughter in a small number of dedicated abattoirs, there is the potential for cases of occupationally acquired *M. bovis* infections to increase, and these may not be evident for some years due to the recognised latency period of this infection in people.

Nevertheless, regular testing of cattle means that most reactor cattle will be slaughtered at an early stage of infection, when the potentially lower numbers of bacteria may make the chances of transmission less likely. Thus, when assessing the risk of infection in reactor abattoirs, the proportion of cases that were microbiologically confirmed or had detectable lesions (around 50% in 2010-2013 (Animal Health and Veterinary Laboratories Agency, 2014b)) could be considered more important than the number of reactors, as these confirmed cases are more likely to have a high bacterial burden. Several stakeholders argued that of equal importance in terms of risk of human infection were slaughterhouse cases in non-reactor abattoirs: such cases, while much less common, were unexpected and often had more advanced disease (and were therefore potentially more infectious) than cases seen in reactor abattoirs.

Based on their knowledge of abattoirs, a visit to an abattoir and other evidence, the Working Group assessed the risk of *M. bovis* infection at each stage of the slaughter line. The assessments are qualitative, as there are insufficient data to carry out quantitative risk assessments. Details can be found in Appendix 8. Possible routes of infection with *M. bovis* in an abattoir were identified as inhalation of aerosols, or exposure through splashes or cuts. None of the stages of the process were assessed to have anything greater than a low risk in reactor abattoirs, or a very low risk in other abattoirs. However, those stages with relatively higher risk are: stunning; removal, dressing and inspection of heads; ‘pluck’\(^4\) inspection and wash-down of the abattoir.

The possible risk during stunning comes from close contact with respiratory secretions: depending on where the operator is standing, (s)he may be exposed to respiratory aerosols from the animal. When processing heads, the most hazardous activities are removal and washing of the tongue and harvesting of the cheeks, when there may be exposure to aerosols from the upper respiratory tract. In addition, during inspection of the head lymph nodes, there is a risk of infection through cuts. Because of the characteristics of the lesions, it is considered unlikely that cutting lymph nodes will produce an infectious aerosol.

Pluck inspection carries the risk of inhalation of aerosol from the respiratory tract when inspecting the lungs, and cutaneous infection if cutting an infected lymph node. There is further potential for creating aerosols if the trachea and hyoid musculature are harvested and washed. Finally, when washing down

\(^4\) For the purposes of this report, ‘pluck’ is defined as: larynx, trachea, heart, lungs and liver.
the abattoir using high pressure hoses, aerosols will be created. However, there is only a low risk of creating infectious aerosols since the degree of *M. bovis* contamination of the abattoir floor is likely to be low.

**Management of risk**

A list of legislation and regulations relevant to workers handling infected cattle can be found in Appendix 9. Responsibility for the health and safety of workers is placed with their employers. Given that reactor cattle are slaughtered in abattoirs and, subject to the number and distribution of lesions, enter the human food chain, then there are three main approaches to managing the risk of zoonotic transmission of bTB. The first is engineering solutions such as ventilation arrangements, avoidance of producing aerosols (e.g., no use of power hoses) and design of the line to ensure that contaminated material is rapidly removed.

The second is personal protective equipment (PPE). Some PPE is already worn routinely by abattoir workers: for example, those workers at particular risk of cuts wear chainmail gloves. To reduce any possible risk of infection through inhalation of aerosols, respiratory protective equipment (RPE) can be utilised, as stated in the Public Health England (PHE) guidance on the management of the public health consequences of tuberculosis in cattle (Public Health England, 2014). The British Meat Processors Association’s guidance on health and safety (British Meat Processors Association, 2014) states that when reactor cattle are being processed, “*masks (EN 149 FFP3 standard) will be issued for certain tasks*, and some abattoirs have local rules that require the use of RPE for certain activities when processing reactor cattle. APHA provided the Working Group with a summary of an internal report, ‘Risk assessment for human infection with *M. bovis* through occupational exposure in post-mortem rooms and field activities’, which assesses the risk of zoonotic infection with *M. bovis* arising from occupational exposure of APHA employees in the course of their specific activities. While none of the procedures covered in the risk assessment were directly comparable to those carried out by reactor abattoir workers, it is worth noting that if APHA employees undertake a post-mortem inspection of a tuberculous animal at a regional laboratory, they employ additional controls including the use of RPE.

