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Heat inactivation of Mptb in lamb meat

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Abstract

Mycobacterium avium subsp. *paratuberculosis,* which causes Johne's disease in livestock, can be present in red meat from infected animals. Because of a perceived link between this organism and Crohn's disease in humans, its inactivation during cooking was investigated. Moderate to high levels of contamination are most likely to be eliminated during domestic cooking of lamb as inactivation of 10^5 organisms was observed after 4 to 5 mins at 65 °C or 10 to 15 sec at 70 °C. The lamb roast was used as a model cooking method in this study, but the results are generally applicable to other cooking methods and types of red meat. When combined with hygienic food handling practices, consumers can have a high degree of confidence that cooking of red meat affords a very low risk of viable *M. avium paratuberculosis* being consumed.

Executive summary

In 2005 MLA commissioned the NSW Department of Primary Industries in collaboration with the University of Sydney to conduct project PRMS.044 “Potential pathogens in Australian red meat; Presence of *Mycobacterium avium* subsp. *paratuberculosis* in red meat”. The results indicated that Mptb may be present in meat and peripheral lymph nodes associated with red meat in sheep from properties with ovine Johne's disease. The concentration of Mptb in meat was determined to be in the range $10^{0.7}$ to $10^{1.4}$ viable cells / gram and in lymph node $10^{1.4}$ to $10^{1.8}$ viable cells / gram. The highest value recorded was about 10^{5} viable cells / gram. It is likely that these were underestimates of viable count due to partial inactivation of the organism during decontamination of samples prior to culture. There were similar findings in a small number of cattle. In experiments in which Mptb was introduced into the blood stream of normal sheep, the organisms were distributed to most tissues and were detected in red meat 48 hours later.

Because of perceived public health risk and the finding that Mptb may be present in Australian red meat entering the human food chain the next logical step was to determine the cooking conditions required to inactivate Mptb if it was present in red meat. There are no published data on the survival of Mptb within red meat during the cooking process. However, the dairy industry has already completed food safety research for its products.

Surprisingly there were few scientific data on temperatures attained in red meat during conventional cooking. Recommendations for cooking meat to ensure that it is microbiologically safe for humans appear to be uncommon and relatively inaccessible. Many contemporary scientific studies of the inactivation of various viral and bacterial pathogens in meat defer to the USDA Food Safety Inspection Service guideline target internal temperature of 71.1 °C.

In this study the thermal profiles (temperature by time) were measured at the core of 16 lamb roasts (mean wt 1803 g; range 1520 to 2664 g) that were baked in domestic ovens by volunteers representing a range of family and cultural origins (USA, Canada, The Netherlands, New Zealand, Italy, Australia, Germany, Britain, as well as several combinations). The recipes used by the volunteers varied greatly in detail: the cooking style; the baking container; the use of additives such as herbs and garlic; whether or not vegetables were co-baked, and; other factors. The results indicated a substantial variation between the oven set temperature and the actual maximum oven temperature that was recorded. Only five of 14 ovens provided an actual maximum temperature within 10°C of the set temperature. The actual temperatures were as much as 50°C above or below the set temperature. The maximum core temperature of the roasts during cooking ranged from a low of 59.6°C to a high of 98.3°C. Thirteen of the 16 roasts had maximum core temperatures $\geq$ 70°C for at least 1 min. Of the remaining three, one reached 69.5°C for at least 10 mins, another reached 67.6°C for at least several minutes while the third reached 59.6°C for at least 2 mins. These data are consistent with findings from a large New Zealand questionnaire survey where only 5.3% of respondents nominated “rare” as their preferred degree of doneness. However, a substantial proportion of New Zealand consumers would prefer roast meat to have not been cooked to the degree recommended by the USDA. This knowledge informed the design of the thermal inactivation experiments conducted in this study.

The times and temperatures required to ensure inactivation of Mptb by a minimum of 5 logs were investigated and found to be in the range obtained during domestic cooking of lamb (inactivation after 4 to 5 mins at 65C; 10 to 15 sec at 70C). To determine the degree of inactivation that is likely to be achieved by different cooking methods, D values for the heating of Mptb in lamb homogenate

fluid were determined at 55, 60, 65 and 70 °C, which covered the most common range of core temperature that was measured during baking. D values in lamb homogenate fluid ranged from 90 mins at 55 °C to 1.5 seconds at 75 °C. These values were similar to those reported for Mptb in substrates such as milk or lactate. Z values determined for this temperature range were 4.3 to 4.5 °C. Data were determined for both common strains of Mptb, S and C, and were similar.

The impact of these findings for the meat and livestock industry are impossible to predict because the links between Mptb and human health remain unclear, have not received much public attention in popular media and appear not to have led to a change in consumer behaviour. If putative links between Crohn's disease and Mptb were to receive public attention in the future, the results of this study can be used to provide consumers with assurance that routine cooking of meat will destroy Mptb if it is present. The data were determined using baking as a model cooking method, but they are generally applicable. Core temperatures during baking are lower than surface temperatures during baking, grilling or frying. Therefore these other cooking processes will destroy Mptb quite effectively. In absolute terms, moderate to high levels of contamination will be destroyed provided that the meat reaches 65 °C for 5 mins or 70 °C for 15 seconds; such temperatures are readily achieved in domestic and commercial cooking without altering commonly used techniques; these may be conservative recommendations. These data should provide consumers with a high degree of confidence that there is very low risk of viable Mptb being consumed with cooked red meat. However, to be more effective cooking needs to be combined with hygienic food handling practices. In particular, uncooked meat or utensils should not come into contact with cooked meat.

Recommendations

1. MLA should consider promotion of cooking practices that ensure adequate decontamination of red meat. Such cooking practices may be common but it is difficult to find recommendations in Australia from an authoritative Australian source.

2. Although the results of this study should provide consumers with a high degree of confidence that there is a very low risk of viable Mptb being consumed with cooked red meat, it is important that other aspects of food hygiene are also addressed, particularly not bringing uncooked meat, or utensils or surfaces used to prepare uncooked meat, into contact with cooked meat.

Contents

1 Background

1.1 Mptb and red meat

1.1.1 Publicly available information

There is a large amount of evidence for extra-intestinal spread of Mptb; it occurs most commonly in advanced sub-clinically or clinically affected animals. Mptb has been found in extra-intestinal lymph nodes, milk, liver, spleen, semen, testes, epididymis, seminal vesicle and other parenchymous organs of cattle (3, 4, 30). There is an extensive literature on the presence of Mptb in bovine milk [for example see (11)]. Haematogenous or lymphatic spread are possible routes for movement of the organism to extra-intestinal sites. Indeed, the organism has been found in peripheral blood (4, 6, 7, 23). There have been similar findings in tissues, blood and milk of sheep, goats, wild ruminants and primates (10, 12, 17, 18, 21, 24, 25, 27, 29, 33, 42, 43). As Johne's disease has a systemic component the developing fetus is at risk of infection.

Surveys of red meat for Mptb have been rare. In one study 200 ground beef samples in the USA were tested using PCR with negative results (20). The first papers to report the presence of Mptb in or on red meat or in peripheral lymph nodes associated with red meat appeared in 2008. In one US study, which used an insensitive culture method, the organism was recovered from prescapular and popliteal lymph nodes, but not from two skeletal muscles from several infected cows (1). In the second study, Mptb DNA was detected using PCR in swabs taken from the anal region of some bovine carcases (26).

2 Project Objectives

I. Investigate times and temperatures required to ensure inactivation of Mptb by a minimum of five logs.

2. Relate the inactivation of Mptb to cooking methods used for sheep and lamb.

3. Determine the degree of inactivation that is likely to be achieved by different cooking methods

For operational reasons objective 2 was addressed first. This enabled objectives 1 and 2 to be closely aligned with greatest public health benefit and made relevant to domestic cooking scenarios.

3 Methodology

3.1 Literature review on cooking temperatures for meat

A literature review was undertaken to ascertain the likely temperature reached within red meat during various stages of the cooking process by various methods.

3.2 Temperatures reached in red meat during cooking

In discussion with MLA it was resolved to use leg of lamb as the most relevant example of red meat from sheep and to use baking in a convection oven as the standard cooking method.

Sixteen leg of lamb roasts (on the bone) were purchased from a retail butcher in Camden, NSW one to two days prior to intended cooking and stored at 4°C in a domestic refrigerator. The weight of each roast and its external dimensions (length, circumference at each end and at 2 cm intervals along the length) were measured.

On the day of cooking, a food-grade electronic temperature probe was inserted to the approximate geometric centre of the roast at two sites in such a way that the probes did not contact bone. A third probe was supplied to record oven temperature. The probes were connected to a data logger (CENTRE 309 data logger thermometer with K type thermocouples, Instrument & Sensor, Skye). The data logger was programmed to collect data at 5 minute (roasts numbers 1 to 6) or 1 minute (roast numbers 7 to 16) intervals. The roasts were wrapped in clear food grade polyethylene film, and packed in an insulated container at approximately 4°C for transport.

Each leg of lamb roast was issued to a different volunteer, chosen to represent a range of family cultural backgrounds, each with prior experience in the cooking of lamb roast. The prepared lamb was transported for up to 60 mins in an insulated box and kept at 4°C in a domestic refrigerator until it was required for cooking.

The sixteen different volunteers then cooked their roast using their usual family recipe. They were asked to record details of the recipe and the cooking process on a standard form. The information requested included the baking container, the oven make, model and set temperature, the basting and the turning routine, the standing time prior to carving and the appearance of the meat before and after cooking. Each volunteer was asked to specify their preference for the degree of doneness prior to being issued with a roast, and were asked to assess this after cooking. The temperature probes were kept in position during the standing of the meat for at least 10 mins after removal from the oven, as it was known that the internal temperature can increase over that time.

Each volunteer was asked to carve their roast transversely and the degree of doneness was estimated by comparison of the cut surface with a colour chart (Meat Standards Australia).

3.3 General microbiological methods

3.3.1 Isolates of Mptb

In vitro grown Mptb strain Telford 9.2, a pure culture of S strain defined by RFLP analysis and IS*1311* PCR-REA was used (S strain). A lyophilized seed stock was reconstituted in water, inoculated into modified BACTEC 12B medium, incubated for 3 weeks, then subcultured to modified Middlebrook 7H10 agar and incubated for 4 weeks. Colonies from 40 plates were harvested in 1ml of PBS with 0.1%v/v Tween 20 (PBST), vortexed for 1 min to remove clumps, diluted 1:10 in PBST, passed through a 26g needle, filtered through an 8 um filter and examined microscopically at x 400 magnification to confirm that the suspension consisted predominantly of single cells; the suspension

was dispensed in 1 ml aliquots and frozen at -80°C. Prior data indicated that the viable cell count is maintained when the frozen suspension is thawed.

A parallel culture of Mptb strain CM00/016, which is a field isolate of a C strain, non-clonal, was also prepared using the same method, except that slopes were incubated for 6 weeks (C strain).

The target concentration of these suspensions was 10^7 cells per ml. The viable cell count was determined by end point titration in BACTEC 12B using the most probable number method as described below.

3.3.2 Enumeration of Mptb

Most probable number method (MPN). A standard method was used to provide an accurate estimate of the viable count/ml of suspension (2). Briefly, a single ten fold dilution series of the parent suspension was prepared from 10^0 to 10^{-10}. An aliquot of 50 ul of each dilution was added to each of three BACTEC 12B vials, to enable a three tube MPN count. Tubes were incubated at 37°C and read at weekly intervals for 12 weeks. The viable count per 50ul was estimated as the MPN index read from standard MPN tables multiplied by the dilution factor. The dilution series were prepared using both PBS and lamb homogenate fluid as diluents (see below).

Cumulative growth index. The viable count was also determined by MPN approaches in a second series of dilutions. Three separate 10 fold dilution series were prepared, and 50 ul of each was inoculated into a single BACTEC vial. BACTEC vials were read twice weekly until a positive GI was recorded; when daily readings were taken for up to 59 days post inoculation. MPN was determined from 3 tube tables as above. The dilution series were prepared using both PBS and lamb homogenate fluid as diluents (see below).

The time in days required for the cumulative growth index (CGI) to reach 1000 in BACTEC12B medium is correlated with inoculum size (32). GI was summed across days and the number of days for CGI to reach 1000 was estimated to the nearest 0.5 days. A standard curve with 95% confidence limits was generated using simple least squares linear regression of the dilution (or log10 viable count) and days to CGI1000 for each dilution, using Genstat (GenStat Release 10.1 2007, Lawes Agricultural Trust, Rothamsted Experimental Station). For experiments on heat inactivation, the inoculum size following each treatment was predicted from the regression equation based on its observed CGI1000.

3.3.3 Preparation of lamb muscle homogenate fluid

Two aliquots of 100g of fresh lamb meat, each mixed with 400 ml of sterile saline, were homogenised in a food blender, filtered through sterile cotton cloth and a 100 to 150um mesh filter. The filtrate was allowed to settle for 30 min and clarified by centrifugation at 2,800 x g for 20 min. The supernatant was passed through an 8 um filter then sterilised by passing through a 0.2 um filter. The lamb homogenate fluid was stored at 4°C until required for use.

3.3.4 BACTEC culture method for Mptb

The culture of Mptb in modified BACTEC medium and identification of the organism using IS*900* PCR was conducted as described previously (41).

3.4 Inactivation of Mptb by 5 logs

To determine the conditions that are required for the complete inactivation of Mptb in a substrate that resembles red meat, suspensions of Mptb were diluted ten fold in lamb homogenate fluid; this resulted in final concentrations of 1.5 x 10^6/ml and 5.8 x 10^6/ml for the S strain and the C strain, respectively. Aliquots of 100 ul were used for the heat inactivation experiments; the aliquots contained 1.5 x 10^5 and 5.8 x 10^5 viable Mptb for the S strain and the C strain, respectively (see table 3).

Triplicate 100 ul aliquots of suspensions were placed in thin-walled 200 ul capped plastic tubes in a PCR thermocycler that was programmed to provide temperatures ranging from 55 °C to 75 °C for set times. After heating, an aliquot of 50 ul from each tube was inoculated into a BACTEC vial and incubated at 37 °C for up to 56 days. Vials were checked for later growth at 12 weeks. The maximum duration of survival among the three replicates at each temperature was recorded.

3.5 Inactivation achieved by different cooking methods

It was not practical to expose Mptb to every possible cooking method. Therefore, the decimal reduction time of Mptb suspensions prepared in lamb homogenate fluid was determined. This was done over the temperature range likely to occur within a lamb roast, which was deemed to be broadly representative of internal temperatures during cooking of whole muscle cuts. This was concurrent with the research described in section 3.4.

3.5.1 Determination of D value

D value is the time required at a constant temperature for a 1 log (or 90%) reduction in viable count, determined by linear regression of experimental data, where D = -1/regression coefficient. An attempt was made to calculate D value at the following temperatures: 55, 60, 65, 70 and 75 °C.

To determine D values in a substrate that resembles red meat, suspensions of Mptb were diluted 10 fold in lamb homogenate fluid to a final concentration of 1.5 x 10^6/ml and 5.8 x 10^6/ml for the S strain and the C strain, respectively. Aliquots of 100 ul were used for the heat inactivation experiments; the aliquots contained 1.5 x 10^5 and 5.8 x 10^5 viable Mptb for the S strain and the C strain, respectively (see table 3). Triplicate 100 ul aliquots of suspensions were placed in thin-walled 200 ul capped plastic tubes in a PCR thermocycler that was programmed to provide temperatures ranging from 55 °C to 75 °C for set times. After heating, an aliquot of 50ul from each tube was inoculated into a BACTEC vial and incubated at 37 °C for up to 56 days. Vials were checked for later growth at 12 weeks. The number of days to CGI1000 was estimated to the nearest 0.5 days. The viable count after heating for each time was determined by prediction from the linear regression equation, using

an average MPN count (that from the single dilution series, and that from the triple dilution series, in section 3.3.2) as the viable count for the parent (unheated) suspension (see Table 3).

