ACM/1021

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

DISCUSSION PAPER

THE POSSIBLE HEALTH RISKS TO CONSUMERS ASSOCIATED WITH *Mycobacterium bovis* AND MILK

PART II – UNPASTEURISED MILK AND MILK PRODUCTS

Introduction

1. This is the second of a two part assessment of the possible health risks to consumers associated with *Mycobacterium bovis* and milk and milk products. Part I addressed the risks from pasteurised milk and milk products and was considered by the ACMSF in September 2010.

2. The purpose of this paper is to seek the views of members on:

   - the potential for **unpasteurised milk and milk products** contaminated with *M. bovis* to enter the food chain and the risk associated with these products

   - whether the risk has changed in light of the increase in *M. bovis* infection in cattle in the UK.

Background

3. In autumn 2009, the FSA Board requested that the Agency review the potential risks to consumers of meat and milk from cattle with *M.bovis* infection. The request was made as a number of years have passed since the ACMSF last considered the issue in 2001 and since that time the incidence o*f M. bovis* infection in the UK cattle population has increased. The Board wished for reassurance that the current controls on meat and dairy products are adequate to protect human health given this rise.

4. Consequently, in March 2010 the ACMSF reviewed changes in the hygiene regulations and disease incidence in cattle and humans which have taken place over the last 10 years. The Committee confirmed the result of its earlier 2001 risk assessment on meat and concluded that the risk remained very low.

5. In its 2002 Report on *M. bovis*, the Committee concluded that there were no concerns in relation to milk and dairy products as the exposure pathway seemed well protected by the existing legislation and controls. In light of the FSA Boards' request that the Agency re-consider the risks to consumers from milk and milk products, the Committee assessed the potential for pasteurised milk and milk products contaminated with *M. bovis* to enter the food chain and whether the risk has changed in light of the increase in *M. bovis* infection in cattle in the UK. Their conclusion in September 2010 was that the risk from pasteurised milk and milk

products contaminated with *M. bovis* has changed but in milk that is properly pasteurised the risk remains acceptably low.

6. The results of the meat risk assessment were reported back to the FSA Board in July 2010 and a further report is planned in autumn 2011, following completion of the risk assessments on milk and milk products.

Paper outline

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Exposure assessment

7. Since 1994, the HPA and its partner agencies have carried out a national surveillance programme for TB in humans[1]. Over this time, despite a rise in TB in cattle, only a very small proportion of human TB cases each year in the UK (25 or 0.5% of culture confirmed cases) are due to *M. bovis* and the vast majority have been caused by *M. tuberculosis*. Most human cases due to *M. bovis* occur in people born in the UK before 1960, suggesting reactivation of old infection that was acquired when the prevalence of *M. bovis* in the UK cattle population was greater and when pasteurisation of milk and cattle testing programmes were not so widespread. About 20% of cases occur in non-UK born persons (suggesting infection contracted abroad). A small number of human cases attributed to direct occupational contact with infected animals have occurred. There is no evidence that any of the recorded UK cases of human *M. bovis* infection which have occurred since 1994 have been acquired through recent consumption of contaminated meat or dairy products derived from *M. bovis* infected animals in the UK.

8. The infectious dose of *M. bovis* for humans is high, estimated to be in the region of millions of organisms by the gastrointestinal route. If infection does occur, approximately 10% of individuals will progress to active tuberculosis, with about half of these occurring within two years. Children are generally at greatest risk of infection with individuals aged 15-44 years having the highest rate of cases of TB (20.8 per 100,000) due to *M. tuberculosis*. The immunosuppressed and very young infants are most at risk of acute infection (miliary tuberculosis). Human to human transmission of *M. bovis* does occur but is very rare in the UK and has only been documented twice in last 20 years.

9. The BCG vaccine is regarded as being 70 – 80% effective at preventing disease due to *M. tuberculosis* and is likely to have a similar level of efficacy against *M. bovis.* Most UK adults will have been vaccinated under a school vaccination programme for all tuberculin skin test negative children introduced in 1953. However, in 2005 the policy was changed and vaccination is now only offered to babies at birth who live in areas with high rates of TB or whose parents or grandparents were born in a country with a high prevalence of TB.