The third approach is immunisation with Bacillus Calmette–Guérin (BCG), a vaccine against TB prepared from attenuated *M. bovis*. Until 2005, BCG was administered universally in the UK to school aged children, but this is no longer the case. As a result, younger generations will not routinely be immunised against TB; furthermore, an increasing number of abattoir workers are migrant workers who may not have received the BCG vaccine in their country of origin. The Working Group heard conflicting views on the use of immunisation.

Control Of Substances Hazardous to Health (COSHH) Regulation 7(6)(f) requires the employer, where appropriate, to make available effective vaccines for those employees who are not already immune to the biological agent to which they are exposed or are liable to be exposed. Information for
public health professionals on immunisation is provided by the Department of Health and PHE in the ‘Green Book’ (Public Health England, 2013) and chapter 32 covers guidance for tuberculosis. The Green Book recommends that unvaccinated tuberculin-negative abattoir workers and veterinary staff under the age of 35 receive the BCG vaccination. With respect to BCG vaccination of over 35 year olds, chapter 32 of the Green Book states that: “There are few data on the protection afforded by BCG vaccine when it is given to adults (aged 16 years or over), and virtually no data for persons aged 35 years or over. BCG is not usually recommended for people aged over 16 years, unless the risk of exposure is great (e.g. healthcare or laboratory workers at occupational risk)”. Therefore, with respect to BCG vaccination of abattoir workers over the age of 35, the COSHH regulations and the guidance given in the ‘Green Book’ appear to be less well-aligned; various bodies have interpreted the regulations and guidance in different ways in their occupational health policies and there is disagreement and uncertainty about the issue of BCG vaccination for over 35 year olds. The Working Group does not have the expertise to make a decision on this issue; it needs to be assessed by the Joint Committee on Vaccination and Immunisation, which is the competent authority to determine whether it is appropriate to recommend BCG vaccination for abattoir workers over the age of 35 years.

Logistical considerations
The Working Group heard the views of several representatives from within the meat industry. There is some opposition to increasing the use of PPE, on logistical grounds. The working conditions in an abattoir are hot and the work is very physical in nature, with workers processing animals at a fast pace. Workers already wear PPE, and the general view is that increasing the amount of cumbersome equipment would hamper their ability to work. While there is less resistance to RPE than to other forms of PPE, the possibility was raised that wearing RPE in hot conditions could lead to discomfort and that donning and doffing reusable RPE may increase the risk of bacterial infection; however, the Working Group recognises that RPE is routinely used in other sectors with comparable working conditions. Furthermore, the point was raised that a requirement for additional control measures could impact on consumer confidence.

Conclusions and recommendations
After taking into consideration all of the available evidence and opinions presented, the Working Group concluded that the overall occupational risk of infection with \textit{M. bovis} in abattoir workers is at most low. However, the consequences of infection, should it occur, are serious. As the probability of exposure varies depending on the particular task being carried out, each stage of the line was considered independently. The Group’s risk assessment for each stage and the recommendations for control measures for bTB infection in addition to those already in place are laid out in Appendix 8 and summarised below. As the hazard (\textit{i.e.} infection with \textit{M. bovis}) remains the same throughout, the risk descriptors used indicate the probability of infection occurring.
The Working Group’s remit was to consider bovine abattoirs, and therefore other premises that handle carcases were not risk assessed. Furthermore, the Group’s recommendations are aimed specifically at reducing the risk of infection with *M. bovis*, but the suggested measures may also be beneficial for protection from other pathogens.