3.5.2 Determination of z value

The z value is the temperature change that results in a ten-fold change in D value. The z value was calculated by linear regression of D values, where z = -1/regression coefficient. The z value was calculated using data derived from the determination of D values at 55, 60, 65 and 70 °C.

3.5.3 Determination of F_p values during cooking

An alternative method for assessing the adequacy of the cooking process also was employed. This technique is commonly used by the food processing industry when establishing process schedules for their pasteurised and sterilised packaged foods and relies upon calculation of the cumulative lethal effect of all time-temperature combinations at the points of temperature measurement in the product (in these instances 16 legs of lamb) throughout a thermal process. Following their conversion into excel format the data from each of the 16 cooking trials were imported into proprietary software (DWC FoodTech Thermal Analyser, DWC FoodTech Pty. Ltd., Melbourne) for calculation of the, so-called, F_p values via the General Method as described by Gaze (1992), Hersom and Hulland (1980), Stumbo (1973) and Warne *et a*l (2009). The F_p value of a thermal process quantifies the total heating effect and expresses it in equivalent minutes at a reference temperature for microorganisms with known z values. In this series of trials the reference temperature was 70 ^oC and the z values of the target microorganisms were 4.21 and 4.51 C^o, for Mptb strains S and C, respectively. The results of F_p calculation for each of two thermocouple probes in each of the 16 trials are shown in Table 6.

The reason for determining F_p values is that they provide a direct and quantifiable measure of the severity of the various heat treatments that were used to cook the legs of lamb in this series of trials. In addition, the severity of these processes can be compared with that achieved by the USDA Food Safety Inspection Service guideline of 71.1 °C at which temperature sufficient lethality of the target pathogens is considered to be achieved instantaneously.

3.5.4 Estimation of the decimal reductions in Mptb counts achieved during cooking

An extension of the method for quantifying (via F_p values) the severity of a heat treatment is to calculate the number of decimal reductions the process delivers with respect to destruction of specified target microorganisms, in this case strains S and C of Mptb. In this manner the total F_p value for the entire cooking/baking process (including heating, holding and cooling) when divided by the D value of the target microorganism yields the number of decimal reductions achieved. This approach has been applied to the data in Table 6 and the results are presented in Table 7.

4 Results and Discussion

4.1 Literature review

A literature review was undertaken to ascertain the likely temperature reached within red meat during various stages of the cooking process by various methods. Surprisingly there were few scientific data on temperatures attained in any of the cuts or forms of lamb or beef during conventional cooking. Therefore it was necessary to extrapolate data from a number of sources, including lay publications, and to include information related to cooking of ground meat and other meat products.

The review is subdivided into a number of sections. The first covers the recommended cooking temperatures, which can be viewed as the industry targets based on objective food quality and safety considerations. Objective measurements of the surface and the internal temperatures during cooking are then summarised to determine whether the technical targets are commonly met, and the temperature gradients between surface and core are discussed to ascertain how measurements should be taken. Finally consumer cooking preferences are reviewed to ascertain whether the technical recommendations and the actual temperatures obtained are likely to be met in domestic cooking environments.

4.1.1 Recommended cooking temperatures

Recommendations for cooking meat to ensure that it is microbiologically safe for humans appear to be uncommon and relatively inaccessible. Several on-line resources from reliable industry or government organizations in Australia were found and are summarised in Table 1. The United States Department of Agriculture(USDA) has made recommendations for cooking meat products, specifying the use of a thermometer to measure core temperature and advising consumers for example to cook beef patties to at least 71°C (5). A similar recommendation was made by the US Food and Drug Administration in 2001 (Food and Drug Administration, 2001. Food Code. U.S. Public Health Service, Food and Drug Administration, Washington D.C. cited by (34)). Many contemporary scientific studies of the inactivation of various viral and bacterial pathogens in meat defer to the USDA Food Safety Inspection Service guideline target internal temperature of 71.1°C (for example (31, 34, 40)). The provenance of this “all purpose” recommendation was not pursued in this literature review.

A detailed set of recommendations is available from the USDA specifically for cooked and roast beef. These recommendations include temperature and time interactions and are reproduced as Appendix 1.

One recent scientific study (8) noted that the current recommendation was to cook at 70°C for 2 mins and cited “Guidance note on Approval and Operation of Independent Meat Production Units under EC Meat Leglislation, Meat Products, Minced Meat & Meat Preparations” which was published in 2001 by the Food Safety Authority of Ireland, Dublin.

In summary, there is a lack of consistency in the scientific literature in relation to the source of recommendations about cooking temperatures. However, it appears that a target temperature of 71°C would not be inconsistent with most recommendations.

Table 1. Recommendations for cooking of meat available on-line from industry or government food organizations in Australia

Organisation	Recommendation	URL
Food Standards Australia and New Zealand (FSANZ)	Cook chicken, sausages and hamburgers until juices run clear – steaks can be cooked to preference	http://www.foodstandards.gov.au/newsroom/factsheets/foodsafetyfactsheets/charitiesandcommunityorganisationsfactsheets/preparingandcookingf1479.cfm; accessed 26th February 2008
Safemeat	When cooking mince, sausages, hamburger patties, rolled or stuffed roasts, ensure they are cooked evenly throughout. It is a good idea to check the internal temperature of these meats during cooking with a meat thermometer – aim for a temperature of 75°C. There should be no pink meat visible and juices should run clear. Cooking of steaks and primal cut roasts is often a matter of preference and can range from 'rare' to 'well done'. Regardless of degree of doneness, the meat surface should always be brown in case bacteria are lurking on the surface. Since the meat below the surface has not been exposed to air or bacteria, it is safe to only cook these cuts rarely such that the internal meat colour will be a deep pink colour.	http://www.safemeat.com.au/English/Meat_Safety/Consumers/Hygeine+tips+and+tools/for+Consumers.htm
Meat & Livestock Australia	Oven roast cuts are suitable for roasting in a moderate oven (180°C). Accurate cooking is best determined using a meat thermometer. Internal temperatures should be as follows for the different degrees of doneness: rare 35°C, medium rare 45°C, medium 55°C, medium well 65°C & well done 75°C. When the roast is removed from the oven allow it to rest for 10 minutes prior to carving. (identical recommendations were provided for sheepmeat and beef)	Tips and Tools. Meat Standards Australia Sheepmeat Information Kit. http://www.mla.com.au/NR/rdonlyres/62F855B9-5AF2-4CAD-BD32-517D50E577C2/0/Theeffectofcutandcookingmethodonsheepmeatquality.pdf

4.1.2 Measurements of surface temperatures of meat during cooking

Lamb meat patties were fried on a hotplate at various heat settings to study mutagen formation; the temperature at the hotplate surface was monitored using a thermocouple and set for cooking in the range 100°C to 300°C (36). In another study the temperatures of a grill were specified to be 163°C, with a separation of 2.16 cm from lamb chops, suggesting that surface temperatures would be similar (37). Surface temperatures exceeded 200°C after 10 min when 150-200 g chicken leg quarters were baked in an air/steam impingement oven for 32 to 35 min at 232°C. (28).

4.1.3 Measurements of internal temperatures of meat during cooking

The temperature at the geometric centre of 2.54cm thick lamb chops was measured immediately after cooking on an industrial belt grill. The grill had the following specifications: top heat 163°C, bottom heat 163°C, preheat 149°C, height gap 2.16 cm, cooking time 5.3 min. These were designed to achieve a final internal temperature of 71°C. The actual internal temperatures were not reported, but the maximum was reached 2 mins after the end of cooking (37). The same authors measured internal temperatures in chops cooked in an open hearth electric broiler, with the same target of 71°C.

The internal temperature of Tekirdag meatballs, a product made in Turkey from ground veal, was measured immediately after cooking and found to be 71°C when grilled for 5 mins, 79°C when baked in a conventional oven at 160°C for 6 mins and 97°C when cooked in a microwave oven at 800W for 5 mins (44).

The internal temperatures of ground beef patties cooked on gas grills at 260°C to 288°C or electric grills at 163°C were measured using thermocouples and removed from the grill after several minutes cooking time when internal temperatures reached 66.1°C or 68.3°C, to allow for a post cooking temperature rise estimated at 2.5°C in cited studies; some patties did not reach 71°C internally in their thickest regions, but the rate of cooling in these regions was lower than in the thin regions of the pattie (37). In another study the time required for the internal temperature of meat patties to reach 71.1°C ranged from 2.7 to 10.9 minutes depending on the type of grill and the cooking method (34).

When 150-200 g chicken leg quarters were baked in an air/steam impingement oven for 32 to 35 min at 232°C, internal temperatures reached 70°C to 75°C (28). In a similar study with chicken breast cooked for 10 min at 177°C and 200°C, internal temperatures reached 79°C and 82.6°C, respectively (31).

4.1.4 Temperature gradient

The magnitude of the difference between the surface temperature and the internal temperature in the above studies where both were measured implies that a substantial temperature gradient exists. Therefore the conductive properties of the meat, its dimensions, and the cooking method including apparatus, temperature and time will be critical variables to consider in any study of pathogen inactivation within red meat.

4.1.5 Consumer cooking preferences

In a large New Zealand study the participants in a questionnaire survey were asked to indicate their preference for the degree of cooking of lamb. Of 300 respondents, 43.3% nominated “medium” and 46% “well done”; only 5.3% nominated “rare” (13).

Based on these data and the figures provided by Meat & Livestock Australia for temperatures corresponding to various degrees of doneness (Table 1), it is reasonable to conclude that the majority of consumers in New Zealand would prefer to bake lamb to a degree of doneness such that internal temperatures reach 55°C to 75°C.

This interpretation suggests that a substantial proportion of New Zealand consumers would prefer roast meat to have not been cooked to the degree recommended by the USDA. The situation in Australia and other markets for Australian red meat should be ascertained if not already known to marketing authorities.

4.1.6 Survival of Mptb in red meat during cooking

There are no published data on the survival of Mptb within red meat during the cooking process. However, there are published data on the survival of the organism at a range of temperatures in substrates such as milk and water. Details are provided in Appendix 2. The data suggest a low potential for survival of Mptb at temperatures over about 60°C provided that such temperatures are maintained for about 30 minutes; survival at 70°C is for only a few minutes.

4.2 Temperatures reached in red meat during cooking

The red meat cooking volunteers represented a range of family and cultural origins including North American (both USA and Canada), Dutch, New Zealand, Italian, Australian, German, British as well as several combinations. Consequently the recipes used by the volunteers varied greatly in detail: the cooking style; the baking container; the use of additives such as herbs and garlic; whether or not vegetables were co-baked, and; other factors (Appendix 3, Table A3.1). The intended degree of doneness ranged from rare to well done, with most volunteers preferring doneness to an extent grater than medium-rare (13 of 16 volunteers). The actual degree of doneness was slightly greater than this, with most volunteers recording doneness $\geq$ medium (14 of 16 volunteers).

The dimensions of the roasts were: length mean 244 $\pm$ 22 mm (range 225 to 305 mm); weight mean 1803 $\pm$ 325 g (range 1520 to 2664 g). Data for individual roasts are provided in Table A3.2.

Objective data on oven set temperatures, the duration of cooking and the actual temperatures recorded are provided in Table A3.3. One oven did not provide for accurate setting of temperature and the oven probe for another was faulty. However, there was substantial variation between the oven set temperature and the actual maximum oven temperature recorded. Only five of 14 ovens provided an actual maximum temperature within 10°C of the set temperature. The actual temperatures were as much as 50°C above or below the set temperature.

An example of the actual data (core internal temperatures) for one roast is shown in figure 1. The data were recorded in a proprietary data logger format and required translation into excel (Microsoft) prior to more detailed analysis. The maximum core temperature of the roasts during cooking ranged from a low of 59.6°C to a high of 98.3°C.

Thirteen of the 16 roasts had maximum core temperatures $\geq$ 70°C for at least 1 min at both probe sites. Only three lamb roasts had maximum core temperatures less than 70°C at one or both probe sites. The intended degrees of doneness for these were rare, medium-rare or medium-well done. One roast reached 69.5°C at one of the probe sites and maintained this for at least 10 mins, and exceeded 70° at the other probe site. Another roast reached 69.2°C and 67.6°C at the two probe sites, respectively, and maintained such temperatures for at least several minutes. The third reached 59.6°C at one probe site for at least 2 mins and 65.9°C at the other, exceeding 60°C at the latter site for at least 22 mins. Considering 60°C as a cut-off, the maximum core temperatures of 15 of 16 roasts exceeded this temperature for $\geq$20 mins at both probe sites, and usually for much longer.

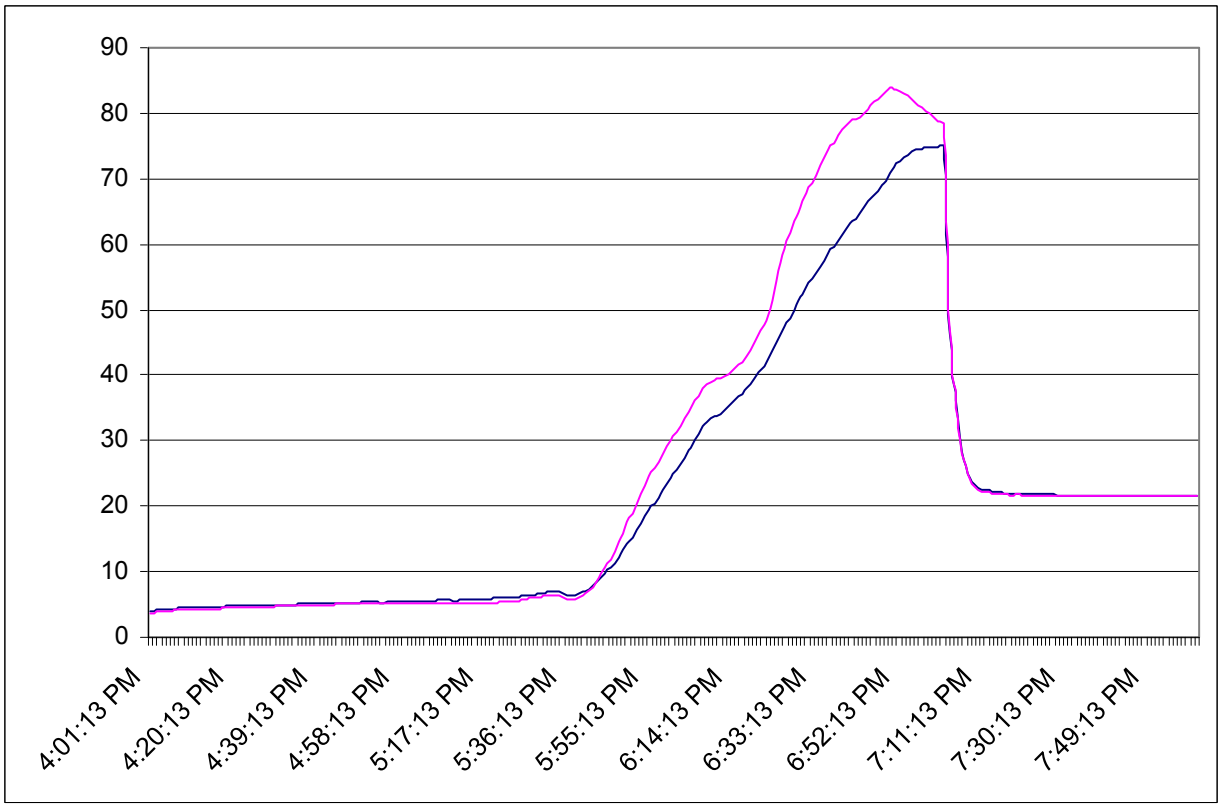


Figure 1. An example of the output from one data logger for two probes that were inserted within a lamb roast during baking.