10. There have been no reported human outbreaks of *M. bovis* infection associated with unpasteurised milk or unpasteurised milk products in the UK for at least the last four decades. The last reported outbreak of foodborne disease associated with unpasteurised milk in England and Wales was in 2002 and the last reported outbreak associated with unpasteurised milk products was in 2009, neither of these outbreaks were due to *M. bovis* infection (HPA).

[1] Annual report on tuberculosis surveillance in the UK 2010

Unpasteurised cows' and buffaloes' milk for direct human consumption

11. The sections below outline the main components of the bovine TB control programme in the UK which apply to cows and buffaloes, controls on sales to the consumer and the potential for *M. bovis* to be present in unpasteurised cow's and buffaloes' milk for direct human consumption.

A: TB controls in cows and buffaloes

12. The current bovine TB control programme in the UK, which applies to cows and buffaloes, is designed to detect *M. bovis* infection in the national herd by routine testing of herds at variable intervals, additional targeted testing of herds and animals at risk and by post mortem inspection of all animals at slaughter by FSA meat inspectors and official veterinarians.

13. The cornerstone of the TB control programme is the routine tuberculin skin testing of cow and buffalo herds performed according to a frequency (annual to 4-yearly) dictated by the local incidence of TB herd breakdowns. All herds in Wales are annually tested. In Northern Ireland, the interval is dependent on risk assessment, with a maximum interval of one year and over 25% of herds tested more frequently. In Scotland, which was declared an Officially TB Free (OTF) region of the UK in October 2009 by the European Commission, all herds are tested every four years. In England, the area and number of herds under annual testing has markedly increased since the beginning of 2010 relative to previous years. As a result of this change, 47% of all English herds are now annually tested and 11% are tested every two years.

14. If TB is detected in a herd, either by use of the skin test or through routine post-mortem meat inspection, the herd will automatically lose its OTF status. All tuberculin skin test reactors and any at-risk direct contacts are required to be isolated and are compulsorily removed and slaughtered by the Animal Health and Veterinary Laboratories Agency (AHVLA) in GB. Herds with test reactors and/or slaughterhouse cases undergo movement restrictions, epidemiological investigations and whole herd testing at 60 to 90-day intervals in order to regain their OTF designation. The more sensitive gamma interferon blood test is deployed as an ancillary parallel test in some herds with culture-confirmed *M. bovis* infection that fulfil certain criteria. Depopulation of whole herds or groups of severely infected animals takes place very occasionally.

B: Controls on unpasteurised milk sales to the consumer

15. Sale of unpasteurised cows' milk direct to the consumer is permitted in England and Wales, however fewer than 100 retailers now exist compared to several hundred in 2001. The sale of unpasteurised cows' milk to consumers is confined to farm gate sales, farm catering, milk rounds and farmers markets. It is also known that two producers offer internet sales. All such milk must carry the health warning 'This milk has not been heat treated and may therefore contain organisms harmful to health'. In Wales, the warning also highlights the risks to vulnerable groups.

16. In England and Wales there are no restrictions on sales of unpasteurised buffaloes' milk to the consumer and in Wales is there a requirement for the milk to carry the health warning.

17. Sales of raw drinking milk and cream from cows and buffaloes are banned in Scotland. Northern Ireland has similar controls to England and Wales but there are no known sales.

18. For cattle herds in England and Wales whose milk is sold unpasteurised direct to the consumer, public health protection is provided through Regulation (EC) 853/2004, which requires that raw cows' and buffaloes' milk for human consumption shall only come from animals belonging to an OTF herd, i.e. where there is no evidence of *M. bovis* infection. Additionally, although not part of the food hygiene regulations, it has been a long standing policy of AHVLA in England and Wales to place all dairy herds that are known to sell unpasteurised cows' milk directly to the consumer under an annual TB testing regime, regardless of the default routine frequency for other cattle herds in their locality.