**Risk control measures**

It should be noted that practises differ between establishments, and therefore local risk assessments (LRAs) must be carried out. This is particularly important for non-reactor abattoirs, in which the probability of encountering infected animals will vary depending on the geographical location of the premises.

The first stage of the line is the lairage, where animals are held after transport and inspected ante-mortem, leading to the possibility of inhalation of respiratory aerosols or nasal secretions from the cattle (there is little risk from contact with milk, as tuberculous udders are found only in very advanced disease and are therefore very rare in the UK). The main risk is thought to be from the generation of aerosols during power washing of the area, which may contain contaminated faeces. The risk from this stage of the line was considered to be very low in reactor abattoirs and negligible to very low in non-reactor abattoirs; no extra control measures are recommended.

The next stage of the line is stunning. In some establishments, the operator stands in front of the animal for this procedure, leading to close contact with respiratory secretions; in such cases, the Working Group recommends the use of RPE in reactor abattoirs (low risk), and its consideration in non-reactor abattoirs (very low risk). Since different establishments will have different practises, LRAs are essential.

The next stages to be considered were shackle and hoist, stick and bleed and rod and ring the oesophagus. Each of these operations may have some associated possibility of close contact with the head and therefore with respiratory secretions; however, the risk was deemed to be very low for reactor abattoirs and negligible or negligible to very low for non-reactor abattoirs, and no extra control measures are recommended. Similarly, no extra control measures are considered necessary for legging, where no potential exposure to *M. bovis* was identified.

Removal, dressing and inspection of the head involves the possibility of exposure to aerosols from the upper respiratory tract, and inspecting the head lymph nodes carries the possibility of infection through cuts. The risk in reactor abattoirs was assessed as low and the Working Group recommends the use of RPE and cut-resistant gloves; the risk in non-reactor abattoirs is very low and RPE and cut-resistant gloves should be considered in the LRA.

No hazardous activities were identified during hide removal or evisceration. During inspection of the green offal and associated lymph nodes, the main risk was assessed to be from cuts. The risk was considered to be very low in all abattoirs, and the Working Group recommends the use of cut-resistant
gloves for this procedure. During the following stage, opening of the thorax and removal of the pluck, the lungs are not incised and there is no close contact with the airways, so there is very little risk of aerosol inhalation: the risk of infection at this stage was deemed to be negligible to very low, and no extra control measures are recommended.

Inspection of the pluck carries a low risk in reactor abattoirs and a very low risk in non-reactor abattoirs. The main risks at this stage are from respiratory tract aerosols and from cutaneous infections when cutting the lymph nodes and lung tissue. Thus, the Working Group recommends the use of RPE and cut-resistant gloves for this stage of the line in reactor abattoirs; RPE should be considered in the LRA in reactor abattoirs. No risk of exposure was identified for the next stage, splitting the carcase, whereas during inspection of the carcase and of the lymph nodes, the main risk is again from cutaneous infections if cutting the lymph nodes, with a very low risk in reactor abattoirs and a negligible to very low risk in non-reactor abattoirs. No recommendations for extra control measures are deemed necessary.

At the end of the each slaughter session, the abattoir is washed down. Aerosols may be generated by power washing; however, the levels of environmental contamination are low, and therefore the risk of infection from such aerosols was assessed to be low in reactor abattoirs and very low to low in non-reactor abattoirs. The Working Group recommends that RPE should be used for this stage in reactor abattoirs, and considered in the LRA for non-reactor abattoirs. Finally, no increased risks of exposure were identified in the gut room.

For some stages of the process, the use of a disinfectant that is known to be effective against *M. bovis* would be a useful risk management tool. Several such disinfectants have been identified by Defra. However, the disinfectant would have to be suitable for use in a food-preparation environment – in other words, it would have to be non-toxic to humans and non-tainting. The Working Group is not aware of the existence of any disinfectant that is both effective against *M. bovis* and food-safe.