Conclusions

The significant findings from this small survey included the following:

- Most lamb roasts were cooked to a degree that should ensure destruction of specified microbial hazards as temperatures approached or exceeded those recommended in the USDA standards (71°C).
- There was considerable inaccuracy in set temperatures in domestic ovens.
- It would be appropriate to determine D values for bacterial pathogens using a temperature range from 55°C to 75°C because this temperature range (rounded to the nearest 5 degrees) encompasses those seen in lamb roasts cooked to a low degree of doneness.

4.3 Inactivation of Mptb by 5 logs

The temperature and time combinations that were used in this experiment are given in Table 2. The raw data from this experiment are provided in Appendix 4, table A.4.1.

The results indicated that a reduction of at least 5 logs can be obtained by heating Mptb in a substrate of lamb tissue homogenate at 65 °C for 5 minutes or 70 °C for 15 seconds. The C strain appeared to be slightly more durable than the S strain, but this might have been due to the slightly higher inoculum for the C strain.

Table 2. The temperature and time combinations that were used to determine the complete inactivation of Mptb suspensions containing about 10^5 viable organisms in 100 ul.

Temperature	Duration of survival*	Recommended time points for sampling	Maximum duration of survival observed among three replicates	
			S strain	C strain
55	unknown	0, 15, 30, 45, 60, 75, 90 min	>90 min	>90 min
60	28 to 30 min	0, 5, 10, 15, 20, 25, 30, 35 min	>35 min	> 35 min
65	unknown	0, 1, 2, 3, 4, 5, 6, 7 min	4 mins (1 of 3 reps)	5 min (1 of 3 reps) 7 mins (1 of 3 reps)
70	30 to 35 sec	0, 5, 10, 15, 20, 25, 30, 35 sec	10 sec (3 of 3 reps)	15 sec (3 of 3 reps)
75	unknown	0, 5, 10, 15, 20, 25, 30, 35 sec	< 5 sec	< 5 sec

* actual data for suspensions of 10^6/ml from (16).

4.4 Inactivation achieved by different cooking methods

The temperature and time combinations that were used in this experiment are given in Table 2. The raw data from this experiment are provided in Appendix 4, table A.4.1.

4.4.1 Linear regression of MPN counts and days to CGI1000

The viable counts of Mptb in the parent suspensions were calculated using an MPN method. Two diluents were used: PBS and lamb homogenate fluid (Table 3). The latter diluent was associated with MPN counts 1 to 2 logs higher than those associated with PBS diluent. The reasons might include declumping of Mptb in the lamb homogenate fluid, but were not investigated in this study.

Table 3. Most probable number counts for the S and the C strain of Mptb in two diluents, and inoculum size used in the in heat inactivation experiments in sections 4.3 and 4.4

Strain and buffer	**MPN from 1 dilution series; 3 BACTEC vials inoculated with each dilution**	**MPN from 3 dilution series; 1 BACTEC vial inoculated with each dilution**	**MPN used in calculations & regression analysis**	**MPN when diluted 1:10 in lamb homogenate fluid**	**MPN per 100 ul aliquot used in heat inactivation experiments**
S strain in PBS	**4.3×10^5 /50ul**	**4.3×10^6 /50ul**			
S Strain in lamb homogenate fluid	**1.5×10^7 /50ul**	**1.5×10^7 /50ul**	**1.5×10^7 /50ul**	**1.5×10^6 /ml**	**1.5×10^5**
C strain in PBS	**9.3×10^6 /50ul**	**2.3×10^6 /50ul**			
C strain in lamb homogenate fluid	**9.3×10^7 /50ul**	**2.3×10^7 /50ul**	**5.8×10^7 /50ul**	**5.8×10^6 /ml**	**5.8×10^5**

*50 ul is the standard inoculum size for BACTEC vials

The linear relationships between $\log_{10}$ inoculum size (which was calculated from MPN data in Table 3 and dilution rates for the dilution series) and days to cumulative growth index 1000 for S strain and C strain are shown in Figures 2 and 3, respectively. These relationships were used to predict viable counts in suspensions that had been exposed to the various heat treatments (shown in Table 2) as part of the procedure for determination of D values described in section 4.4.2..

Figure 2. The linear regression of log_{10} inoculum (y axis) and cumulative growth index 1000 (x axis) for the S strain of Mptb.

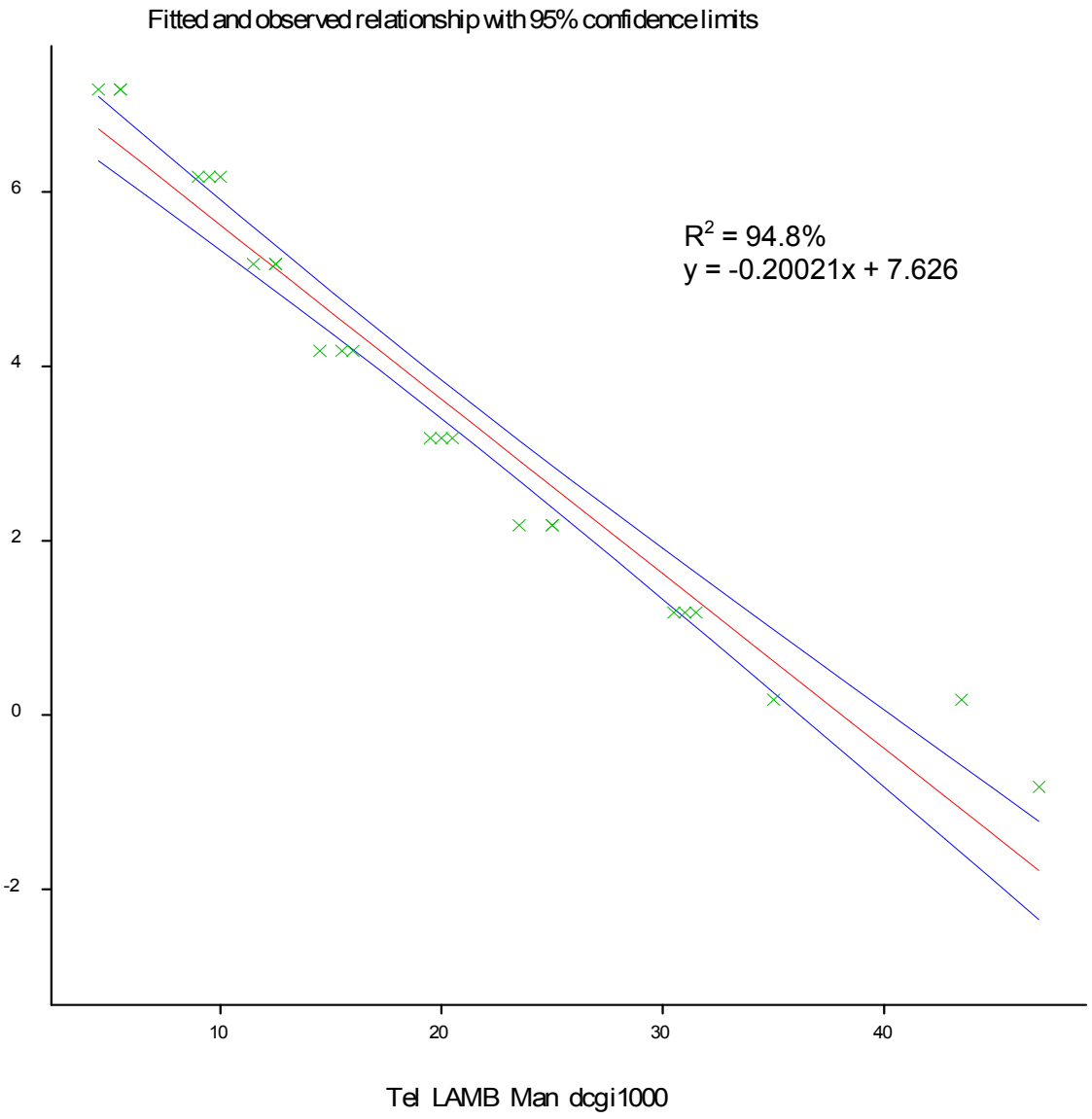
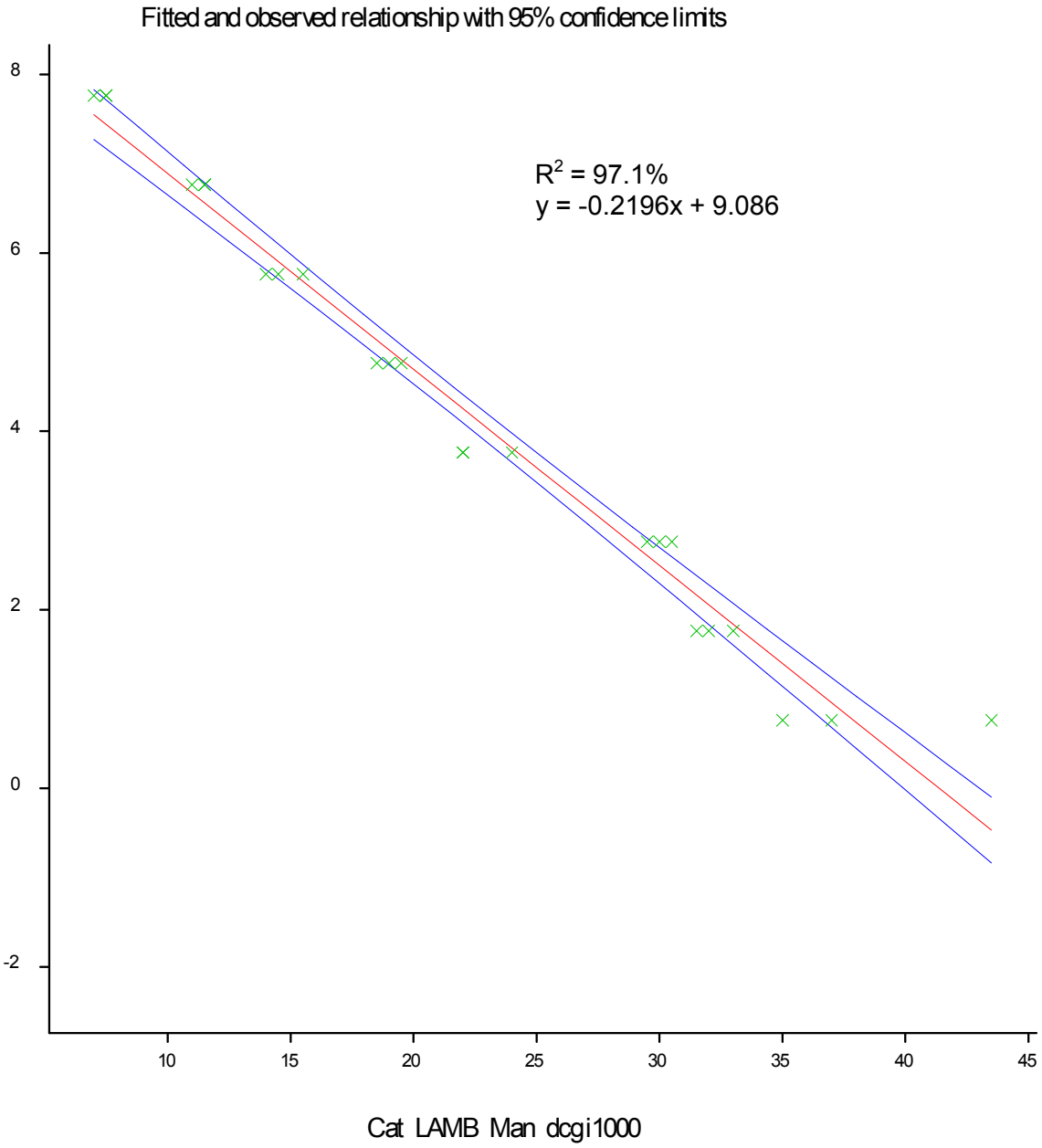


Figure 3. The linear regression of log_{10} inoculum (y axis) and cumulative growth index 1000 (x axis) for the C strain of Mptb



4.4.2 D value

D value is the time required at a constant temperature for a 1 log (or 90%) reduction in viable count.

D values were determined for each temperature using a linear regression of the viable count on time. The regression data and D values are shown in Table 4. There were too few observations of growth after heating at 75 °C to determine a D value for this temperature.

Table 4. The results of linear regression of $\log_{10}$ viable count of Mptb on the duration of heating

Temperature	Unit	Co-efficient	Constant	R-squared	D value	D value (sec)
S strain						
55	minutes	-0.011202	6.071	90.1	89.27	5356.2
60	minutes	-0.12966	5.9828	98.4	7.71	462.7
65	minutes	-2.271	6.486	95.9	0.44	26.4
70	seconds	-0.6507	6.264	98.2	1.54	1.5
75	seconds	not valid				
C strain						
55	minutes	-0.017864	7.7143	95	55.98	3358.7
60	minutes	-0.09028	7.042	84.2	11.08	664.6
65	minutes	-1.721	6.918	84.7	0.58	34.9
70	seconds	-0.5497	7.892	87.9	1.82	1.8
75	seconds	not valid				

Each regression takes the form: $\log_{10}$ viable count = co-efficient x time + constant. D value is the absolute value of the reciprocal of the co-efficient, expressed in units of time at a constant temperature. In this manner the temperature at which D values were determined is indicated by the subscript, so that D_{70} refers to the D value determined at 70 ^{o}C. The D values in Table 7 are specific for the temperatures shown and for the specific medium used, lamb homogenate fluid.

There are no published data on the D_{55} value of Mptb. However, a previously reported D_{55} value of 53 mins for *M. avium* in water (35) is similar to that obtained here for Mptb at 55 °C. Previously reported D_{60} values for Mptb suspended in milk or lactate were 2 to 14 min (22, 39), again similar to the values obtained here. D_{65} values of 56 to 71 sec were obtained in milk or lactate in a previous study (39); values in this study were slightly lower. D_{71} values of 13 to17 sec were obtained in milk or lactate (39), whereas D_{70} values obtained in this study in lamb homogenate fluid were lower.

4.4.3 The z value

The z value is the temperature change that results in a ten-fold change in D value. z was estimated using four temperatures across the range 55 to 70 °C using linear regression (Figures 4 and 5, Table 5).

Table 5. Estimated z values for the two common strains of Mptb diluted in lamb homogenate fluid

Strain	Co-efficient	Constant	R-squared	z value (C^o)
S	-0.2374	16.838	99.8	4.21
C	-0.2216	15.89	97.6	4.51

Each regression takes the form: $\log_{10}$ D value = co-efficient x temperature + constant. The z value is the absolute value of the reciprocal of the co-efficient, expressed in Celsius degrees (C^o).