19. If the OTF status of a dairy herd is suspended or withdrawn (e.g. when test reactors or slaughterhouse cases are detected or when a TB test becomes overdue), AHVLA will immediately notify the relevant Local Food Authority, who are responsible for enforcing compliance with the food hygiene regulations. At the time of suspension of OTF status, the affected herd owner (food business operator) is also informed by AHVLA in writing of their legal obligations in respect of the marketing of milk from their herds. Milk from animals giving a positive reaction to the tuberculin test is not permitted to go for human consumption. Milk from other animals in the herd must undergo pasteurisation (minimum 72°C for 15s) until the OTF status is restored. The control programme in Northern Ireland works on broadly similar principles.

C: The potential for *M. bovis* to be present in unpasteurised milk for direct human consumption

20. There are two aspects of the biology of TB infection in cattle that need to be understood when considering the likelihood that unpasteurised milk for direct human consumption might contain *M. bovis*. The first is the route by which milk could become contaminated with *M. bovis* and the occurrence of tuberculous mastitis. The second is the potential for infected cows to remain undetected by the skin test so that their milk continues to enter the food chain.

21. Where there is infection in the herd, either detected or undetected, routes that could lead to contamination of raw milk with *M. bovis* include via faeces and from the environment but the main risk arises from direct contamination of the milk in the udder. Although this can occur before the animal tests positive on the skin test or before clinical signs of infection are apparent, this is rare. It is most likely to occur when infection becomes disseminated and there is tuberculous mastitis. In such cases large numbers of bacteria can be shed in the milk. However, evidence suggests that such cases are rare in the UK nowadays.

22. In 1934, before the adoption of milk pasteurisation and compulsory tuberculin skin testing of cattle, it was reported that more than 40% of dairy cows in Great Britain were infected with *M. bovis* and 0.5% suffered from TB of the udder. Despite the resurgence of bovine TB in the cattle population since the late 1980s, the percentage of dairy cows infected is much lower than it was in the 1930s and tuberculous mastitis nowadays is rarely seen in cows in the UK. This is believed to be due to the fact that the current statutory bovine TB surveillance programme removes infected animals before the disease becomes disseminated to the udder. The proportion of TB test reactors and slaughterhouse cases presenting with visible tuberculous lesions in the udder or the mammary lymph nodes in the course of post-mortem examination is very small (<1%) (see Annex 1). Although it is not possible to accurately estimate the frequency with which *M. bovis* is shed in infected cows' milk in the UK (and whether there has been an upward or downward trend in recent years), it is somewhat reassuring to note that only a small number of incidents of TB in dairy calves associated with exposure to contaminated milk from tuberculous cows are reported by AHVLA every year (Monies and Head 1999, Houlihan et al. 2008).

23. On the second point, there is the potential for dairy cows that are infected to go undetected by the skin test and therefore for their milk to continue to enter the food supply while the herd remains OTF. This may happen because animals become exposed, infected and subsequently infectious in the intervening period between two routine herd tests and/or because of infected cattle escaping detection by *ante mortem* TB testing. Although these animals are likely to be small in numbers it is important to be aware of this possibility. The tuberculin skin test is not 100% sensitive and the bovine TB control programme cannot be expected to detect every infected animal. There may be a number of reasons why an animal is 'anergic', i.e. has visible evidence of TB at slaughter but fails to show a cutaneous response to tuberculin (see Annex 2).

24. In summary, the potential for *M. bovis* to be present in unpasteurised milk destined for human consumption is minimised through rapid detection and removal of infected cattle and exclusion of reactor milk from the food chain. The risk cannot be totally eliminated due to the possibility of the presence of anergic cattle, which go undetected by the skin test, may develop tuberculous mastitis and whose milk may continue to enter the food supply. However, anergic animals are believed to be rare and it seems unlikely that a herd would have one infected cow that is anergic and for there to be no other positive animals. So even if there is an anergic cow present in a herd, other infected animals would give a positive reaction to the tuberculin test, resulting in a loss of OTF status and the milk would not be permitted to be sold unpasteurised for direct human consumption.