**Recommendations**

In summary, the Working Group’s recommendations for reducing the occupational risk of infection with *M. bovis* in abattoirs are as follows:

1. Existing guidance in the Green Book, which recommends that unvaccinated tuberculin-negative abattoir workers and veterinary staff under the age of 35 receive the BCG vaccination, should be followed.

2. COSHH regulations and guidance from PHE and ACDP (Advisory Committee on Dangerous Pathogens, 2003, Public Health England, 2014) which state that employers should provide suitable health surveillance, should be followed.

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3. In establishments in which the operator stands in front of the animal during the process of stunning, RPE should be used in reactor abattoirs and considered in the LRA in non-reactor abattoirs.

4. During removal, dressing and inspection of the head, RPE and cut-resistant gloves should be used in reactor abattoirs and considered in the LRA in non-reactor abattoirs.

5. Cut-resistant gloves should be used during inspection of the green offal and associated lymph nodes.

6. When inspecting the pluck, RPE and cut-resistant gloves should be used in reactor abattoirs, and RPE should be considered in the LRA in non-reactor abattoirs.

7. During washdown at the end of each slaughter session, RPE should be used in reactor abattoirs and considered in the LRA in non-reactor abattoirs.

**Final comments**

The Working Group found that there is occupational risk, albeit low, of *M. bovis* infection in abattoirs, and endorses full compliance with existing guidance and health and safety legislation. However, for most stages of the processing line there is negligible or very low risk and recommending the use of extra PPE would be disproportionate. For some operations in which the risk is relatively higher, the Working Group has recommended the use of additional control measures, most commonly the use of RPE, at certain points on the slaughter line. Additional information on the selection and use of RPE to control exposure to biological agents is included in Appendix 10. While the Group recognises that there could be practical issues to be considered when using RPE in the abattoir setting, RPE is routinely used in numerous other occupational settings when undertaking hard, physical work activities in challenging environments.

In addition to the recommended control measures outlined above, there is a need for better education of abattoir workers and owners. This will ensure that they understand the probability and the consequences of infection with *M. bovis*, increasing the likelihood of them complying with any extra control measures. It is also important to provide support for abattoirs and industry bodies in undertaking risk assessments and devising risk management strategies.

It is important that more genotyping work be done to identify the sources of human infection. At present it is difficult to compare human and bovine isolates in the UK because different genotyping techniques are routinely used for the two. APHA and PHE should be encouraged to work together to standardise and compare the subtypes of bovine and human isolates.
The recommendations of this report will need to be reviewed should the situation change, for example should the bTB epidemic expand further, or should there be more spillover into other species.
Appendix 1

*M. bovis* is a member of the *Mycobacterium tuberculosis* complex, a group of closely related *Mycobacterium* species that can cause tuberculosis in humans and other animals. It is a Gram-positive, rod-shaped aerobic bacterium with a slow doubling time. It has a single chromosome carrying approximately four thousand genes (Garnier *et al.*, 2003).

The infectious dose in cattle has been reported to be as low as one colony-forming unit (CFU) (Dean *et al.*, 2005). The infectious dose in humans is not known, but has been estimated to be in the region of tens to hundreds of organisms by the respiratory route (Ashford *et al.*, 2001).

**Genotyping M. bovis**

There are several means of genotyping *M. bovis* isolates, each of which has advantages and disadvantages in terms of discrimination, interpretation and cost.

- Spoligotyping (Spacer Oligonucleotide Typing) is the genotyping method used by APHA. It detects patterns in numbers and types of spacer sequences between directly repeated sequences, and is routinely used to compare *M. bovis* isolates from animals nationally as each spoligotype appears to have a geographic 'home range' (see Appendix 5, Map 4).
- VNTR (variable number tandem repeats, also known as mycobacterial interspersed repetitive units or MIRU) typing determines patterns in tandem repeat sequences across a series of loci. It is the genotyping method carried out by PHE on isolates from humans.