Figure 4. The linear regression of $\log_{10}$ D value (y axis) on temperature (x axis) for the S strain of Mptb

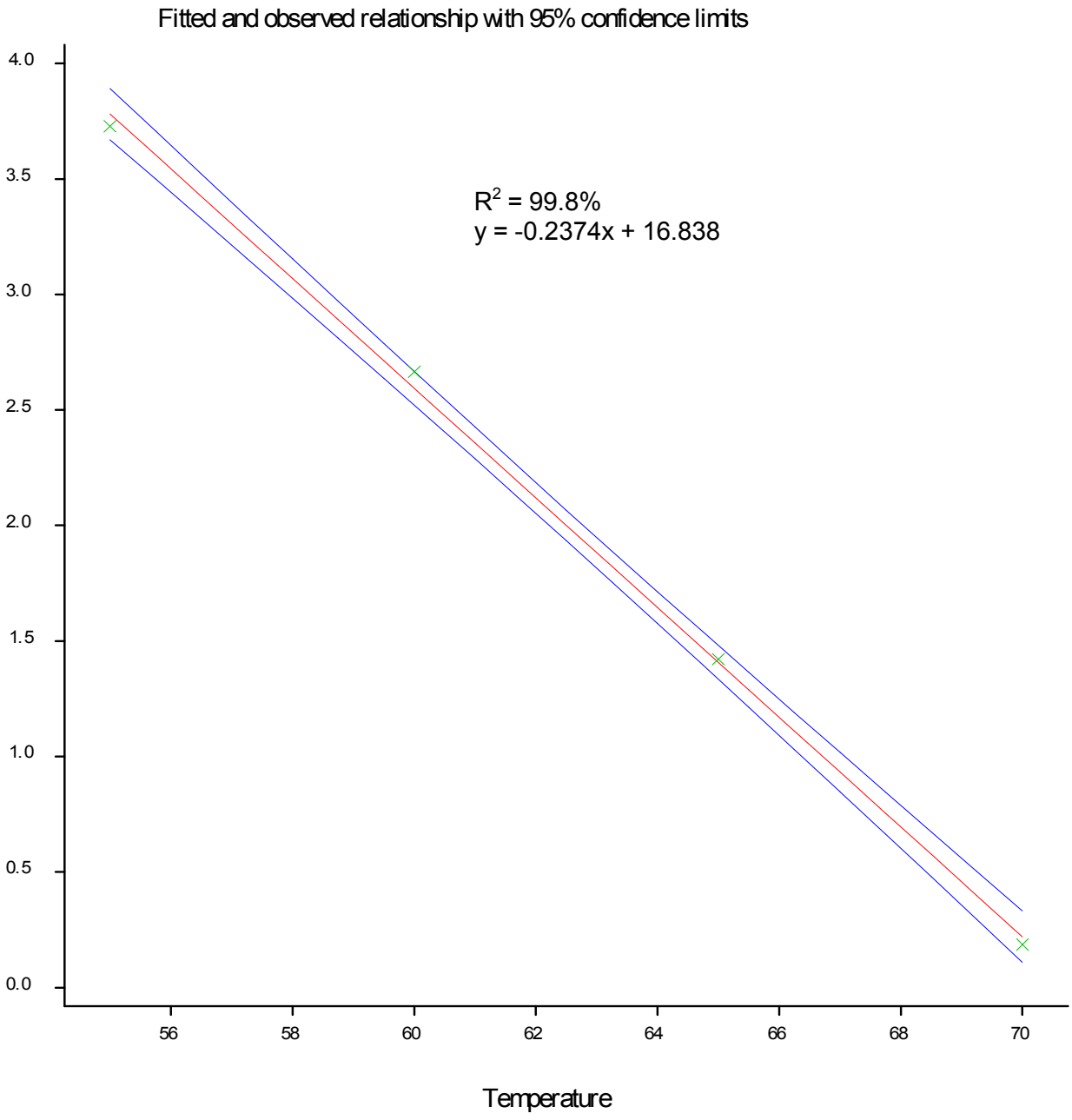
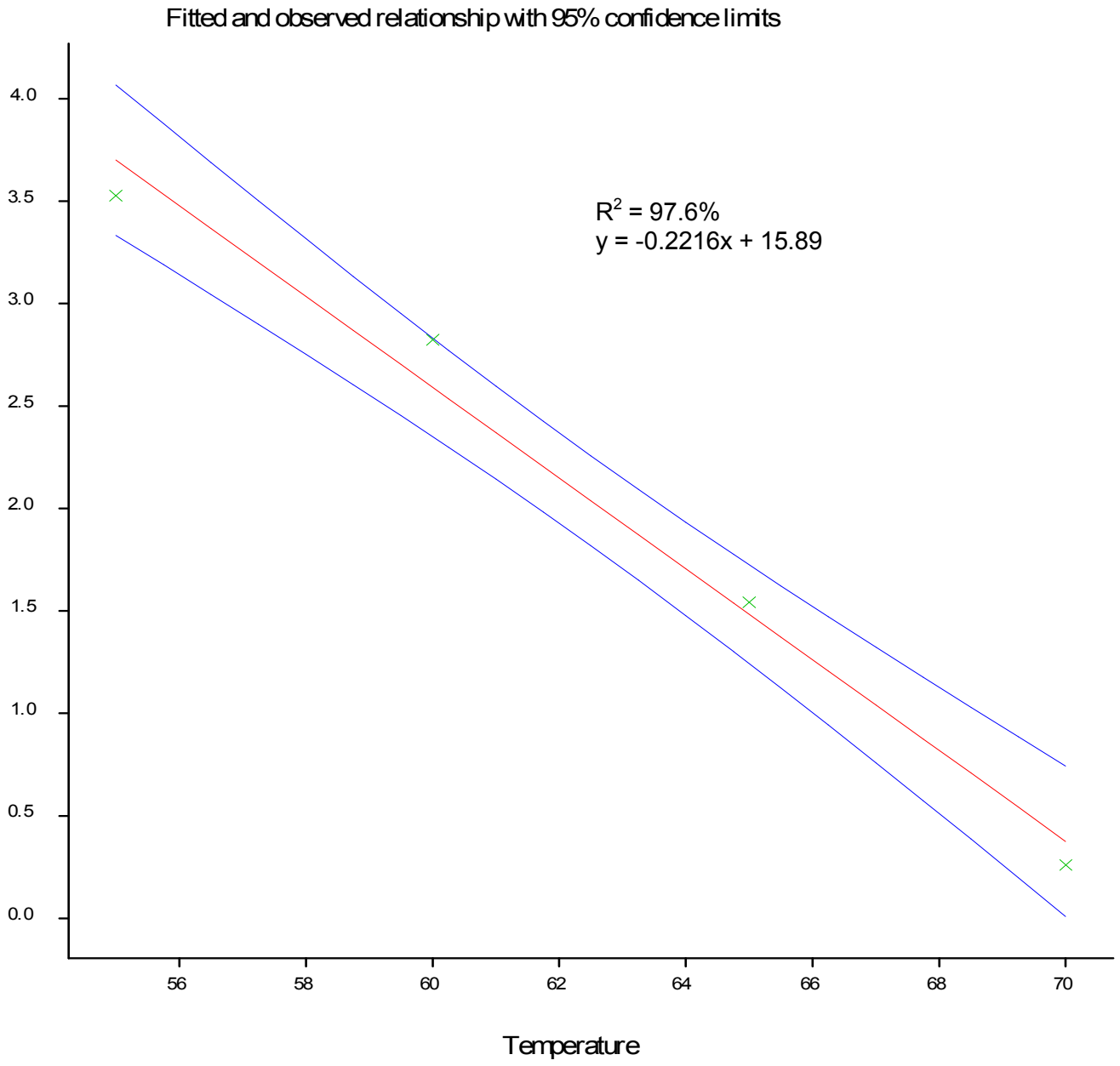


Figure 5. The linear regression of $\log_{10}$ D value (y axis) on temperature (x axis) for the C strain of Mptb



4.4.4 The F_p values achieved during cooking

The results of the calculation of F_p values achieved during each of the cooking trials are shown in Table 6 and may be summarised as follows:

- With the exception of sample 070408/1, for which minimum F_p values recorded by Probe 1 were zero for both strains, and for Probe 2 were 0.6 and 0.7 min for S and C strains respectively, all other legs of lamb had cooking processes which delivered F_p values ≥ 2.4 and 2.7 min, for S and C strains, respectively. This means that with the exception of sample 070408/1, all processes were at least equivalent to 144 s (2.4 min) and 162 s (2.7 min) at 70°C for S and C strains respectively. When the severity of these heat treatments is converted to equivalent time at 71.1 °C (to enable comparison with the USDA standard) the least processes (excluding sample 070408/1) are equivalent to 78.9 and 92.4 s for the S and C strains, respectively. In both instances therefore, the minimum processes are well in excess of the recommended USDA standard which only requires that the product reach 71.1°C rather than be maintained at that temperature for a specified time.
- The data for sample 070408/1 for Probe 2 indicate that the heat treatment was equivalent to 0.6 min (36 s) and 0.7 min (42 s) at 70 °C, or 19.7 and 24.0 s at 71.1 °C for S and C strains respectively. Whilst these treatments exceed the recommended USDA standard they are close to the values of 30 and 35 s at 70 °C recorded for the maximum duration of survival of 4.3×10^6 viable cells of S and C strains, respectively, of Mptb/mL as reported by Gumber (2007); see Appendix Table A2.2.
- The sole example for which the severity of the heat treatment was marginal and less than the USDA standard, and also less than the necessary exposure determined by Gumber (2007), is that calculated for Probe 1 with sample 070408/1. In this instance F_p values of zero were recorded for both strains of Mptb. The maximum temperature recorded by Probe 1 was 59.6°C at which temperature the rate of destruction for the S and C strains, respectively, of Mptb would be < 0.003 and < 0.005 times that achieved at 70 °C.
- Whilst the heat treatment for Probe 1 with sample 070408/1 was marginal, the severity of the heat process received by the other 15 legs of lamb were more than sufficient to eliminate all S and C strains of Mptb.
- The wide range in the final F_p values delivered by the various cooking processes can be attributed to a combination of the following,
  i. Average oven temperatures ranging from 129 to 230 °C
  ii. Cook times ranging from 66 to 134 min
  iii. The logarithmic rate of change in accumulation of F_p value across a temperature range of z C°. For instance, whereas the lowest of the maximum temperature was 59.6 °C (Probe 1 with sample 070408/1) the highest of the maximum core temperatures was 98.3 °C (Probe 1 with sample 080408/3). At these two extremes the lethal rates of destruction (relative to that at 70 °C) range from 0.003 to 5,273,394 and from 0.005 to 1,883,409 for strains S and C, respectively. Notwithstanding that extrapolation over such broad temperature ranges (or multiples of z values) is not usual practice when comparing maximum lethal rates of destruction for “like” samples, the results nevertheless demonstrate the impact that widely different core temperatures can have on final F_p values.

Table 6. Summary of average oven temperatures, cooking times and final F_p values achieved in domestic trials by volunteers

Leg of lamb ID	Weight of leg (kg)	Average[1] oven temp (^oC)	Cook[2] time (min)	Final F_p value[3] for S strain		Final F_p value for C strain	
				Probe 1 (min)	Probe 2 (min)	Probe 1 (min)	Probe 2 (min)
020408/1	1.520	220	120	425.8	1,067.2	424.5	965.2
020408/2	1.530	175	95	31.9	21.5	30.6	20.9
020408/3	1.530	154	120	104.5	144.1	104.6	143.3
030408/1	1.706	186	130	318.8	1,892.2	319.1	1,892.4
030408/2	1.774	169	120	12.1	36.7	12.7	36.6
030408/3	1.742	181	125	700,756.1	10,374,153.5	700,756.2	10,374,153.6
070408/1	1.659	193	66	0.0	0.6	0.0	0.7
080408/1	1.780	183	139	195,042.1	663,984.9	193,876.0	662,671.7
080408/2	1.656	157	141	19.3	34.1	19.3	34.1
080408/3	1.666	230	105	17,691,756.2	25,308.8	17,668,607.9	25,304.7
090408/1	1.718	148	110	132.2	65.3	132.4	65.5
090408/2	1.722	192	80	108.5	9,417.8	107.5	9,407.1
090408/3	1.786	201	81	6.1	2.4	6.5	2.7
100408/1	2.664	NA	98	10.8	134.8	11.2	135.1
100408/2	2.524	149	134	7,446,173.7	475,436.4	7,380,747.9	457,077.8
110408/1	1.878	129	97	193.8	22.2	194.1	22.9

1. The average of the actual temperatures recorded by the thermocouple in the oven after it had reached cooking temperature.
2. The elapsed time that the leg was held in the oven, as indicated by the time-temperature data gathered by the thermocouple recording oven temperature.
3. F_p value is the severity of the heating process expressed as equivalent time in minutes at a reference temperature of 70 ^oC and with z values of 4.21 and 4.51 C^o for type S and type C Mptb strains, respectively.
4. The final F_p value is the accumulated F_p value after the leg of lamb had cooled.

4.4.5 The decimal reductions in Mptb counts achieved during cooking

The results of the calculation of decimal reductions achieved during each of the cooking trials are shown in Table 7 and may be summarised as follows:

- The data show that all treatments other than that for Probe 1 with sample 070408/1 were more than satisfactory to satisfy even the most stringent demands of Good Manufacturing Practice.
- Of those processes that can be considered adequate, the least process (Probe 2 with sample 070408/1) delivered 24.0 and 23.3 log reductions for strain S and C Mptb, respectively.
- To place these findings in a broader context, the least severe nevertheless adequate process (Probe 2 with sample 070408/1) lies between 3.4 and 3.6 times that required by FSIS with respect to elimination of *Salmonella* in ready-to-eat beef products. Furthermore, it delivers more than twice the decimal reductions required for elimination of mesophilic *Clostridium botulinum* in commercial heat processed shelf-stable low-acid canned foods.
- The data indicate that the least severe of all processes (Probe 1 with sample 070408/1) for which the maximum core temperature was 59.6 ^{o}C failed to bring about a single log reduction in the population of the S strain Mptb.
- As a corollary of the discussion in Section 4.4.4 and mirroring the wide range in F_p values delivered by the cooking processes, there was a correspondingly wide range in the number of log reductions achieved for both strains of Mptb.

Table 7. Estimated number of log reductions[1] achieved in baking processes, calculated as Total F_p value/ D_{70} value for S and C strains of Mptb

Leg of lamb ID	No. log reductions for S strain		No. log reductions for C strain	
	Probe 1	Probe 2	Probe 1	Probe 2
020408/1	17,032.0	42,688.0	14,150.0	32,173.3
020408/2	1,276.0	859.1	1,020.0	696.3
020408/3	4,180.0	5,765.0	3,486.7	4,775.4
030408/1	12,752.0	75,688.6	10,636.7	63,080.6
030408/2	484.0	1,467.8	423.3	1,220.6
030408/3	28,030,244.0	414,966,138.7	23,358,540.1	345,805,118.6
070408/1	0.0	24.0	0.0	23.3
080408/1	7,801,685.2	26,559,394.9	6,462,533.4	22,089,055.1
080408/2	771.7	1,362.9	643.3	1,136.7
080408/3	707,670,248.0	1,012,352.4	588,953,596.3	843,488.9
090408/1	5,287.1	2,610.5	4,413.4	2,183.8
090408/2	4,339.6	376,710.2	3,584.0	313,570.5
090408/3	243.2	96.3	217.6	90.3
100408/1	432.7	5,392.1	372.3	4,504.0
100408/2	297,846,949.6	19,017,456.8	246,024,930.0	15,235,927.6
110408/1	7,751.8	887.2	6,470.4	762.0

1. Log reductions of Mptb calculated using experimentally determined (See Section 4.4.2 Table 4) D_{70} values of 1.5 s and 1.8 s for the S and C strains, respectively.