Unpasteurised milk from non-bovine species for direct human consumption

25. The sections below outline the main components of the TB controls for sheep and goats in the UK, controls on sales to the consumer and the potential for *M. bovis* to be present in unpasteurised sheep and goats' milk for direct human consumption.

A: TB Controls in sheep and goats

26. Sporadic incidents of TB caused by *M. bovis* arise in non-bovine dairy species (sheep and goats) and occur almost invariably in areas of endemic high incidence of TB in cattle and wildlife. In GB, TB in farmed animals other than cattle is also notifiable and when a culture-confirmed episode of *M. bovis* infection is disclosed in those species, movement restrictions are immediately applied on the herd/flock of origin and a veterinary risk assessment of the premises is carried out by AHVLA to inform the need for further action. As with all confirmed incidents of *M. bovis* infection in cattle, AHVLA will also inform the Consultant in Communicable Disease Control of the Local Health Protection Unit. If dairy goats or sheep are involved, the Local Food Authority is also notified. Movement restrictions remain in place until the entire affected herd or epidemiological group has been slaughtered or after repeat tuberculin skin testing as required by AHVLA has been completed with removal of any test reactor animals. In England, *ante mortem* TB testing of these species is voluntary and there is no compensation to herd owners for the slaughter of test reactors. Similarly in Wales there is no routine *ante mortem* testing of sheep and goats although powers to require TB testing are in place as well as statutory compensation for any goats removed as test reactors.

27. In NI, bovine TB in other species is notifiable. No action is taken in respect of movement restriction, disease control or testing and compensation in these species outside the risk they pose to bovines. If they are considered significant in a bovine episode, restrictions on movements and disease control measures are placed on the cattle herd as required.

B: Controls on unpasteurised milk sales to the consumer

28. In England and Wales there are no restrictions on sales of unpasteurised sheep or goats' milk to the consumer but the milk is required to carry the same health warning as unpasteurised cows' milk. Production is small scale with 27 producers registered with AHVLA known to sell raw goats drinking milk and 3 known to sell sheep milk. Sales of raw drinking milk and cream from any species are banned in Scotland. Northern Ireland has similar controls to England and Wales but there are no known sales.

29. The legislative requirements in Regulation (EC) 853/2004 for species other than cows and buffaloes which are susceptible to TB requires that raw milk from sheep and goats must come from herds which are regularly checked for this disease under a control plan that the competent authority has approved. A control plan to enact this legislative provision is under development by the FSA in discussion with the agriculture departments of the UK. In the meantime, AHVLA

will only test goats for TB whenever *M. bovis* infection is found in a co-located cattle herd or *M. bovis* infection is confirmed in a goat herd. DARD has no control programme directed at disease control in other species.

C: The potential for *M. bovis* to be present in unpasteurised milk for direct human consumption

30. When considering the potential for *M. bovis* to be present in sheep and goats milk there is significantly less information available on the level of infection in these animals because they are not currently routinely tested under a control programme. However, as there are only sporadic reported incidents of TB in such flocks/herds, this suggests that the incidence is low and therefore the likelihood of animals with disseminated disease supplying contaminated milk into the foodchain is also low.

Unpasteurised milk products from bovine and non-bovine species

A: Controls on unpasteurised milk products

31. For cattle herds in England and Wales whose milk is used to produce unpasteurised milk products, public health protection is provided through Regulation (EC) 853/2004, which requires that raw cows' and buffaloes' milk for human consumption shall only come from animals belonging to an OTF herd, i.e. where there is no evidence of *M. bovis* infection. The frequency at which such herds are tested will be dictated by the local incidence of TB but AHVLA place individual herds on an annual testing regime, where they have knowledge that the milk is used to produce unpasteurised milk products.