VNTR is more discriminatory than spoligotyping, hence spoligotyping and VNTR are increasingly used together in order to detect transmission clusters. Comparison of whole genome sequences (WGS) is becoming the gold standard for comparing isolates, however, and is considered of particular use, especially for outbreaks of human TB, in local-scale studies of microevolution and who-infests-whom.
Appendix 2

*Mycobacterium bovis* Working Group

Terms of Reference

Purpose & Scope

The current evidence of work related incidence to *M. bovis* is largely based on historically low numbers of reactor cattle processed for slaughter in such abattoirs. In the near future, there will however, be a significant rise in numbers of reactor cattle processed in this way and as a consequence, an increase in potential exposure of abattoir workers, which may not be evident for some years due to latency of infection.

A recent investigation has established that, out of the reactor cattle slaughtered, approximately 50% were confirmed as having the disease. Therefore, from the perspective of occupational risk, it can be concluded that *M. bovis* is present in large numbers of reactor cattle, and that the process of slaughter is likely to generate conditions for the dissemination of the organism into the work environment with a potential for exposure to the abattoir workforce.

Perceived low incidence of infection, together with an absence of qualitative and quantitative data to accurately inform risk have been used as the basis for industry risk assessments resulting in the conclusion that risk is low and controls are not needed beyond the basic hygiene measures that are currently in place in any abattoir. Therefore, the purpose and scope of the group is to attempt to quantify the risks of *M. bovis* transmissibility for those who work in abattoirs, particularly (reactor) abattoirs and other occupational settings. This will help in providing an evidence base for future ACDP guidance on transmission risk and inform as to what controls are needed for this occupational setting.

Objectives

The objectives of the working group are to:

- Develop a project plan;
- Review available information relating to:
  - Prevalence of bovine TB in GB cattle;
  - Infection in occupational setting (*i.e.* abattoirs)
- Attempt to quantify the risks of *M. bovis* transmissibility for those who work in abattoirs, particularly (reactor) abattoirs;
• Produce a report for ACDP to consider that will provide an evidence base for future ACDP guidance on risk and inform as to what controls are needed

Meeting arrangements

The initial meeting of the group will be used to scope out the tasks required to put in place mechanisms for assessing the risks from *M. bovis* in an occupational setting.

Further meetings will be scheduled every two months to progress against milestones in the project plan.

Experts will be invited to present evidence to the core Working Group to inform this exercise.

Minutes and reporting

Minutes will be taken by the secretary and circulated to the group for approval within one week of each scheduled meeting. A progress update will be provided to ACDP at February and June 2014 meetings.
Appendix 3

Membership of the *M. bovis* Working Group:

Professor Bill Reilly (Chair)  University of Glasgow
Professor Ibrahim Abubakar  University College London/
                            Public Health England
Professor Malcolm Bennett   University of Liverpool
Professor Dominic Mellor    University of Glasgow
Professor Dilya Morgan      Public Health England
Mr John Newbold             Health and Safety Executive
Professor Sarah O’Brien     University of Liverpool
Dr Keith Stephenson         Health and Safety Executive

Secretariat:

Dr Ginny Belson             Public Health England
Dr Mariam Orme              Public Health England
Mr Lee Wilson               Health and Safety Executive
Appendix 4

The Working Group heard expert evidence and opinion from the following organisations and individuals

Mr Fred Beeson, Beesons Limited
Mr Ed Beeson, Beesons Limited
Mr Angus Beeson, Beesons Limited
Other abattoir and Food Standards Agency staff on site at Beesons Limited

Mr Noel Sykes, Food Standards Agency
Mr Martin Evans, Food Standards Agency
Mr Jose Camara-Diaz, Food Standards Agency

Dr Musarrat Afza, Public Health England
Dr Dominik Zenner, Public Health England

Mr David Bryant, Animal Health and Veterinary Laboratories Agency
Dr Amie Adkin, Animal Health and Veterinary Laboratories Agency
Dr Ricardo De la Rua-Domenech, Animal Health and Veterinary Laboratories Agency