5 Success in Achieving Objectives

The objectives of this project were achieved. The times and temperatures required to ensure inactivation of Mptb by a minimum of 5 logs were investigated and found to be in the range obtained during domestic cooking of lamb (inactivation after 4 to 5 mins at 65 °C; 10 to 15 sec at 70 °C). To determine the degree of inactivation that is likely to be achieved by different cooking methods, D values for the heating of Mptb in lamb homogenate fluid were determined at 55, 60, 65 and 70 °C, which covered the range of core temperatures that was measured during baking. Z values were determined for this temperature range. Data were determined for both common strains of Mptb, S and C.

6 Impact on Meat and Livestock Industry

The impact of these findings for the meat and livestock industry are impossible to predict because the links between Mptb and human health remain unclear, have not received much public attention in popular media and appear not to have led to a change in consumer behaviour.

If the putative links between Crohn's disease and Mptb were to receive public attention in the future, the results of this study can be used to provide consumers with assurance that routine cooking of meat will destroy Mptb if it is present. The data were determined using baking as a model cooking method, but they are generally applicable. Core temperatures during baking are lower than surface temperatures during baking, grilling or frying. Therefore these other cooking processes will destroy Mptb very effectively. In absolute terms, quite high levels of contamination will be destroyed provided that the meat reaches 65 °C for 5 mins, or 70 °C for 15 seconds; this is readily achieved in domestic and commercial cooking without modifying the commonly used techniques. These data should provide consumers with a high degree of confidence that there is very low risk of viable Mptb being consumed with cooked red meat. Cooking must be combined with hygienic food handling practices, particularly not bringing uncooked meat, or utensils or surfaces used to prepare uncooked meat, into contact with cooked meat.

7 Conclusions and Recommendations

7.1 Conclusions

Routine cooking of meat will destroy Mptb organisms if they are present in moderate to high levels of contamination. The data determined using baking as a model cooking method are generally applicable as core temperatures during baking are lower than surface temperatures during baking, grilling or frying. In absolute terms, quite high levels of contamination will be destroyed provided that the meat reaches 65 °C for 5 mins, or 70 °C for 15 seconds; this is readily achieved in domestic and commercial cooking without modifying the commonly used techniques; these may be conservative recommendations.

7.2 Recommendations

1. MLA should consider promotion of cooking practices that ensure adequate decontamination of red meat. Such cooking practices may be commonplace but it is difficult to find recommendations in Australia from an authoritative Australian source.

2. Although the results of this study should provide consumers with a high degree of confidence that there is very low risk of viable Mptb being consumed with cooked red meat, it is important that other aspects of food hygiene are also addressed, particularly not bringing uncooked meat, or utensils or surfaces used to prepare uncooked meat, into contact with cooked meat.

7.3 Acknowledgments

Thanks are due to Mrs Anna Waldron for skilled technical assistance throughout this study, and to the volunteers who participated in the cooking study. Dr. Darian Warne, DWC FoodTech Pty Ltd, Hawthon Victoria provided the analyses in sections 3.5.3, 3.5.4, 4.4.4 and 4.4.5 under separate contract to MLA.

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9 Appendices

9.1 Appendix 1 – USDA FSIS Guidelines

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Compliance Guidelines For Meeting Lethality Performance Standards For Certain Meat And Poultry Products

Introduction

Establishments producing ready-to-eat roast beef, cooked beef and corned beef products and certain ready-to-eat poultry products are required by FSIS to meet the lethality performance standards for the reduction of Salmonella contained in §§ 318.17(a)(1) and 381.150(a)(1) of the meat and poultry inspection regulations. Further, FSIS requires meat and poultry establishments, if they are not operating under a HACCP plan, to demonstrate how their processes meet these lethality performance standards within a written process schedule validated for efficacy by a process authority (§§ 318.17(2)(b)and (c) and 381.150 (2)(c) and (d)).

To assist establishments in meeting the lethality requirements, FSIS is issuing these compliance guidelines, which are based upon the time/temperature requirements contained in previous regulations. Establishments may choose to employ these guidelines as their process schedules. FSIS considers these guidelines, if followed precisely, to be validated process schedules, since they contain processing methods already accepted by the Agency as effective.

Also within these guidelines, FSIS has provided discussion regarding disposition of product following heating deviations and advice for the development of customized procedures for meeting the lethality performance standards.

Guidelines for Cooked Beef, Roast Beef, and Cooked Corned Beef

1. Cooked beef and roast beef, including sectioned and formed roasts, chunked and formed roasts, and cooked corned beef can be prepared using one of the following time and temperature combinations to meet either a 6.5-$\log_{10}$ or 7-$\log_{10}$ reduction of Salmonella. The stated temperature is the minimum that must be achieved and maintained in all parts of each piece of meat for a least the stated time:

Minimum Internal Temperature		Minimum processing time in minutes or seconds after minimum temperature is reached	
Degrees Fahrenheit	Degrees Centigrade	6.5-$\log_{10}$ Lethality	7-$\log_{10}$ Lethality
130	54.4	112 min.	121 min.
131	55.0	89 min.	97 min.
132	55.6	71 min.	77 min.
133	56.1	56 min.	62 min.
134	56.7	45 min.	47 min.
135	57.2	36 min.	37 min.
136	57.8	28 min.	32 min.
137	58.4	23 min.	24 min.
138	58.9	18 min.	19 min.
139	59.5	15 min.	15 min.
140	60.0	12 min.	12 min.
141	60.6	9 min.	10 min.
142	61.1	8 min.	8 min.
143	61.7	6 min.	6 min.
144	62.2	5 min.	5 min.
145	62.8	4 min.*	4 min.*
146	63.3	169 sec.	182 sec.
147	63.9	134 sec.	144 sec.
148	64.4	107 sec.	115 sec.
149	65.0	85 sec.	91 sec.
150	65.6	67 sec.	72 sec.
151	66.1	54 sec.	58 sec.
152	66.7	43 sec.	46 sec.
153	67.2	34 sec.	37 sec.
154	67.8	27 sec.	29 sec.
155	68.3	22 sec.	23 sec.
156	68.9	17 sec.	19 sec.
157	69.4	14 sec.	15 sec.
158	70.0	0 sec.**	0 sec.**
159	70.6	0 sec.**	0 sec.**
160	71.1	0 sec **	0 sec.**

* Past regulations have listed the minimum processing time for roast beef cooked to 145°F as "Instantly." However, due to their large size, most of these roasts dwell at 145°F, or even at higher temperatures, for at least 4 minutes after the minimum internal temperature is reached. FSIS has revised this time/temperature table to reflect this and emphasizes that, to better ensure compliance with the performance standard, establishments should ensure a dwell time of at least 4 minutes if 145°F is the minimum internal temperature employed.

**The required lethalities are achieved instantly when the internal temperature of a cooked meat product reaches 158°F or above.

2. Cooked beef, including sectioned and formed roasts and chunked and formed roasts, and cooked corned beef should be moist cooked throughout the process or, in the case of roast beef or corned beef to be roasted, cooked as in paragraph (3) of this compliance guide. The moist cooking may be accomplished by placing the meat in a sealed, moisture impermeable bag, removing the excess air, and cooking; by completely immersing the meat, unbagged in water throughout the entire cooking process; or by using a sealed oven or steam injection to raise the relative humidity above 90 percent throughout the cooking process.

3. Roast beef or corned beef to be roasted can be cooked by one of the following methods:

- Heating roasts of 10 pounds or more in an oven maintained at 250 °F (121 °C) or higher throughout a process achieving one of the time/temperature combinations in (1) above;
- Heating roasts of any size to a minimum internal temperature of 145 °F (62.8 °C) in an oven maintained at any temperature if the relative humidity of the oven is maintained either by continuously introducing steam for 50 percent of the cooking time or by use of a sealed oven for over 50 percent of the cooking time, or if the relative humidity of the oven is maintained at 90 percent or above for at least 25 percent of the total cooking time, but in no case less than 1 hour; or
- Heating roasts of any size in an oven maintained at any temperature that will satisfy the internal temperature and time combinations of the above chart of this compliance guide if the relative humidity of the oven is maintained at 90 percent or above for at least 25 percent of the total cooking time, but in no case less than 1 hour. The relative humidity may be achieved be use of steam injection or sealed ovens capable of producing and maintaining the required relative humidity.

4. Establishments producing cooked beef, roast beef, or cooked corned beef should have sufficient monitoring equipment, including recording devices, to assure that the time (accuracy assured within 1 minute), the temperature (accuracy assured within 1 °F), and relative humidity (accuracy assured within 5 percent) limits of these processes are being met. Data from the recording devices should be made available to FSIS program employees upon request.

Guidelines for Cooked Poultry Rolls and Other Cooked Poultry Products

1. Cooked poultry rolls and other cooked poultry products should reach an internal temperature of at least 160 °F prior to being removed from the cooking medium, except that cured and smoked poultry rolls and other cured and smoked poultry should reach an internal temperature of at least 155 °F prior to being removed from the cooking medium. Cooked ready-to-eat product to which heat will be applied incidental to a subsequent processing procedure may be removed from the media for such processing provided that it is immediately fully cooked to the 160 °F internal temperature.

2. Establishments producing cooked poultry rolls and other cooked poultry products should have sufficient monitoring equipment, including recording devices, to assure that the temperature (accuracy assured within 1 °F) limits of these processes are being met. Data from the recording devices should be made available to FSIS program employees upon request.

Discussion

Heating Deviations and Slow Come Up Time

Determining the appropriate disposition of products following heating deviations can be even more difficult than determining the disposition of product after a cooling deviation. Heating deviations, which most often involve slow come-up time or an inordinate dwell time within the optimum temperature range for microorganism growth, can foster the multiplication of many pathogens. This multiplication sometimes can be so prodigious that even recooking may be ineffective in rendering the product safe. Also, certain toxigenic bacteria can release toxins into the product. Some of these toxins, such as those of Staphylococcus aureus, are extremely heat stable and are not inactivated by normal recooking temperatures.

Further, the sampling of product following a heating deviation may not yield sufficient information to determine the safety of the product in question. Heating deviations can favor the multiplication of many types of bacteria. It would be difficult and expensive to sample for all of them.

Depending on the circumstances, establishments may want to use computer modeling to estimate the relative multiplication of bacteria. For example, in a past incident involving an extreme heating deviation, product was put in an oven in which the temperature was inadvertently set to 95°F for about 12 hours. Computer modeling was easily applied in this case because much of the dwell time was at one temperature. The Agency determined that within a 6 hour time frame (with other growth conditions assumed to be favorable), the relative multiplication of many pathogens of concern could have exceeded five logs. Clearly the product could not be salvaged by reprocessing and was therefore destroyed.

Under changing conditions of temperature, however, computer modeling becomes more difficult. One approach is to average lag/log times over small increments such as 5° and add these times to get an approximation of possible total relative growth over a larger increment of time. Establishments must keep in mind that the population of bacteria before processing is generally unknown and that assumptions in the high range often are used as input parameters in the modeling.

Establishments should ultimately rely upon the expertise of a processing authority to determine the severity of heating deviations and subsequent appropriate disposition of the product in question. Dwell times of greater than 6 hours in the 50°F to 130°F range should be viewed as especially hazardous, as this temperature range can foster substantial growth of many pathogens of concern. And, a knowledge of the specific product and factors that would favor or inhibit the growth of various bacteria is essential.

Computer Modeling Program Availability

The Microbial Food Safety Research Unit of the Eastern Regional Research Center, USDA Agriculture Research Service, has developed a bacterial pathogen modeling program. Entitled "Pathogen Modeling Program-Version 5.1 for Windows," it is available on the Internet from http://www.arserrc.gov/www/. Other programs may be available commercially.

Customized Processes

Although compliance with these guidelines will yield product that meets the lethality performance standards, some establishments may want to develop customized processing procedures that meet the codified lethality performance standards: 6.5 $\log_{10}$ of Salmonella in ready-to-eat beef products and 7 $\log_{10}$ in ready-to-eat poultry products. Establishments also may want to develop and implement processes using alternative lethalities. Keep in mind, however, that all processes also must achieve, throughout the product, an appropriate reduction of other pathogens of concern and their toxins or toxic metabolites.

Establishments or their process authorities may develop customized procedures or alternative lethalities that meet the performance standards by using information obtained from the literature and/or by comparing their methods with established processes. However, statistical calculations on results obtained from sampling alone are not sufficient to demonstrate that product satisfies reduced initial product conditions or that product meets the performance standards. Rather, the demonstration should be based on scientific rationale, supported by experimental data.

One of the most definitive tools at the disposal of an establishment or processing authority is the challenge study. Although challenge studies must be conducted in the laboratory rather than the establishment, they should be designed and conducted to accurately simulate the commercial process. Challenge studies should be undertaken by individuals who have a thorough knowledge of laboratory methods used in salmonellae research. A cocktail of various serotypes of Salmonella should be used in an inoculated pack study to demonstrate that the lethality performance standard is met. Relatively heat resistant pathogenic strains should be included in the cocktail to develop a worst case. The serotypes/strains selected should be among those that have been historically implicated in an appreciable number of outbreaks.