32. In cases where herds providing milk to raw milk cheesemakers lose their TB free status, the local food authority will carry out a risk assessment on the public health implications for any products made prior to the loss of status and any control measures necessary. This assessment is undertaken locally with the Consultant in Communicable Disease Control (CCDC) and Animal Health. Guidance issued to Local Authorities by the FSA sets out detailed information on the factors to take into account when making such a risk assessment e.g. the TB history of the herd, the number of reactors found, whether the reactors were milk producing animals and tissue culture results[2].

33. Advice on the investigation and management of potential human contacts is included in guidance issued to CCDCs and Chief EHOs by the National Institute of Clinical Excellence. Screening is generally recommended only in the case of those less than 16 years old who have not been vaccinated and who may have consumed unpasteurised milk or dairy products from affected animals with proven or possible udder infection.

B: The potential for *M. bovis* to be present in unpasteurised milk products

34. A variety of unpasteurised milk products are produced in the UK including cream, butter, yoghurt, ice cream, whey and cheese. Many are made from cows' milk but a minority are made from buffaloes, sheep or goats' milk. The volume produced is difficult to estimate as production appears to be mainly very small scale except in the case of unpasteurised milk cheeses. Information from membership of the Specialist Cheesemakers Association (www.specialistcheesemakers.co.uk) suggests there are currently as many as 57 manufacturers of raw milk cheeses in the UK and there appears to be a growing market for such products.

35. The parameters under which *M. bovis* may grow outside of an animal are not clearly defined. However, it is very slow growing even under ideal laboratory conditions and it is generally considered that it does not multiply in milk (Sinha,

[2] Food Law Practice Guidance (England) ANNEX 8, APPENDIX 1: Guidance to Food Authorities in England on Officially Tuberculosis Free Status and Dairy Hygiene Legislation (Similar guidance exists in the equivalent documents for Scotland Wales and N. Ireland)

1994).The question is therefore the extent to which it is able to survive the production process and still be viable when the product is consumed.

36. Evidence of survival of *M. bovis* in products such as cream, yoghurt, butter and ice cream is scant as research has tended to concentrate on survival in cheese. Where there has been research undertaken, survival has been seen in butter, cottage cheese and fromage blanc (a product similar to thick yoghurt). In the fromage blanc survival was seen at 14 days but not at 17 days, however by this time the product was not suitable for consumption due to mould contamination. It is therefore possible to suggest that short shelf life products could pose a risk to consumers as, if contaminated, they will be consumed while there are still viable bacteria present.

37. A wider body of work on survival in cheese has included studies on Emmental, Cheddar, Gruyere, Munster, Camembert and Bleu d'Auvergne (a blue cheese). Emmental has been particularly well studied and it has been shown that the production process has a considerable impact on survival of *M. bovis*. This may be due to the scalding process where the curds are heated to 53°C for 30-40 minutes, which would not destroy *M. bovis* but may have affected its ability to survive during maturation of the cheese.

38. The studies on other hard cheeses such as Cheddar show greater variability in the time during which the bacteria may remain viable (approx. 60 days up to >200 days). Since there are few variables in the cheddar making process which might influence survival of *M. bovis* it is likely that this variability reflects differences in concentration of this organism in the milk used for making the cheese. Early experiments only mixed contaminated with uncontaminated milk without measuring the number of *M. bovis* present. Thus these tests would use a much greater inoculum concentration than would be likely from cows which gave a positive result to the tuberculin test but did not exhibit symptoms of tuberculous mastitis. Nevertheless, there is evidence that *M. bovis* will not remain viable indefinitely in hard cheese.

39. Due to an identified need for further data on survival of *M. bovis* in cheese the FSA has been funding a research project on UK produced unpasteurised milk cheese which has investigated the survival of *M. bovis* through the manufacturing process and maturation. The research was designed to produce data on survival which would help inform risk assessments on raw milk products made prior to the loss of OTF status, e.g. maturing cheese not yet placed on the market (as highlighted in para. 32).