Dr Dil Sen, Health and Safety Executive

Mr Dennis Cryer, Association of Independent Meat Suppliers

Mr Christopher Tozer, UNISON

Mr Richard Dilworth, British Meat Processing Association

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6 Now the Animal and Plant Health Agency
Appendix 5

Maps kindly provided by APHA

Map1: density of skin test, IGRA test reactors and slaughterhouse cases in 2013
Map 2: the proportion of *M. bovis* culture positive slaughterhouse submissions for the 238 slaughterhouses with a throughput of >40 animals per year between January 2009 and December 2012.
Map 3: The proportion of *M. bovis* culture positive slaughterhouse submissions to the Animal Health and Veterinary Laboratories Agency (now the Animal and Plant Health Agency) for the 207 slaughterhouses with a throughput >40 animals in 2013.
Map 4: locations of the major genotypes (spoligotypes: see Appendix 1) causing cases in 2013 (spoligotype 25 is grey)
### Appendix 6

Case notifications of *Mycobacterium bovis* in people by country, UK 1999-2013

<table>
<thead>
<tr>
<th>Year</th>
<th>England</th>
<th>Wales</th>
<th>Northern Ireland</th>
<th>Scotland</th>
<th>United Kingdom</th>
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<tbody>
<tr>
<td>1999</td>
<td>16</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>2000</td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>2001</td>
<td>17</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>20</td>
</tr>
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<td>2002</td>
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<td>1</td>
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<td>2</td>
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<td>19</td>
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<td>14</td>
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<td>3</td>
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<td>6</td>
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<td>1</td>
<td>1</td>
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<td>3</td>
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<td>17</td>
<td>4</td>
<td>1</td>
<td>7</td>
<td>29</td>
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<tr>
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<td>30</td>
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<td>1</td>
<td>5</td>
<td>37</td>
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<td>2011</td>
<td>30</td>
<td>0</td>
<td>2</td>
<td>7</td>
<td>39</td>
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<tr>
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<td>30</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>39</td>
</tr>
<tr>
<td>2013</td>
<td>23</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>29</td>
</tr>
</tbody>
</table>

Appendix 7

*M. bovis* cases by age group and place of birth, UK 1999-2013

Sources: Enhanced Tuberculosis Surveillance (ETS), Enhanced Surveillance of Mycobacterial Infections (ESMI), May 2014. Public Health England
### Appendix 8

<table>
<thead>
<tr>
<th>Bovine slaughter probability of infectious exposure</th>
<th>Lairage</th>
<th>Stunning</th>
<th>Shackle and hoist</th>
<th>Stick and bleed</th>
<th>Rod and ring oesophagus</th>
<th>Legging (removal of distal legs)</th>
<th>Remove/dress and inspect head</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure/hazardous activities</td>
<td>Animal handling, cleaning out (including vehicle), especially washing down, ante-mortem inspection (very rarely exposure to milk if dairy cows are being killed, as tuberculous udders are very rare)</td>
<td>Close contact with respiratory secretions</td>
<td>If animals get caught or are kicking, there can be a need to handle them which may include head contact</td>
<td>Close contact with respiratory secretions</td>
<td>At this station in some abattoirs the operative may also free skin over the fact prior to hide removal, which may involve direct contact with the nose and secretions (rather than generation of aerosols)</td>
<td>None identified</td>
<td>Removal and washing/hosing of tongue; harvest of cheeks; inspection of head lymph nodes.</td>
</tr>
<tr>
<td>Personnel exposed</td>
<td>Abattoir employees mainly; Food Standards Agency (FSA) employees/contractors less so</td>
<td>Abattoir employees (FSA employees need to check on this from time to time and may be quite close)</td>
<td>Abattoir employees</td>
<td>Abattoir employees</td>
<td>Abattoir employees</td>
<td>Abattoir employees</td>
<td>Abattoir employees; FSA employees/contractors</td>
</tr>
<tr>
<td>Comments</td>
<td>Main risk is from power washing contaminated environment (respiratory lesions, later stage infection, coughed and swallowed leading to contaminated faeces), possibly plus aerosol from close contact and respiratory aerosols/nasal secretions from cattle.</td>
<td>Main risk is from respiratory aerosol if the operator is standing in front of the animal. Different establishments will have different practices – LRA is essential.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible risk of aerosols from upper respiratory tract, e.g. when removing tongue; also from wounds/cuts especially of infected lymph nodes.</td>
</tr>
<tr>
<td>Reactor abattoirs</td>
<td>Risk*</td>
<td>VL</td>
<td>L</td>
<td>VL</td>
<td>VL</td>
<td>VL</td>
<td>N</td>
</tr>
<tr>
<td>Control measures (extra, beyond boots, overalls etc.)</td>
<td>RPE is appropriate if facing animal when stunning</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RPE is appropriate, also cut-resistant gloves</td>
</tr>
<tr>
<td>Non-reactor abattoirs</td>
<td>Risk*</td>
<td>N/VL</td>
<td>VL</td>
<td>N</td>
<td>N/VL</td>
<td>N/VL</td>
<td>N</td>
</tr>
<tr>
<td>Control measures (extra, beyond boots, overalls etc.)</td>
<td>Consider RPE in LRA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Consider RPE in LRA, also cut-resistant gloves</td>
</tr>
</tbody>
</table>