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9.2 Appendix 2 – published studies of Mptb thermal resistance

Table A2.1. Thermal resistance of *Mycobacterium avium* species (in liquids). Data are from Gumber (2007) (16)

Organism	Strain	Method	Media	Inoculum level/ml	Temp (°C)	Time	100% Inactivation	Log reduction	D value	Reference
Mptb*	OL,914,2276,23182966	PS[1]	Milk	10^{0-5}	68.1-79.1	18s	No	2-6	-	(19)
Mptb	OL,914,2276,23182966	PS	Milk	10^{1-5}	72-75	18-30s	No	3-6	-	(19)
Mptb	OL,914,2276,23182966	PS	Milk	10^{4}	72-90	18-60s	No	4-6	-	(19)
Mptb	Bovine 3737, ATCC19698, Human Linda and Ben	WB[2]	Milk	10^{4}	63	30m	No	<2	-	(9)
Mptb	As above	DB[3]	Milk	10^{4}	72	15s	No	<2	-	(9)
Mptb	ATCC19698, Linda, NCTC8578, B1, B2, B4, M8, M9, USDA1038, USDA1113 and DVL 943	GT[4]	Milk	10^{7}	63.5	30m	No	5-6	-	(14)
Mptb	As above	GT	Milk	10^{4}	63.5	30m	No	2-3.7	-	(14)
Mptb	As above	GT	Milk	10^{4}	71.7	15s	No	2-3.7	-	(14)
Mptb	As above	PU[5]	Milk	10^{7}	71.7	15s	No	4.3-6	-	(14)
Mptb	NCTC8578, B2 and DVL943	PU	Milk	10^{6}	72-90	15s	No	5-6	-	(15)
Mptb	ATCC19698 and Ben	PU	Milk	10^{4-6}	72	15s	Yes	-	-	(38)
Mptb	ATCC19698	PT[6]	Milk	10^{4-6}	72	15s	No	6	-	(38)
Mptb	Ben	PT	Milk	10^{4-6}	72	1m	Yes	-	-	(38)
Mptb	Dominic, Ben and BO45	ST[7]	Milk	10^{6}	62	22.8m	Yes	-	-	(39)
Mptb	As above	ST	Lactate	10^{6}	62	17.91m	Yes	-	-	(39)
Mptb	As above	ST	Lactate	10^{6}	65	265s	Yes	-	-	(39)
Mptb	As above	ST	Milk	10^{6}	65	287s	Yes	-	-	(39)
Mptb	As above	ST	Lactate	10^{6}	68	116s	Yes	-	-	(39)
Mptb	As above	ST	Milk	10^{6}	68	131s	Yes	-	-	(39)
Mptb	As above	ST	Milk	10^{6}	71	70s	Yes	-	-	(39)
Mptb	As above	ST	Lactate	10^{6}	71	73s	Yes	-	-	(39)
Mptb	As above	ST	Lactate	10^{5}	62	14.9m	Yes	-	-	(39)

* Mycobacterium avium subsp. paratuberculosis
1 Pasteurization system
2 Water bath (Holder)
3 Double Boiler (HTST)
4 Stoppered glass test tube in water bath (Holder)
5 Pasteurizing unit in water bath
6 Polystyrene tubes in shaking water bath (Holder)
7 Sealed tube in water bath

Heat inactivation of Mptb in lamb meat

Organism	Strain	Method	Media	Inoculum level/ml	Temp (°C)	Time	100% Inactivation	Log reduction	D value	Reference
Mptb	As above	ST	Milk	10^5	62	19m	Yes	-	-	(39)
Mptb	As above	ST	Lactate	10^5	65	221s	Yes	-	-	(39)
Mptb	As above	ST	Milk	10^5	65	239s	Yes	-	-	(39)
Mptb	As above	ST	Lactate	10^5	68	97s	Yes	-	-	(39)
Mptb	As above	ST	Milk	10^5	68	109s	Yes	-	-	(39)
Mptb	As above	ST	Milk	10^5	71	59s	Yes	-	-	(39)
Mptb	As above	ST	Lactate	10^5	71	62s	Yes	-	-	(39)
Mptb	ATCC19698	ST	Milk	10^6	62	-	No	-	119.9s	(39)
Mptb	ATCC19698	ST	Milk	10^6	65	-	No	-	70.6s	(39)
Mptb	ATCC19698	ST	Milk, Lactate	10^6	68	-	No	-	22.8s-23.8s	(39)
Mptb	ATCC19698	ST	Milk	10^6	71	-	No	-	16.5s	(39)
Mptb	ATCC19698	ST	Lactate	10^6	62	-	No	-	324.6s	(39)
Mptb	ATCC19698	ST	Lactate	10^6	65	-	No	-	55.7s	(39)
Mptb	ATCC19698	ST	Lactate	10^6	71	-	No	-	13.0s	(39)
Mptb	Strain 785991	CT[8]	Milk	10^{6-7}	63	30m	No	>10	-	(22)
Mptb	ATCC 43015 (clumped)	CT	Milk	10^{6-7}	60-63	-	No	-	2.6-11.0m	(22)
Mptb	ATCC 43015(Declumped)	CT	Milk	10^{6-7}	60-63	-	No	-	1.6-11.5m	(22)
Mptb	Bovine isolate 62 (c)	CT	Milk	10^{6-7}	60-63	-	No	-	2.1- 8.6m	(22)
Mptb	Bovine isolate 62 (d)	CT	Milk	10^{6-7}	60-63	-	No	-	1.8-8.2m	(22)
Mptb	Bovine isolate 785991(c)	CT	Milk	10^{6-7}	60	-	No	-	2.9-8.6m	(22)
Mptb	Bovine isolate 785991(d)	CT	Milk	10^{6-7}	60	-	No	-	2.5-14.1m	(22)
M.avium	DSM 43216	GB[9]	Water	10^5	50	-	No	-	16.8h	(35)
M.avium	DSM 43216	GB	Water	10^5	55	-	No	-	53.5m	(35)
M. avium	DSM 43216	GB	Water	10^5	60	-	No	-	240s	(35)
M.avium	DSM 43216	GB	Water	10^5	70	-	No	-	2.3s	(35)

8 Capillary tube in water bath
9 Glass beakers in water bath

Heat inactivation of Mptb in lamb meat

Table A2.2. Impact of constant heating and temperature flux on the viability of Mptb. Data are from Gumber (2007) (16).

Temperature (°C)	Viable cells/mL	Maximum duration of survival at peak temperature [a]	
		S strain	C strain
Constant temperature			
50	4.3×10^{6}	16 h	18 h
60	4.3×10^{6}	28 m	30 m
70	4.3×10^{6}	30 s	35 s
80	4.3×10^{6}	25 s	30 s
Temperature flux*			
10-50	4.3×10^{6}	11 h [c]	11 h [c]
18-50	4.3×10^{6}	11 h 12 m [c]	11 h 12 m [c]
12-60	4.3×10^{6}	9 m [c]	13.5 m [c]
20-60	$4.3\times10^{5-7}$	nd	nd

*regular repeated cycles within the range specified: 4 mins at minimum, 8 min rising; 4 mins at peak; 8 mins descending, repeated many times.

[a] h = hour; m = minute; s = seconds

[c] Total duration for which the organism was exposed to peak temperature

nd = not detected

9.3 Appendix 3 – lamb roast cooking data

Table A3.1. Cooking methods

Leg of Lamb ID	Cultural background/ nationality	Baking container used	Oven Make & Model	Degree of doneness preferred by volunteer	Recipe Details	Record times of basting & how basting done	Record times when leg is turned & how leg is turned	Standing time prior to carving (minutes)	Problems that occurred
020408/1	UK + Australian	Pyrex dish, placed directly into dish, no rack	St George (Very old, see photo)	Medium - Well Done	Baste in garlic, crushed from jar, cover in foil	7.44pm baste in juices with brush, return to oven without foil cover.	Not turned	10 mins	None .
020408/2	Australian	Metal tray with Glad Baking Paper	Simpson Celebrity, Fan Forced	Medium	Rubbed with extra virgin olive oil, crushed garlic and fresh rosemary, sprinkled with ground sea salt	7.00pm to add vegetables, closed at 7.02pm. No basting.	No turning.	8 mins - 8.10pm - 8.18pm	
020408/3	British	Metal rack on metal baking dish	Chef Select Fan Forced	Well done	Cover with minced garlic, pour oil over top	Not done	Not done	10 mins	Make sure 3rd probe not touching baking container
030408/1	Australian	Deep oven tray	Chef Gas Fan Forced	Medium	Coat with olive oil & rub salt into skin	6.15pm take out and add vegetables. 6.20pm put back in oven.	7.18pm take out & turn vegetables, 7.23pm put back in, 7.38 take out & cover with foil	12 mins	Too late putting vegetables in & over-cooked lamb
030408/2	Australian	10cm deep enamel baking dish - no lid	Westinghouse 650 (Gas)	Medium - Rare	Slits made in lamb with knife, 1 clove of garlic sliced into 10 slices + pushed into 10 slits, sprinkled with coarse black pepper, rock salt & oregano. 1/2 cup of vegetable oil poured over top.	No basting	5.30pm, turned back at 6.10pm	Covered with foil while resting on a plate for 20 mins	
030408/3	Australian	Baking metal tray/Glad Oven Bag	Whirlpool Model VR86ADP	Well done	Nothing added	No basting	No turning	10 mins	None - was lovely!
070408/1	Australian + German	Stainless steel 18/10	BBQ Galore with Hood + Thermometer (Gas)	Rare	Multiple stab wounds with 4 cloves garlis & 10 grams fresh picked rosemary, salted, basted red wine and olive oil	6.10pm onions in oil & red wine added	Not turned	10 mins	
080408/1	Australian	Vitrious enamel baking dish	Miele H248 BP-KAT	Medium - Well Done	Just baked it!	n/a	n/a	25 mins	5.20pm Oven temp dropped due to door not being closed properly
080408/2	NZ + Australian	Roasting dish with lid	Simpson Nova 502 - not fan force	Medium - Well Done	Basted with garlic infused olive oil, lighlty sprinkled with rock salt & added a sprig of rosemary, in a covered dish	6.25pm open oven, took lid off. 6.32pm opened oven, added vegetables. 7.05pm increased oven temp to 210°C. 7.20pm opened oven, removed vegetables, turned & put back in oven. 7.34pm tested meat with knife, blood still ran out. Temp to 180°C.	Not turned	10 mins	
080408/3	Australian	Romertof Clay Cooking Pot	DeLonghi D926 GWF Dual XX??	Rare-Medium	Rub with olive oil, insert slivers of garlic in slits, sprinkle with basil & oregano	7.00pm Open door to check & bast with juices, add baked vegetables. 7.19pm check vegetables, 7.27-7.29 pm turn vegetables, 7.41pm, vegetables out	Not turned	2 mins	None - was lovely!
090408/1	Italian	Bessemer non-stick roasting pan	Chef Coronet Integra	Medium	Placed slivers of garlic into meat, rubbed roast all over with salt, papper & Italian herb mix. Drizzled with olive oil & placed in pre-heated oven tray.	4.27pm Baste & add potatoes, reduce to 180°C for 1-1.5 hrs. 4.57pm tray tilted to collect juices & then spoon over leg at 5.40pm	4.27pm leg turned from 1 side to other (sensor on top & then on the bottom). 4.57pm same as before, sensors now on top	25 mins	
090408/2	Canadian/Dutch	Metal roasting pan	Chef Select Plus (no fan)	Medium		6.00pm + vegetables/foil	6.45pm rest in foil	15 mins	No
090408/3	Australian	Metal Tray	Westinghouse Freestyle 678	Medium - Well Done			6.36pm Checked & opened door	10 mins	
100408/1	Australian	Cast iron baking dish with aluminium lid	Chef Classic Extra - Electric Fan Force	Medium	Leg on flat rack in dish, half cup of water. Rack removed when turned,sprinkled with lemon pepper.	Not basted	7.12pm, turned over	2 mins	Probe 3 was resting on lid prior to turning
100408/2	American + NZ	Enamel	Westinghouse	Medium	Coat in oil, sprinkle with herbs, cover	Add potatoes 7.45pm	Add carrots & onion 8.26pm	20 mins	
110408/1	American	Roasting rack	Chef 600 Gas	Med to Med-Well	Stab incisions (10) filled with garlic+rosemary	Not basted	Not turned	30 mins	

Table A.3.2. Dimensions of lamb roasts and placement of temperature probes

Leg of Lamb ID	Length (mm)	Circumference (mm)															Weight (kg)	Datalogger ID	Probe 1	Probe 2	Probe 3	Photographs taken with colour chart a places in the roast as carved
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15						
020408/1	230	404	410	413	390	360	295	270	260	230	180	145	135				1.52	71202783	Meat centre 1	Meat centre 2	Oven	Yes
020408/2	235	411	410	401	375	360	315	310	288	242	200	175					1.53	61201269	Meat centre 1	Meat centre 2 (Bone)	Oven	Yes
020408/3	225	390	387	385	375	360	345	322	305	260	230	210					1.53	71202782	Meat centre 1	Meat centre 2	Oven	Photos but no colour chart
030408/1	235	420	418	409	385	335	290	265	265	230	220	220					1.706	71202782	Meat centre 1	Meat centre 2	Oven	
030408/2	235	400	409	397	378	342						325	277	230			1.774	71202783	Meat centre 1	Meat centre 2	Oven	Yes
030408/3	240	393	400	395	395	385	355	320	305	270	242	210					1.742	61201269	Meat centre 1	Meat centre 2	Oven	
070408/1	230	392	397	395	390	372	341	305	230	195	172	145	140				1.659	71202782	Meat centre 1	Meat centre 2	Oven	Yes
080408/1	245	383	392	396	383	377	345	286	260	228	208	197	155	140			1.78	61201269	Meat centre 1	Meat centre 2	Oven	Yes - Took photos progressively
080408/2	255	385	395	400	370	350	278	240	190	167	150	146	132				1.656	71202783	Meat centre 1	Meat centre 2	Oven	Yes
080408/3	230	385	393	390	372	332	285	238	212	185	175	155					1.666	71202782	Meat centre 1	Meat centre 2	Oven	Yes
090408/1	240	380	384	389	385	373	342	311	265	244		342	207	173	153		1.718	71202783	Meat centre 1	Meat centre 2	Oven	Yes
090408/2	230	395	402	394	370	335	295	266	247	210	187	157	135				1.722	71202782	Meat centre 1	Meat centre 2	Oven	Yes
090408/3	230	400	405	395	367	330	290	254	223	201	185	165	155				1.786	61201269	Meat centre 1	Meat centre 2	Oven	Yes
100408/1	305	404	406	418	425	404	411	366	322	276	260	250	227	200	181	162	2.664	61201269	Meat centre 1	Meat centre 2	Oven	Yes
100408/2	285	390	400	405	407	405	406	395	385	349	320	275	213	190	170		2.524	71202782	Meat centre 1	Meat centre 2	Oven	Yes
110408/1	255	403	410	410	385	354	335	330	283	260	239	225	202	175	163		1.878	71202783	Meat centre 1	Meat centre 2	Oven	1 picture

Heat inactivation of Mptb in lamb meat

Table A3.3. Temperatures recorded during cooking. Data in last six columns are the number of intervals of 5 mins (roasts 1 to 6) or 1 minute (roasts 7 to 16) where the temperature shown was exceeded, with grouping accuracy to 0.5°C (for example temperatures > 70°C are in fact >69.5°C).

Leg of Lamb ID	Cooking time from placing in oven to removal from oven		Standing time prior to carving (minutes)	Degree of doneness preferred by volunteer	Degree of Doneness after cooking	Oven set temperature, not temp on data logger	Max temp	Max temp	Max temp	No. int >60	No. int >60	No. int >65	No. int >65	No. int >70	No. int >70
	Time in	Time out					Oven	Probe 1	Probe 2	Probe 1	Probe 2	Probe 1	Probe 2	Probe 1	Probe 2
020408/1	6.11pm	8.10pm	10 mins	Medium - Well Done	Well done all through	180°C at 6.11pm, opened oven at 7.55pm, oven off at 8.00pm	245	76.3	79.1	12	8	11	6	9	4
020408/2	6.30pm	8.06pm	8 mins - 8.10pm - 8.18pm	Medium	Medium	180°C	181	73.1	72.4	5	5	4	3	2	1
020408/3	5.10pm	7.15pm	10 mins	Well done	Medium	170°C at 5.10pm, 150°C at 5.40pm. Opened door at 6.31 to check oven off at 7.05pm, lamb out 7.15pm	174	73.8	74.2	12	11	10	9	6	6
030408/1	5.33pm	7.38pm	12 mins Covered with foil while resting on a plate for 20 mins	Medium	Well done	200°C	210	76.3	80.2	12	12	10	10	7	8
030408/2	4.30pm	6.30pm		Medium - Rare	Medium	170°C at 4.30pm, 150°C at 4.45pm, 160°C at 5.00pm, 175°C at 6.10pm	205	69.5	70.7	9	11	5	9	3	5
030408/3	6.11pm	8.11pm	10 mins	Well done	Well done	200°C	188.6	91.2	96.7	14	16	13	15	12	14
070408/1	5.40pm	6.40pm	10 mins	Rare	Medium Rare to Rare in centre	300°C plus (maxiumum)	210.2	59.6	65.9	3	23	0	20	0	0
080408/1	3.30pm	5.45pm	25 mins	Medium - Well Done	Well done	180°C (no changes) See Problems	199.2	88.2	90.8	86	90	78	82	68	73
080408/2	5.24pm	7.46pm. 7.56pm Probes removed	10 mins	Medium - Well Done	Medium well - small tinge of blood still there	170°C	174.4	71	71.5	38	45	26	34	12	22
080408/3	5.57pm	7.42pm	2 mins	Rare-Medium	More medium than medium rare	250°C	252	98.3	85.5	71	66	69	64	67	60
090408/1	4.05pm	5.50pm	25 mins	Medium	Medium	220°C initial, 190°C after 35 mins @ 4.40pm	203	74.1	72.5	73	46	47	36	35	24
090408/2	5.30pm	7.00pm	15 mins	Medium	Medium	220°C prewarmed, 190°C at 5.45pm	240.9	74.8	83.8	26	37	20	34	14	31
090408/3	5.30pm	6.48pm	10 mins	Medium - Well Done		180°C	221.5	69.2	67.6	29	22	20	13	3	0
100408/1	5.52pm	8.30pm	2 mins	Medium	Medium - Well Done	240°C (pre heated 20 mins)	70.4	71.5	75.8	33	44	20	33	7	20
100408/2	7.15pm	9.10pm	20 mins	Medium	MediumWell	190°C	170.3	93.9	90.4	91	81	83	73	75	65
110408/1	5.55pm	7.55pm	30 mins	Med to	Top-Well, Bottom-	180°C Constant	131.4	74.4	71.3	61	59	50	33	38	6

Med-Well Medium/Rare

Heat inactivation of Mptb in lamb meat

9.4 Appendix 4 – experimental data

Table A4.1. Experimental data – growth of Mptb following exposure to heat for various times. The first column shows temperature and time; a,b,c are replicates. Data are growth indices.