40. The research undertaken focussed on two types of cheese – Caerphilly and Cheddar. These cheeses were chosen to represent semi-hard and hard cheeses with long maturation periods and because these are the type of cheeses most likely to require a risk assessment if the herd from which the milk came lost its OTF status. The work involved manufacturing the cheese with milk inoculated with *M. bovis* and assessing survival through up to a year of maturation. Caerphilly can be matured for 2 weeks to 4 months and Cheddar for over a year in some cases. Based on the results obtained with high inoculum levels of *M. bovis*, D_{10} values have been calculated of 58 days for Caerphilly and 48 days for

Cheddar, indicating significant die-of during the maturation period. The results will be presented in more detail to the Committee by Queen's University, Belfast who undertook the research project. Further work using low inoculum levels is still underway.

41. In summary, there is little available evidence on survival of *M. bovis* in unpasteurised products other than cheese. What evidence does exist shows *M. bovis* can survive the production process and be present in short shelf life products. The evidence that exists on cheese supports the possibility that if milk contaminated with high levels of *M. bovis* is used to produce unpasteurised milk cheese, the organism can survive the production process through to the maturation stage. The extent to which there is a decline in viability during maturation will be influenced by the physical parameters of the cheese (pH, water activity, salt) and the length of maturation. However, in the case of both short and longer shelf life products evidence suggests that there is little likelihood of *M. bovis* being present in the milk in the first place and if it were present numbers would be low.

Discussion

42. The potential exists for *M. bovis* to be present in unpasteurised milk. The main risk arises from direct contamination of the milk in the udder, which is most likely when infection becomes disseminated and there is tuberculous mastitis. However, despite the resurgence of bovine TB in cattle since the late 1980's, tuberculous mastitis is rarely seen in the UK as the surveillance programme means that infected animals tend to be removed from the herd in the relatively early stages of infection. The TB controls on sheep and goats are less stringent but incidents of *M. bovis* infection in these species are only sporadic and a control plan is under development.

43. There is a theoretical risk to human health from consumption of *M. bovis* contaminated milk or milk products if there is *M. bovis* infection in a dairy herd producing raw milk for direct human consumption or for the manufacture of unpasteurised milk products. There are however several factors which contribute to minimising this risk.

- The proportion of TB reactors presenting with visible lesions in the udder or mammary lymph nodes at post mortem examination is very small (<1%).
- Annual TB testing is carried out in cow and buffalo herds supplying milk for direct human consumption or manufacture into unpasteurised products. Any positive results exclude the producer from unpasteurised sales. Shedding in the milk occurs only in advanced cases, and presence of infection is unlikely to remain undetected in a herd undergoing repeated annual tests.
- Any contaminated milk would be diluted with milk from healthy animals in the herd and often with milk from other herds.

- *M.bovis* is reported not to multiply in milk.
- There is likely to be a reduction in *M. bovis* contamination during the production process for unpasteurised milk cheeses, including loss in the whey and die off during maturation.
- The infectious dose of *M. bovis* for humans is estimated to be in the region of millions of organisms by the gastrointestinal route.
- Should any viable bacteria remain and human infection be established, the chance of active TB developing is approximately 10%, as for any TB infection.
- The BCG vaccine is regarded as providing some level of protection against *M. bovis* and most UK adults will have been vaccinated as children.

ACMSF action:

The Committee is requested to consider and give a view on:

- **The potential for:**

  a) unpasteurised milk

  b) unpasteurised milk products

  contaminated with *M. bovis* to enter the food chain and therefore the risk to human health associated with these products.

- **Whether the risk has changed in light of the increase in *M. bovis* infection in cattle in the UK.**

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Sinha R. N. (1994) *Mycobacterium bovis*. In: The significance of pathogenic microorganisms in raw milk. International Dairy Federation, Brussels, Belgium 141-166.

