QUANTITATIVE RISK ASSESSMENT OF INTRODUCTION OF FOOT AND MOUTH VIRUS INTO THE U.S. THROUGH CHEESE IMPORTATION

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ABSTRACT

A quantitative risk assessment was conducted to determine the risk of introduction of foot and mouth disease (FMD) virus into the United States via cheese importation. This risk assessment does not address the consequences of FMD virus introduction, its establishment, spread and the related economic, environmental and epidemiological consequences after introduction. A risk pathway scenario tree was developed to examine the most critical points in cheese production where FMD virus may be introduced or survive. At each node of the scenario tree the FMD viral infectivity was tracked and followed through the next node until the total FMD viral infectivity was determined at the final node. Assuming worst-case scenario that all cheese imported from FMD endemic countries is infected, the total risk of expected FMD infectivity in terms of ID_{50}, in case of all mitigations failure, was determined for six ageing periods of 15, 30, 60, 75, 90, and 120 days. The values for cheese made from pasteurized milk and aged for 15 and 30 days are 363.13 and 8.12 ID_{50}, respectively and for 60, 75, 90, and 120 days the amount is 0 ID_{50}. For cheese made from un-pasteurized milk the values are 2.13 x 10^5, 1.41 x 10^4, 9.25 x 10^2, 3.85 x 10^2, 1.88 x 10^2, and 61 ID_{50}, respectively for the six ageing periods. It is concluded, based upon the number of infected dozes, that the imported cheese, regardless of its origin, kind of milk used, and ageing period, has an extremely low risk of FMD virus introduction into the United States.

Key words: risk assessment, FMD in cheese, pasteurization, pH, ageing.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cattle, swine, and other ruminants with a potential to disrupt the entire economy in the event of an outbreak. An FMD outbreak will not only result in direct loss of productivity in livestock but the indirect cost, due to trade barriers with trickle down effects on all associated industries and on tourism, will be monumental. The 2001 United Kingdom FMD outbreak had a devastating impact on its economy with four million slaughters (DEFRA, 2003 and Royal Society Report, 2003) and a price tab of over $6.58 billion (BBC, 2003). Given that the United States has ten times the livestock population of the United Kingdom and is a major exporter of livestock products; any FMD outbreak in the United States will be catastrophic (Ekboir, 1999; Paarlberg et al., 2002). The United States has not had an outbreak of FMD since 1929 mainly due to the stringent measures adopted. These measures includes monitoring of foreign diseases internationally with strict surveillance to thwart their entry into the United States, inspection of animal products at borders, maintaining a strong veterinary infrastructure and surveillance, and a strong emergency response to foreign diseases (Animal Disease Risk Assessment, Prevention, and Control Act of 2001).

For a long time this strategy has been effective, however, recent European FMD outbreaks have raised new concerns.

One potential avenue of infection is through the importation of cheese, as currently the United States is importing cheese from all parts of the world including the FMD affected areas. The work presented in this research paper has helped USDA-APHIS decision makers (and similar other regulatory agencies) to reassess their rule making with respect to cheese importation under Title 9, Code of Federal Regulations Parts 94-98 (US-CFR, 1999). This quantitative risk assessment (QRA) has evaluated the likelihood of FMD virus introduction into the United States through cheese importation. The imported cheese considered in this QRA study may have come from any region of the world regardless of its FMD status and may have been produced from pasteurized or unpasteurized milk.

The introduction of FMD virus, through cheese importation, into the United States depends upon survival of FMD virus at three critical stages of the cheese production process (Figure 1): 1) heat treatment or

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pasteurization of the milk used for cheese production; 2) ‘acidic’ or ‘alkaline’ state, i.e., pH during the cheese production process; and 3) survival of FMD virus during curing and ageing of the cheese. These critical stages were determined from the cheese production procedure and various empirical studies. Blackwell, (1976) observed that the FMD virus survival in cheese depends upon the pasteurization of milk and subsequent acidification during the cheese manufacturing process. Schjeming-Thiesen, (1979) could not detect the infectious FMD virus in cheddar cheese two weeks after processing the milk samples (that contained FMD virus- $10^{3.5}$ - $10^{6.5}$ mouse ID_{50}/ml) at 63ºC for 15 min before processed into the cheese.

This QRA study has determined the probability of FMD virus introduction into the United States based on four likely factors. Those four key factors are:

1. that the cheese is imported from an FMD-endemic country
2. that it had been made either from pasteurized or unpasteurized milk
3. that it had not been matured to pH 6.0 or below that it had not been aged to 120 days or more.