Name of isolate: Telford 2636 Suspension ID: T08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
55-0a	217	210	228	316	721	496	506	765	722	908	742		999		999	825	786										
55-0b	240	244	273	318	638	481	475	717	645	851	692		892		999	826	736										
55-0c	224	238	257	286	587	423	474	739	719	926	760		853		999	836	722										
55-15a	143	119	131	170	533	412	552	822	885	999	999		999		999	907	767										
55-15b	92	81	82	112	388	328	398	635	677	913	868		771		952	794	739										
55-15c	120	102	109	142	423	320	366	595	616	838	843		826		999	934	893										
55-30a	118	87	78	112	443	346	410	680	703	904	824		882		999	984	930										
55-30b	123	90	85	126	420	329	365	616	636	887	827		997		999	999	999										
55-30c	129	97	87	134	468	367	402	697	686	953	881		999		999	999	999										
55-45a	107	77	68	95	333	279	296	499	476	690	648		960		999	999	890										
55-45b	98	75	66	94	330	137	292	532	499	720	607		984		999	925	794										
55-45c	101	77	74	93	326	265	309	547	517	795	672		977		999	903	824										
55-60a	94	60	53	60	286	247	337	642	655	999	893		999		999	942	797										

Heat inactivation of Mptb in lamb meat

55-60b	**106**	**68**	**58**	**68**	**326**	**285**	**421**	**686**	**791**	**999**	**999**		**999**		**999**	**969**	**834**									
55-60c	**106**	**69**	**55**	**66**	**312**	**258**	**339**	**634**	**710**	**999**	**999**		**940**		**999**	**986**	**906**									
55-75a	**82**	**50**	**36**	**41**	**197**	**178**	**228**	**419**	**455**	**715**	**761**		**744**		**999**	**989**	**935**									
55-75b	**73**	**42**	**33**	**37**	**173**	**158**	**197**	**409**	**413**	**670**	**716**		**777**		**999**	**999**	**999**									
55-75c	**80**	**47**	**35**	**41**	**203**	**185**	**218**	**399**	**410**	**614**	**615**		**796**		**999**	**999**	**999**									
55-90a	**66**	**35**	**21**	**25**	**115**	**106**	**163**	**384**	**365**	**628**	**629**		**992**		**999**	**999**	**999**									
55-90b	**68**	**40**	**27**	**30**	**139**	**130**	**184**	**416**	**385**	**670**	**655**		**999**		**999**	**999**	**999**									
55-90c	**70**	**40**	**27**	**29**	**132**	**126**	**182**	**405**	**380**	**672**	**697**		**999**		**999**	**999**	**984**									

Heat inactivation of Mptb in lamb meat

Name of isolate: Telford 2636 Suspension ID: T08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
60-0a	214	224	255	280	562	395	503	762	705	857	751	868	898		999	791	680	999									
60-0b	180	181	206	253	528	387	547	779	786	931	817	720	776		942	747	644	999									
60-0c	220	222	232	266	586	415	551	833	889	999	999	956	923		999	851	743	999									
60-5a	84	54	44	56	272	218	279	463	489	825	833	802	820		999	999	976	999									
60-5b	83	56	48	61	276	221	282	466	488	772	761	789	810		999	999	999	999									
60-5c	85	61	54	69	306	248	282	470	461	715	692	900	915		999	999	990	999									
60-10a	50	27	17	13	55	54	89	297	307	646	686	850	999		999	999	999	999									
60-10b	47	26	16	15	61	58	96	296	288	543	548	884	886		999	999	999	999									
60-10c	46	25	14	14	60	59	96	303	297	532	557	888	916		999	971	857	999									
60-15a	25	12	6	3		9		34	42	209	292	323	327		901	886	846	999									
60-15b	28	13	6	4		8		43	51	242	372	373	385		653	865	838	999									
60-15c	27	13	6	0		9		52	61	245	360	320	320		853	844	827	999									
60-20a	21	11	4	0		3		5	8	50	113	130	171	240	420	828	966	999									
60-20b	19	10	3	1		2		7	9	50	119	134	174	225	486	845	999	999									
60-	22	10	4	1		3		7	13	60	137	169	222	266	594	999	999	999									

Heat inactivation of Mptb in lamb meat

20c																									
60-25a	**20**	**10**	**4**	**1**		**1**		**1**	**1**		**34**	**35**	**55**	**150**	**342**	**742**	**999**	**999**							
60-25b	**19**	**9**	**4**	**2**		**4**		**2**	**2**		**47**	**51**	**75**	**167**	**375**	**822**	**999**	**999**							
60-25c	**18**	**7**	**4**	**2**		**1**		**3**	**3**		**49**	**58**	**81**	**124**	**320**	**632**	**844**	**999**							
60-30a	**14**	**7**	**1**	**2**		**0**		**0**	**1**		**8**	**9**	**14**	**66**	**124**	**396**	**620**	**794**	**999**						
60-30b	**18**	**7**	**2**	**1**		**1**		**0**	**0**		**13**	**14**	**20**	**72**	**182**	**479**	**746**	**879**	**999**						
60-30c	**17**	**8**	**3**	**2**		**2**		**0**	**0**		**15**	**16**	**23**	**74**	**186**	**472**	**735**	**863**	**999**						
60-35a	**10**	**3**	**1**	**0**		**1**		**0**	**1**		**4**	**4**	**7**	**10**	**107**	**323**	**643**	**905**	**999**						
60-35b	**14**	**4**	**5**	**2**		**1**		**0**	**0**		**5**	**2**	**5**	**8**	**107**	**268**	**512**	**706**	**999**						
60-35c	**13**	**5**	**3**	**1**		**1**		**0**	**1**		**6**	**1**	**9**	**10**	**117**	**298**	**559**	**778**	**999**						

Heat inactivation of Mptb in lamb meat

Name of isolate: Telford 2636 Suspension ID: T08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
65-0a	210	192	223	280	662	515	550	760	755	999	999		999	999	999	999	845										
65-0b	214	214	245	303	698	558	537	774	733	999	999		999	999	999	976	791										
65-0c	209	212	247	307	735	558	495	774	741	999	999		999	999	999	946	740										
65-1a	33	14	7	7	43	55	82	273	290	537	577		932	999	999	999	953										
65-1b	43	24	17	18	89	91	148	367	365	637	659		953	999	999	999	999										
65-1c	38	24	16	16	81	83	144	349	368	520	587		808	999	999	999	999										
65-2a	11	7	1	1		0		0	0	12	7		0	10	144	342	598	849									
65-2b	10	6	2	0		2		2	0	5	7		10	32	118	446	831	999									
65-2c	11	5	2	0		2		3	1	7	18		41	102	251	623	961	999									
65-3a	7	4	0	0		1		0	1		2		0		1	3	5	2	39	237	376	483	863	999	999	999	999
65-3b	8	3	1	0		1		1	0		3		1		1	5	6	14	17	30	29	18	224	553	745	999	999
65-3c	8	4	1	0		0		0	0		3		3		2	7	19	87	148	503	503	528	786	999	999	999	999
65-4a	7	4	2	0		0		0	0		1		1		4	3	0	0	2	0	8	0	0	0	0	0	0
65-4b	7	3	0	0		0		2	0		4		0		0	3	0	5	5	10	12	12	124	369	568	999	999
65-	8	4	1	1		1		2	0		3		2		0	4	0	1	0	0	0	0	0	0	0	0	0

Heat inactivation of Mptb in lamb meat

4c																									
65-5a	**7**	**3**	**2**	**2**		**0**		**0**	**0**		**4**		**1**		**1**	**2**	**1**	**1**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**1**
65-5b	**6**	**3**	**0**	**0**		**0**		**0**	**1**		**0**		**1**		**1**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
65-5c	**7**	**2**	**1**	**0**		**0**		**1**	**0**		**4**		**1**		**0**	**2**	**0**	**0**	**2**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**
65-6a	**4**	**2**	**0**	**0**		**0**		**0**	**0**		**0**		**1**		**0**	**1**	**0**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
65-6b	**5**	**2**	**0**	**0**		**1**		**1**	**0**		**3**		**0**		**0**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
65-6c	**8**	**4**	**0**	**0**		**0**		**1**	**0**		**2**		**0**		**2**	**3**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
65-7a	**5**	**2**	**0**	**0**		**0**		**1**	**0**		**3**		**0**		**0**	**3**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
65-7b	**6**	**0**	**1**	**0**		**0**		**0**	**0**		**0**		**1**		**0**	**0**	**0**	**3**	**0**	**0**	**149**	**236**	**255**	**223**	**190**	**163**	**110**
65-7c	**5**	**1**	**0**	**0**		**0**		**1**	**1**		**1**		**1**		**0**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

The underlined growth indices were attributed to growth of irrelevant microorganisms based on negative results in IS*900* PCR

Heat inactivation of Mptb in lamb meat

Name of isolate: Telford 2636 Suspension ID: T08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
70-0a	182	191	225	273	618	498	574	777	686	959	818	999	999		887	841	816	999									
70-0b	247	263	292	338	668	523	592	795	693	999	849	999	999		927	860	815	999									
70-0c	186	197	226	282	660	527	605	819	726	999	869	999	999		932	890	826	999									
70-5a	8	6	0	0		2		0	0	75	151	185	550		761	999	999	999									
70-5b	9	5	2	1		2		9	11	62	151	189	382		584	781	944	999									
70-5c	12	5	3	1		0		11	13	69	157	169	431		644	836	950	999									
70-10a	6	1	1	0		0		1	0		0		0		5	7	15	63	115	525	545	580	824	999	999	999	999
70-10b	9	3	1	0		0		0	1		3		0		4	13	36	138	240	635	620	657	725	999	999	999	999
70-10c	4	2	0	0		0		0	0		1		1		3	4	8	18	32	121	237	423	697	999	999	999	999
70-15a	3	1	0	1		0		1	1		4		0		2	0	1	0	1	3	2	0	0	0	0	0	0
70-15b	6	2	0	0		0		0	0		2		0		0	1	1	2	0	0	1	0	0	0	0	1	1
70-15c	5	3	1	0		0		1	0		2		0		1	1	0	1	1	1	0	0	0	0	0	0	0
70-20a	4	1	0	0		0		0	1		0		0		0	0	0	0	0	1	0	0	1	0	0	1	1
70-20b	0	0	0	0		0		1	1		0		2		0	0	0	1	0	0	2	0	0	0	0	1	0
70-	5	2	0	1		0		1	1		0		1		0	1	0	1	0	1	2	0	0	1	0	0	0

Heat inactivation of Mptb in lamb meat

20c																									
70-25a	6	2	1	0		0		1	1		0		1		0	0	0	0	0	1	0	1	0	0	0	0	2
70-25b	6	0	0	0		0		1	1		1		1		0	0	0	1	0	0	0	0	0	0	0	0	2
70-25c	6	0	1	0		0		1	1		0		2		0	0	0	1	1	1	1	0	1	0	0	0	2
70-30a	6	1	1	0		0		0	0		0		1		0	1	0	0	1	1	2	0	0	0	0	0	0
70-30b	7	1	1	0		0		2	0		0		1		1	0	2	1	1	2	2	0	0	0	0	0	1
70-30c	8	1	2	0		0		0	2		0		1		0	0	0	1	4	0	1	1	0	0	0	0	1
70-35a	4	2	2	0		0		0	0		0		0		2	2	1	2	0	0	2	1	0	0	0	0	2
70-35b	4	1	0	0		0		2	2		6		2		2	3	1	2	1	2	0	0	0	0	0	0	0
70-35c	2	0	0	1		0		3	0		2		3		1	4	1	3	0	2	0	0	0	0	0	0	1

Heat inactivation of Mptb in lamb meat

Name of isolate: Telford 2636 Suspension ID: T08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
75-0a	199	191	216	254	544	415	449	721	588	743	699	999	999		787	819	822										
75-0b	233	222	237	270	614	476	474	815	646	841	750	999	999		787	762	740										
75-0c	228	206	231	285	631	527	485	833	654	901	811	999	999		835	753	698										
75-5a	5	2	0	0		1		0	0		5		0		6	5	1	1	0	1	1	0	0	0	0	0	0
75-5b	6	2	2	0		0		1	0		5		0		2	0	1	0	0	1	3	0	0	0	0	0	0
75-5c	7	2	2	0		1		0	0		2		1		4	2	2	0	2	3	1	0	0	0	0	1	2
75-10a	5	2	1	1		1		2	1		2		1		4	1	1	0	1	1	2	0	1	0	0	0	3
75-10b	6	1	3	0		0		2	0		3		2		3	0	0	1	2	4	3	1	0	0	0	0	2
75-10c	7	2	1	0		0		1	1		2		1		0	2	0	2	1	1	2	1	0	0	0	0	2
75-15a	2	0	0	0		0		1	0		3		1		3	1	0	3	2	3	0	0	0	1	0	0	3
75-15b	6	3	1	1		0		0	0		3		1		2	0	0	3	1	2	1	0	0	0	1	1	1
75-15c	7	2	3	1		0		1	0		3		2		4	0	0	2	2	2	0	0	1	0	0	1	2
75-20a	6	2	3	2		0		0	0		3		0		4	0	1	1	1	0	4	0	0	0	0	0	1
75-20b	5	2	2	1		0		1	0		2		1		3	0	0	1	1	1	0	0	0	0	0	0	1
75-	7	4	1	1		1		1	0		5		1		3	0	0	1	2	4	0	1	0	0	0	0	2