An assessment of the potential for *M. bovis* to contaminate raw milk cheese in the UK. Communication from the Specialist Cheesemakers Association, 2000

Annex 1

Total tissue samples from TB reactor cattle and slaughterhouse cases submitted to VLA for mycobacterial culture (2003-July 2010)

Sex	Lesions	**Total**	2003	2004	2005	2006	2007	2008	2009	2010
Undefined	Total	**10**	1	1	2	1	1	1	3	
	NVL	**2**			1	1				
	VL	**8**	1	1	1		1	1	3	
Male	Total	**19862**	525	2997	3491	2611	2649	3589	2600	1400
M	NVL	**10005**	166	1626	2001	1304	1306	1904	1161	537
M	VL	**9857**	359	1371	1490	1307	1343	1685	1439	863
Female	Total	**92287**	2930	15878	16040	11580	12965	15780	11647	5467
F	NVL	**62601**	1441	11572	11706	7726	8979	10879	7308	2990
F	VL, split as follows:	**29686**	1489	4306	4334	3854	3986	4901	4339	2477
	Lymph nodes	**27920**	1392	4026	4092	3657	3762	4626	4038	2327
	(of which *M.bovis* positive)	**26059**	1216	3724	3829	3464	3503	4330	3772	2221
	Organs	**1473**	67	231	197	170	192	223	260	133
	(of which *M.bovis* positive)	**1262**	55	189	162	153	168	190	225	120
	Other	**161**	6	33	17	22	19	37	17	10
	(of which *M.bovis* positive)	**87**	5	14	13	9	5	21	13	7
	Udder	**58**	1	4	22	2	4	7	13	5
	(of which *M.bovis* positive)	**20**	1	2	4	2	3		6	2
	Mammary lymph nodes	**74**	23	12	6	3	9	8	11	2
	(of which *M.bovis* positive)	**48**	20	8	2	2	5	5	5	1

Notes:
1. Data only go back to the advent of the current TB Culture System at VLA. Prior to that tissues were not being recorded between 2000 and November 2003. 'Year' is the year processed at VLA.
2. Only sample references that appear in the VetNet database and thus enable retrieval of the animal's sex are included.
3. These figures only represent the animals sampled by AH or the MHS and submitted to VLA for examination and culture. In multiple-reactor breakdowns only a representative number of animals are sampled for culture (normally up to 3 reactors with visible lesions). Less animals per breakdown have been submitted since 2009.
4. 'NVL' = no visible lesions of TB, 'VL' = typical visible lesions of TB recorded.
5. One VL animal may present with multiple TB lesions and thus have several tissues recorded against it.

Annex 2

Anergic Cattle

Under the widest definition of anergic, this may be the result of a poor skin testing technique, use of tuberculin of reduced potency, desensitisation after injection of tuberculin, immunosuppression during early post-partum, due to the administration of certain drugs or co-infections with certain parasites and viruses. A proportion of those anergic animals will be detected by the gamma-interferon blood test.

The narrower definition of anergy is when visibly infected cattle fail to react to the tuberculin test due to changes in the host's immunopathological response in the advanced disease stages of TB, when the bacteria "break out" of the primary lesions at their point of entry and disseminate throughout the body. In those cases, the cell-mediated immune response measured by the skin and gamma-interferon tests is gradually replaced by circulating antibodies against *M. bovis* and this requires a different type of immunological test (so-called serological or antibody assays).

The serum antibody assay currently available for bovine TB diagnosis in GB is the Chembio Stat-PAK rapid test. This assay has been validated in cattle, but it has a low sensitivity (other than for animals with advanced TB lesions) and is only used by AHVLA in very exceptional circumstances and with the herd owner's consent. Other antibody-based tests have been developed in the USA and Ireland and are undergoing validation by AHVLA. However, for the time being antibody tests are not officially approved by the EU, either for routine TB screening of cattle or as ancillary tests.

By definition, it is difficult to know when a herd contains anergic animals due to advanced TB and, by implication, when to deploy the serological tests but, in general, it is believed that those cases are quite rare and AHVLA will use serology only when there is evidence of ongoing cattle-to-cattle spread in a chronically infected herd despite the repeated application of the tuberculin and gamma-interferon tests (e.g. herds with a high incidence of TB in young calves associated to milk-borne spread from a tuberculous mastitic cow). The antibody assay may be used in NI where considered beneficial for disease control in a herd, but this is infrequent.