METHODOLOGY

Epidemiology of a given disease lays out the comprehensive structure for risk pathways. The epidemiologic problem oriented approach (EPOA) is a systematic epidemiologic study of a problem emphasizing the problem identification and management-mitigations triads (Figure 2). This methodology of problem decomposition in epidemiologic modeling consists of two generic problem-solving steps: problem identification-definition; and problem solution-management-mitigations. The problem identification-definition triad consists of the host, agent and environment as the pillars of the triad. The problem solution-management-mitigations triad consists of therapeutics, prevention-control and health maintenance-promotion as the pillars. The two triads are linked together by diagnostic procedure linkages.

Using the above-mentioned EPOA for FMD virus and the critical control points of the cheese production process (Figure 1), a scenario tree was developed that describe the risk pathways ending in the introduction of FMD virus into the United States via imported cheese (Figure 3). The risk pathways scenario tree describes the pathways of risk that is initiated by the decision to import cheese from any country in the world and ends in the possible introduction of FMD virus into the United States. The first node addressed in this scenario tree “Is the cheese imported from an FMD-endemic country?” begins the dichotomous risk pathway process. If the cheese is imported from an FMD-endemic country, then the risk of FMD virus is propagated to the next level of risk determination. If the cheese is not imported from an FMD-endemic country then there is no risk. In case the cheese is imported from an FMD-endemic country, the second node determines the first mitigation in place, i.e. pasteurization. If the imported cheese from an FMD-endemic country is made from un-pasteurized milk, then the risk of FMD virus is further propagated. However, if the cheese from an FMD-endemic country is made from pasteurized milk it reduces the FMD infectivity from $4x10^9$ to $7.9x10^2$ ID_{50} per liter (Donaldson, 1997). This reduction in FMD virus infectivity is based on the assumption that pasteurization was performed at desired temperature, duration and without process failure, both mechanical and intentional, to curtail the effects of FMD virus survival. The reduction in FMD viral infectivity due to pasteurization is then taken into account for the final infectivity determination.

If the imported cheese from an FMD-endemic country is made from un-pasteurized or inadequately pasteurized milk and had not been matured to pH 6 or below, then the FMD virus introduction is further propagated down the risk pathway in node 3. However, if the cheese is matured to pH 6 or below, then there is 63.8% reduction in FMD virus infectivity determined from the work of Donaldson, (1997) and Hagan and Bruner, (1988).

If the cheese from an FMD-endemic country, made from un-pasteurized or inadequately pasteurized milk, had not been matured to pH 6 or below, and had not been aged to 120 or more days (node 4), then the FMD virus will be introduced into the United States via importation of such cheese.

Scientific evidence for various parameters of the scenario tree was collected from published literature, official databases, and personal communications (Blackwell, 1976; FAS, 2003a, b; Henning, 2002; OIE, 2003a, b and Sellers, 1969). From such data, appropriate parameters were computed, underline distributions were generated and the results were simulated with Monte Carlo simulations using simulation software (@RISK) on a Gateway Pentium IV computer.

While developing this QRA model the following assumptions were made:

1. That cheese is imported from any parts/region of the world regardless of the FMD status.
2. That all the imported cheese from FMD-endemic countries is presumed to be produced from FMD contaminated milk.
3. That the imported cheese is made from pasteurized as well as un-pasteurized milk of domesticated animals such as cow, goat, camel, buffalo, etc.
4. That Pasteurization does not completely eliminate the FMD virus.

5. That the milk collected for the cheese production is pooled from various dairy farms and other locations.
6. That the FMD infectivity in cheese will be 10 times less than milk infectivity given a 10:1 milk to cheese production ratio.
7. Detailed information about the data and parameter estimation for each node is described below:

Parameter: Initiating Event:

Cheese importation into the United States: The initiating event in this QRA study is "importation of cheese" (into the United States from various parts of the world). The initiating event is quantified as metric tons of all varieties of cheese imported into the United States each year from various parts of the world. During 1992 to 2001 the imported cheese quantity ranged between 133,310 to 202,950 metric tons per given year as reported by Foreign Agricultural Service of the US Department of Agriculture (FAS, 2003a).

Parameter: **Is the Cheese Imported From FMD Country? (Node 1):**

The quantity of cheese, in kilograms, imported from FMD-endemic countries is determined using FAS-USDA and OIE classification databases (FAS, 2003a; OIE, 2003a). During 1992 to 2001 the quantity of imported cheese from FMD-endemic countries ranged between 61,269,000 to 97,821,000 kg per given year. Based on the 2001 FMD status of OIE, 46% of the total imported cheese was from FMD endemic countries. Minimum, average and maximum amount of all varieties of cheeses that were imported to the United States from FMD-endemic countries per year during 1992-2001 were determined and are given in Table 1, cells B2-D2. These values were used to find out the corresponding imported cheese quantity distribution using RiskPert function of @Risk® software, and values are given, in cell E2, row 2 of Table 1. Using the distribution thus generated, the expected amount of cheese in kg that was coming from FMD-endemic countries is given in cell G2.