Heat inactivation of Mptb in lamb meat

20c																									
75-25a	0	2	1	3		0		1	0		4		0		4	1	2	2	2	2	2	1	0	0	0	0	2
75-25b	5	2	1	0		0		0	0		4		2		3	4	1	121	276	591	540	582	725	999	999	999	951
75-25c	3	2	1	0		1		1	1		3		1		4	2	0	1	1	3	16	0	1	0	0	0	0
75-30a	6	2	1	0		1		1	0		4		1		1	1	1	2	1	2	0	0	0	0	0	0	0
75-30b	3	0	0	1		0		1	0		0		2		0	1	0	1	1	1	1	0	0	0	0	0	1
75-30c	6	2	1	0		1		0	0		3		2		0	1	0	1	0	1	2	1	1	0	0	1	2
75-35a	6	1	1	2		0		2	0		1		1		2	0	0	2	1	2	0	0	0	0	0	0	2
75-35b	6	0	0	2		2		1	0		2		1		1	0	0	4	2	0	0	1	0	0	0	0	3
75-35c	6	0	2	2		2		1	1		3		0		2	0	0	2	0	0	0	0	0	0	0	0	1

Heat inactivation of Mptb in lamb meat

Name of isolate: Cattle CM00/416 Suspension ID: C08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
55-0a	354	319	342	409	861	596	681	923	786	999	919		979		999	825	418										
55-0b	239	218	244	283	589	444	507	623	573	768	677		774		999	804	461										
55-0c	339	287	309	362	760	555	657	872	732	999	855		950		999	835	457										
55-15a	267	240	271	330	721	529	655	889	798	999	967		999		999	919	487										
55-15b	248	227	258	327	730	501	622	799	680	948	782		883		999	866	498										
55-15c	263	236	250	307	674	495	615	831	734	999	893		982		999	912	509										
55-30a	116	106	124	173	529	391	504	755	690	999	929		999		999	981	522										
55-30b	123	115	136	194	550	409	531	781	729	999	920		999		999	973	516										
55-30c	97	97	106	156	384	289	375	536	501	714	650		761		999	847	486										
55-45a	81	76	85	130	469	351	473	761	688	999	958		999		999	999	551										
55-45b	69	66	75	112	389	309	372	534	495	748	712		813		999	861	471										
55-45c	68	66	72	108	387	301	374	555	509	812	725		856		999	905	502										
55-60a	51	46	52	78	345	271	414	696	638	988	857		995		999	999	545										
55-60b	43	33	43	62	290	224	310	507	472	840	806		928		999	999	563										
55-	44	35	38	59	269	213	307	521	465	832	796		916		999	999	561										

Heat inactivation of Mptb in lamb meat

60c																										
55-75a	**34**	**27**	**28**	**46**	**212**	**171**	**297**	**588**	**531**	**980**	**921**		**999**		**999**	**999**	**572**										
55-75b	**33**	**29**	**30**	**50**	**242**	**196**	**308**	**569**	**532**	**954**	**895**		**999**		**999**	**999**	**571**										
55-75c	**28**	**23**	**24**	**33**	**155**	**127**	**186**	**363**	**346**	**621**	**630**		**799**		**999**	**955**	**550**										
55-90a	**22**	**17**	**17**	**23**	**109**	**86**	**144**	**305**	**273**	**527**	**564**		**709**		**999**	**855**	**493**										
55-90b	**24**	**18**	**19**	**28**	**125**	**107**	**154**	**344**	**317**	**592**	**630**		**861**		**999**	**999**	**611**										
55-90c	**25**	**17**	**19**	**31**	**134**	**115**	**206**	**423**	**407**	**751**	**773**		**966**		**999**	**999**	**611**										

Heat inactivation of Mptb in lamb meat

Name of isolate: Cattle CM00/416 Suspension ID: C08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
60-0a	395	334	320	378	786	577	702	914	714	999	846	953	883		999	790	426										
60-0b	430	359	331	398	769	545	669	859	685	945	786	852	871		999	825	466										
60-0c	418	352	350	421	919	625	762	986	745	983	776	799	820		999	767	416										
60-5a	37	31	30	45	193	148	207	357	335	614	622	743	759		999	927	529										
60-5b	39	30	27	45	209	163	243	425	366	680	638	725	765		999	862	492										
60-5c	33	26	26	36	175	135	218	396	338	629	616	756	804		999	872	496										
60-10a	16	11	12	19	93	77	146	411	383	798	825	899	999		999	999	633										
60-10b	18	14	8	17	79	67	119	316	304	609	667	801	891		999	999	655										
60-10c	21	11	8	10	71	60	113	303	311	658	721	899	951		999	999	671										
60-15a	13	7	7	5		27		109	110	328	425	383	335		963	877	536										
60-15b	9	5	4	4		21		113	110	401	504	436	401		999	999	647										
60-15c	10	4	3	6		17		83	81	314	381	342	337		931	854	527										
60-20a	9	6	3	5		11		49	47	225	332	311	315		933	917	539										
60-20b	12	6	5	4		13		48	44	228	325	298	291		858	826	516										
60-	10	5	5	4		10		51	48	252	360	359	353		999	999	604										

Heat inactivation of Mptb in lamb meat

20c																									
60-25a	**8**	**6**	**6**	**2**		**7**		**30**	**28**	**147**	**267**	**276**	**297**	**344**	**570**	**919**	**575**									
60-25b	**9**	**7**	**2**	**3**		**10**		**30**	**27**	**157**	**271**	**286**	**309**	**450**	**515**	**987**	**632**									
60-25c	**11**	**4**	**3**	**6**		**10**		**31**	**30**	**158**	**296**	**339**	**368**	**470**	**529**	**999**	**773**									
60-30a	**12**	**5**	**3**	**7**		**5**		**19**	**20**	**102**	**221**	**256**	**305**	**459**	**540**	**999**	**738**	**999**								
60-30b	**12**	**6**	**6**	**2**		**6**		**12**	**13**	**65**	**124**	**145**	**174**	**256**	**743**	**778**	**531**	**999**								
60-30c	**10**	**5**	**4**	**2**		**4**		**10**	**11**	**57**	**113**	**147**	**173**	**289**	**385**	**774**	**495**	**999**								
60-35a	**11**	**6**	**3**	**4**		**3**		**11**	**11**	**72**	**155**	**169**	**385**	**446**	**554**	**999**	**666**	**999**								
60-35b	**10**	**4**	**4**	**4**		**3**		**10**	**10**	**48**	**105**	**134**	**236**	**302**	**463**	**845**	**585**	**999**								
60-35c	**8**	**5**	**3**	**4**		**3**		**8**	**9**	**47**	**109**	**130**	**270**	**335**	**490**	**891**	**604**	**999**								

Heat inactivation of Mptb in lamb meat

Name of isolate: Cattle CM00/416 Suspension ID: C08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
65-0a	349	302	286	338	693	527	632	862	633	890	706		871		999	857	481										
65-0b	357	324	325	377	764	594	687	897	736	952	775		917		999	825	460										
65-0c	315	295	315	399	814	616	715	917	796	987	872		987		999	817	478										
65-1a	13	12	6	9		64	76	208	235	519	617		855		999	999	677										
65-1b	15	9	10	12		73	84	246	249	546	613		867		999	999	679										
65-1c	16	10	9	2		78	88	283	280	627	683		922		999	999	655										
65-2a	11	5	5	4		2		0	6		12		47	100	298	649	480	999									
65-2b	10	6	3	4		1		1	2		15		43	80	306	694	518	999									
65-2c	11	3	3	4		4		3	4		10		19	59	199	514	408	960									
65-3a	10	6	2	3		5		1	1		0		0		0	7	19	561	711	999	804	780	999	999	999	999	999
65-3b	7	6	2	3		4		1	0		1		0		3	5	7	54	71	311	471	609	855	999	999	999	999
65-3c	5	2	3	2		3		1	2		0		0		2	2	3	31	43	158	308	534	765	956	999	999	999
65-4a	6	2	1	4		4		0	1		0		0		0	1	1	4	5	16	26	78	207	522	825	999	999
65-4b	8	7	3	4		0		0	1		0		1		0	2	1	23	30	205	428	615	824	999	999	999	999
65-	7	5	3	0		3		0	1		0		1		0	1	0	0	1	1	0	0	0	124	336	763	999

Heat inactivation of Mptb in lamb meat

4c																									
65-5a	8	4	6	3		2		0	0		1		0		0	0	0	1	1	2	2	3	10	223	255	324	385
65-5b	3	3	2	4		3		1	1		0		0		0	1	0	1	0	0	0	0	0	0	0	0	9
65-5c	9	5	3	4		2		3	0		0		0		0	0	0	2	0	0	0	0	0	0	0	0	1
65-6a	11	3	3	3		2		1	0		0		1		0	0	0	0	0	0	0	0	0	0	0	0	3
65-6b	9	6	4	3		2		1	0		0		0		0	0	0	0	0	0	0	0	0	0	0	0	0
65-6c	6	5	4	2		2		1	2		0		1		0	0	0	1	0	0	0	0	0	0	0	0	0
65-7a	9	3	3	2		1		1	2		0		0		1	0	0	2	3	13	47	169	254	426	843	999	999
65-7b	8	0	1	0		0		2	2		0		2		1	1	0	0	1	0	0	0	0	0	0	10	53
65-7c	8	4	0	0		0		0	1		0		0		0	1	1	0	0	0	0	0	0	0		1	1

Heat inactivation of Mptb in lamb meat

Name of isolate: Cattle CM00/416 Suspension ID: C08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
70-0a	370	330	321	406	865	664	730	980	811	999	750	800	870		999	747	404	999									
70-0b	383	330	327	366	812	607	681	843	693	887	710	789	812		999	776	457	999									
70-0c	359	311	306	345	683	547	604	780	665	810	590	666	742		966	756	455	999									
70-5a	23	19	19	27		197	212	393	377	653	565	500	439		999	952	577	999									
70-5b	10	6	5	3		12		17	25	87	137	166	182	200	381	590	420	999									
70-5c	13	6	4	1		1		37	36	181	302	516	370	445	554	999	786	999									
70-10a	10	4	3	0		0		1	0	0	0		0		12	86	123	806	999								
70-10b	11	7	5	4		8		35	32	141	274	338	597	604	999	999	773	999	999								
70-10c	10	5	3	2		1		1	2		0		0		28	155	206	894	999								
70-15a	9	5	4	4		1		2	1		0		0		0	0	0	0	1	0	0	2	17	118	399	798	999
70-15b	9	5	0	0		0		1	0		1		0		1	6	9	249	388	738	625	641	999	999	999	999	999
70-15c	8	0	2	1		3		1	0		1		1		0	0	1	0	0	7	47	164	255	398	699	999	999
70-20a	11	4	1	0		0		0	0		0		0		0	0	0	0	0	0	0	0	0	0	0	4	8
70-20b	8	2	1	0		0		0	1		0		1		1	1	0	0	0	0	0	0	0	0	0	0	1
70-	9	1	0	1		0		0	0		0		0		0	0	1	0	0	0	0	0	0	0	0	0	1

Heat inactivation of Mptb in lamb meat

20c																									
70-25a	10	2	0	0		1		0	0		0		0		0	0	1	0	0	0	0	0	0	0	0	4
70-25b	10	2	0	0		0		0	0		0		0		1	0	0	1	2	0	0	0	0	1	0	0	0
70-25c	11	4	1	0		0		2	0		0		0		0	0	0	0	0	0	0	0	0	0	0	0	0
70-30a	5	1	0	0		0		1	0		0		0		0	0	1	1	0	0	0	0	0	0	1	0	0
70-30b	8	1	2	0		0		0	0		1		0		0	0	0	0	2	0	0	0	0	0	0	0	0
70-30c	3	0	0	0		0		1	0		2		0		0	0	0	0	0	0	0	0	0	0	0	0	0
70-35a	6	4	2	0		2		0	0		1		0		0	2	0	0	1	0	0	0	0	0	0	0	0
70-35b	9	1	0	1		2		1	0		1		0		0	0	0	0	0	0	0	0	0	0	**20**	**56**	**118**
70-35c	5	1	1	4		5		2	0		0		0		0	1	1	0	0	0	0	0	0	0	0	0	3

The underlined growth indices were attributed to growth of irrelevant microorganisms based on negative results in IS*900* PCR

Heat inactivation of Mptb in lamb meat

Name of isolate: Cattle CM00/416 Suspension ID: C08/1 Dilution Buffer: LAMB Date CGI set up: 21.8.08 Bactec batch: Q911 Officer: Anna

# of Days	4	5	6	7	9	10	11	13	14	16	18	19	20	21	24	27	29	33	35	38	40	42	45	47	49	52	56
Date	26.8	27.8	28.8	29.8	31.8	01.9	02.9	04.9	05.9	07.9	09.9	10.9	11.9	12.9	15.9	18.9	20.9	24.9	26.9	29.9	1.10	3.10	6.10	8.10	10.10	13.10	17.10
75-0a	339	323	319	398	934	694	793	999	902	999	738		858		950	754	680										
75-0b	397	372	356	466	999	761	854	999	958	999	697		817		859	694	648										
75-0c	344	308	323	434	911	697	811	999	951	999	759		852		910	737	685										
75-5a	9	0	6	3		4		0	5		0		0		0	0	1	0	0	0	0	0	1	0	0	1	3
75-5b	10	4	0	0		0		3	0		1		0		1	2	0	0	0	0	0	0	0	0	0	0	0
75-5c	9	3	0	3		1		1	0		2		0		0	0	0	0	0	0	0	0	0	0	0	0	0
75-10a	8	3	0	0		0		0	0		1		0		1	0	0	0	0	0	0	0	1	0	0	1	0
75-10b	9	2	0	0		3		1	0		1		1		1	1	0	0	0	0	0	0	0	0	0	2	0
75-10c	9	3	0	0		0		2	0		1		0		0	0	1	0	0	0	0	0	0	1	0	0	0
75-15a	7	3	3	3		1		1	0		0		0		0	0	0	0	0	0	0	0	0	0	0	0	0
75-15b	6	3	0	0		0		2	0		0		1		0	0	0	0	0	0	0	0	0	0	0	0	0
75-15c	8	3	0	0		0		0	0		1		0		0	0	1	1	0	0	0	0	0	0	0	0	1
75-20a	9	3	0	0		1		1	0		1		0		0	0	0	1	0	0	0	0	0	0	0	1	0
75-20b	5	3	0	0		0		1	0		0		1		0	0	1	0	0	0	0	0	0	0	0	0	0
75-20c	5	2	0	0		2		0	0		0		0		0	0	0	0	0	0	0	0	0	0	0	0	0

Heat inactivation of Mptb in lamb meat

75-25a	6	3	0	0		0		1	1		1		0		0	0	0	0	0	0	0	0	0	1	0	0	0
75-25b	8	3	1	0		0		2	0		0		0		1	0	0	0	0	0	0	0	1	0	0	0	0
75-25c	10	3	0	0		0		2	0		0		0		1	0	1	0	0	0	0	0	0	0	0	0	0
75-30a	10	2	0	1		0		1	0		1		0		0	0	1	0	0	0	0	0	0	0	0	0	0
75-30b	9	3	0	0		0		1	0		0		0		1	1	0	1	0	0	0	0	1	0	0	0	0
75-30c	5	4	0	0		1		0	0		0		0		0	0	0	0	0	0	0	2	0	0	0	0	0
75-35a	6	3	1	2		0		1	0		2		1		0	0	1	0	0	0	0	0	0	0	0	0	0
75-35b	4	3	0	0		0		1	0		1		1		1	0	0	0	0	0	0	0	0	1	0	0	0
75-35c	9	3	0	1		0		0	0		0		0		1	0	1	0	2	0	0	0	0	0	1	0	1