### Evidence base/uncertainty

Satisfactory (based on...)

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* Risk level descriptors:
  - **Negligible (N)**: So rare that it does not merit consideration
  - **Very Low (VL)**: Very rare but cannot be excluded
  - **Low (L)**: Rare but does occur

Note that the hazard (i.e. infection with *M. bovis*) remains the same throughout, so the risk descriptors used indicate the probability of infection occurring.
<table>
<thead>
<tr>
<th>Remove hide</th>
<th>Evisceration</th>
<th>Green offal/lymph node inspection</th>
<th>Open thorax and remove pluck</th>
<th>Pluck inspection</th>
<th>Splitting</th>
<th>Carcase/other lymph node inspection</th>
<th>Washdown of abattoir</th>
<th>Gut room</th>
</tr>
</thead>
<tbody>
<tr>
<td>None identified</td>
<td>None identified</td>
<td>Cut/wound contamination (including knife cuts)</td>
<td>None identified</td>
<td>Possible aerosols from lungs; cut/wound contamination. In some abattoirs there may be harvest of the trachea and hyoid musculature and these tissues may be separated and washed/hosed, potentially creating aerosols</td>
<td>None identified</td>
<td>Cut/wound contamination (including knife cuts)</td>
<td>Aerosols from contaminated flooring etc. during hosedown</td>
<td>None identified</td>
</tr>
<tr>
<td>Abattoir employees</td>
<td>Abattoir employees</td>
<td>FSA employees/contractors</td>
<td>Abattoir employees</td>
<td>FSA employees/contractors</td>
<td>Abattoir employees</td>
<td>FSA employees/contractors</td>
<td>Abattoir employees</td>
<td>Abattoir employees</td>
</tr>
<tr>
<td>Main risk is cuts</td>
<td>Minimal aerosol risk, as the procedure does not involve opening the lungs or close contact with airways</td>
<td>Main risks are aerosol from the respiratory tract during inspection; also cutaneous infections from knife cutting lymph nodes</td>
<td>Main risk is cuts/wounds when cutting lymph nodes</td>
<td>Aerosols are produced by power washing, but low levels of environmental contamination so low risk of infectious aerosols</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>VL</td>
<td>N/VL</td>
<td>L</td>
<td>N</td>
<td>VL</td>
<td>L</td>
<td>VL</td>
</tr>
<tr>
<td>Cut-resistant gloves</td>
<td>RPE is appropriate, also cut-resistant gloves</td>
<td>RPE is appropriate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>VL</td>
<td>N/VL</td>
<td>VL</td>
<td>N</td>
<td>N/VL</td>
<td>VL/L</td>
<td>VL</td>
</tr>
<tr>
<td>Cut-resistant gloves</td>
<td>Consider RPE in LRA</td>
<td>Consider RPE in LRA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(on expert opinion)
Appendix 9