Parameter: Is the Cheese Made From Pasteurized Milk? (Node 2):

If imported cheese was made from pasteurized milk, then FMD infectivity is reduced from 4×10^9 to 7.9×10^2 ID_{50} per liter (Donaldson, 1997). Thus FMD infectivity propagation is processed through node 2a of the scenario tree (Figure 2). Otherwise (for unpasteurized milk) risk is propagated through node 3. Using FAS import database (FAS, 2003b) the amount of different varieties of cheese in kg, based upon their production procedure, were determined. Cheese made from pasteurized milk were differentiated from the cheese made from unpasteurized milk in order to determine each category's amount in total cheese imports to the United States from FMD-endemic countries. Cell G3, Table 1, gives the amount of cheese in kg made from unpasteurized milk and imported from FMD-endemic countries. Subtracting cell G3 from cell G2 (Table 1) gives the amount of cheese made from pasteurized milk and imported from FMD-endemic countries (cell G4).

Parameter: Is the Cheese Made From Pasteurized Milk at Desired Temp, Duration, and Adequate Process? (Node 2a):

Pasteurization does not entirely remove FMD virus from the contaminated milk. FMD infectivity (ID_{50}) that remains after pasteurization is determined and propagated through node 3 along with the infectivity propagated via cheese made from unpasteurized milk (node 2). Donaldson, (1997) and Holsinger et al. (1997) argued that high temperature-short-time (HTST, 72ºC for 15 sec) pasteurization of the milk would produce a $10^{4.0}$ to $10^{5.0}$ ID_{50}/liter reduction in FMD viral infectivity. Therefore, the concentration of virus in the pasteurized milk will be dropped to $10^{1.9}$ to $10^{2.9}$ ID_{50}/liter. Given the milk-to-cheese production ratio of 10:1 (Henning, 2002) this infectivity in cheese is presumed ten times smaller and is given in cell B5 and D5, Table 1. Using RiskUniform function of @Risk, the corresponding cheese infectivity distribution was determined and is given in cell E5 (Table 1). This distribution hence multiplied with the amount of cheese (kg), made from pasteurized milk and imported from FMD-endemic countries (G4), and is given in cell G5. This is FMD viral load attributed to the cheese made from pasteurized milk and imported from FMD-endemic countries.

Total FMD viral load that will propagate to node 3 is determined by combining the FMD viral load from the cheese made from pasteurized milk and from the cheese made from unpasteurized milk. FMD infectivity (viral load) attributed to unpasteurized milk per liter, under various conditions is ascertained from the work of Donaldson, (1997) and Sellers, (1969) and is given in cells B6-D6 (Table 1).

Under normal conditions FMD infectivity in raw unpasteurized milk is $10^{6.6}$ ID_{50}/ml. This infectivity value is converted into ID_{50} /kg of cheese first multiplying by 1000 and then dividing by 10 respectively, for milliliter to liter conversion and for milk to cheese conversion based upon the assumption that FMD infectivity in cheese would be 10 times lesser than milk infectivity (Henning, 2002). Thus the corresponding ID_{50}/kg cheese will be 3.98×10^8. When FMD infected milk is diluted, with other uninfected milk either from the same farm location or from other premises, it reduces the infectivity to 8.0×10^6 /liter, which further reduces to 8.0×10^5 /liter when filtered. Using these three values (3.98×10^8, 8.0×10^6, and 8.0×10^5) respectively as maximum, most likely and minimum in RiskPert function of @Risk, FMD viral infectivity distribution for unpasteurized milk was determined and is given in cell E6, Table 1. Multiplying cell E6 with the amount of cheese made from unpasteurized milk and imported from FMD-endemic countries (cell G3) yields the FMD infectivity (viral load) in cheese made from unpasteurized milk and imported from FMD-endemic countries and is given in cell G6, (Table 1). Adding cells G5 and G6 gives the total FMD viral infectivity in cheese made from both pasteurized as well as unpasteurized milk and is given in cell G7 (Table 1).

Parameter: Is the Cheese Matured to Desired pH (=<6.0)? (Node 3):

If the cheese was matured to pH 6.0 or below then there is a reduction of FMD infectivity (ID_{50}); otherwise risk is propagated through node 4. Empirical studies of Heng and Wilson, (1993) and Hagan and Bruner, (1988) were used to determine the effect of time, temperature, and pH on FMD virus infectivity in processed cheese (Table 2 & 3). Minimum, most likely and maximum infectivity decline at pH = <6.0 were determined and are given in cells B8-D8, Table 1. These three values were used to generate the RiskPert distribution of @Risk to calculate the corresponding distribution (cell E8, Table 1). Using this distribution output and the FMD infectivity in cheese made from unpasteurized and pasteurized milk, the percentage drop in FMD virus infectivity due to pH maturation to 6.0 or below was determined and is given in cell F8. The leftover infectivity (1-F8) was multiplied with the FMD viral infectivity in cheese made from pasteurized and cheese made from unpasteurized milk to yield the FMD infectivity that is propagated to the final node and is given in cells G9 and G10, respectively. These quantities reflects the total FMD viral infectivity in cheese imported from FMD-endemic countries and produced from both pasteurized as well as un-pasteurized milk and matured to pH 6.0 or below.