Legislation and regulations relevant to workers handling infected cattle:

1. Section 2(1) and (2) of the Health and Safety at Work etc. Act 1974, requires employers to ensure, so far as is reasonably practicable, the health, safety and welfare at work of all employees. This includes the provision of plant equipment and systems of work that are safe and without risk to health; the provision of adequate information, instruction and training and supervision as is necessary; and facilities and arrangements for employee welfare at work.

2. Regulation 3(1) of the Management of Health and Safety at Work Regulations 1999 requires every employer to make a suitable and sufficient assessment of the risks to the health and safety of his employees to which they are exposed whilst they are at work.

3. Regulation 6(1) of the Control of Substances Hazardous to Health (COSHH) Regulations 2002 (as amended) requires the employer to make a suitable and sufficient assessment of the risks created by that work to the health of those employees.

4. COSHH Regulation 6(2)(h)(k) requires the employer to include in the risk assessment the results of relevant health surveillance, and the approved classification of any biological agents.

5. The Approved Code of Practice for COSHH Regulation 6 states that “In certain circumstances, for example in medical facilities or livestock farming, the risk assessment should take account uncertainties about the presence of infectious agents in patients and animals.”

6. COSHH Regulation 7(6)(f) requires the employer, where appropriate, to make available effective vaccines to those employees who are not already immune to the biological agent to which they are exposed or are liable to be exposed.

7. COSHH Regulation 11(1) states “Where it is appropriate for the protection of the health of his employees who are, or are liable to be, exposed to a substance hazardous to health, the employer shall ensure that such employees are under suitable health surveillance.”
Appendix 10

Respiratory Protective Equipment

The COSHH Regulations require that exposure of employees to substances hazardous to health, which includes biological agents, should be prevented or adequately controlled. Where Respiratory Protective Equipment (RPE) is to be used to control exposure to biological agents (or other hazardous substances) employers must ensure that the equipment used is suitable for the work undertaken and that the selected RPE has the potential to provide adequate protection for individual wearers.

There is a large variety of RPE available. The type of RPE selected depends on the nature of the work that is being carried out and consideration should be given to the work rate, the length of time that workers will need to wear the RPE, and the environment where the work will be carried out. When in an airborne state, micro-organisms can be classed as particles, so they can usually be removed by filter-type RPE. Where filter-type RPE is to be used, equipment should be chosen that controls exposure down to the lowest levels i.e. the highest efficiency P3 filter.

In deciding which types of RPE are suitable for the task and are capable of providing adequate protection, the following general factors need to be considered:

- the biological agents likely to be present and their routes of transmission;
- the quantity of airborne material likely to be generated and the nature of the contamination;
- the wearer;
- medical fitness;
- thermal strain;
- face to face-piece seal - this should be determined by conducting face-fit testing;
- compatibility with other PPE;
- work-related factors including length of time RPE is worn, physical work rate, mobility, visibility, communication, work environment (environmental and physical) and the use of tools and other equipment.

Furthermore, it is important to ensure that staff are properly trained in its use and that refresher training is undertaken to maintain competence in the use of RPE. Training should also cover the cleaning, maintenance, storage and disposal of such equipment.

Extensive guidance on the selection and use of RPE can be found on the HSE website (http://www.hse.gov.uk/respiratory-protective-equipment/) and in the following HSE guidance document: HSG53 Respiratory Protective Equipment at work – a practical guide, which is available in a free to download form (http://www.hse.gov.uk/pubns/priced/hsg53.pdf).
References


EUROPEAN FOOD SAFETY AUTHORITY 2013. Technical specifications on harmonised epidemiological indicators for biological hazards to be covered by meat inspection of bovine animals. EFSA Journal, 11, 3276.