Parameter: Is the Cheese Aged to 120 Days? (Node 4):

If the cheese was aged to 120 days or more prior to export then there is no risk of FMD virus introduction into the United States; otherwise FMD virus is likely to be introduced into the United States. There is a limited amount of data on the effect of ageing on cheese made from pasteurized or unpasteurized milk. The data is partly quantitative and partly qualitative in nature. Blackwell (1976) qualitative and quantitative data for cheddar and camembert cheese were used to determine the FMD infectivity in cheese made from un-pasteurized and pasteurized milk respectively. In the case of cheese made from unpasteurized milk it was subjected to 1, 30, 60 and 120 days of ageing and on the 120^{th} day no FMD virus was detected. For cheese made from pasteurized milk the effect of ageing was considered for 1, 21 and 35 days ageing and on the 35^{th} day no FMD virus was detected. Using regression equations on the above mentioned data FMD viral infectivity was predicted for six scenarios at 15, 30, 60, 75, 90, and 120 days of ageing both on linear and log scale for cheese made from both pasteurized and unpasteurized milk. Based on these values the multiplicative reduction ratio of the ID_{50} was calculated and the values are given in Table 4.

Using RiskSimtable function of @Risk corresponding distributions for the above-mentioned six ageing scenarios, for cheese made from pasteurized, unpasteurized and from pasteurized and unpasteurized milk, of ID_{50} doses were calculated. This is done by multiplying these effects of ageing on cheese values (the proportion of the reduction of the FMD viral infectivity due to ageing Table 4) by the total infectivity left after all the mitigation including pH mitigation cells (G9, G10, Table 1) in the cheese making process. The corresponding distribution is presented in cell G12, 13 and 14 respectively for cheese made from pasteurized, from un-pasteurized and from both pasteurized and un-pasteurized milk, Table 1).

Ten thousand iterations with six simulations were performed on this infectivity values to produce corresponding distributions of total cheese infectivity using @Risk. Sensitivity analysis was performed to determine the inputs effects on this output.

Cell G12 and G13 present the FMD infectivity in ID_{50}'s, in all the cheese made from pasteurized and un-pasteurized milk respectively, and imported into the US per year. Cell G14 presents the total FMD infectivity in terms of number of ID_{50}'s, in all the cheese made from both pasteurized and un-pasteurized milk, and imported into the United States per year.

RESULTS AND DISCUSSION

Detailed results of this QRA study are given in Table 1. Total FMD infectivity or viral load is expressed in ID_{50} that may likely be introduced into the United States, via all kinds of cheese imports per year. The six ageing scenarios considered for cheese maturation are 15, 30, 60, 75, 90 and 120 days. Assuming a worst-case scenario, of all cheese imported from FMD endemic countries is infected, the total risk of expected FMD infectivity, in ID_{50}, assuming complete mitigation failure, was determined for the six ageing periods. The values for cheese made from pasteurized milk and aged for 15 and 30 days are 363.13 and 8.12 ID_{50}, respectively and for 60, 75, 90, and 120 days the amount is 0 ID_{50} (Table 1 using prediction equation of Table 4). For cheese made from un-pasteurized milk the values are 2.13×10^5, 1.41×10^4, 9.25×10^2, 3.85×10^2, 1.88×10^2, and 61 ID_{50}, respectively for 15, 30, 60, 75, 90 and 120 days (Table 1 using prediction equation of Table 4). For the six scenarios of ageing, the total expected FMD infectivity in terms of ID_{50} from all cheese imported from FMD endemic countries are 2.14 x 10^5, 1.41 x 10^4, 9.25 x 10^2, 3.85 x 10^2, 1.88 x 10^2, and 61 ID_{50} respectively for 15, 30, 60, 75, 90 and 120 days (Table 1 line 14, Table 4; Figure 4&5). This conclusion is reached based upon the above described 4-node risk pathway scenario tree (Figure 3, Table 1). The first node of this risk pathway scenario tree classified the imported cheeses from FMD-endemic or FMD-free countries based upon the OIE-FMD status of the exporting countries. Given annual average imports of about 75,785 (FAS 2003, OIE 2003) metric tons of cheese (from FMD-endemic countries) the FMD viral load linearly increases as cheese coming from FMD-endemic countries increases. On the other hand if cheese is imported from FMD free countries there is no risk associated. The @Risk model accommodates the varying amounts of imported cheese along with the changing proportion of FMD endemic countries to reflect the updated risk of FMD contamination, as the new data become available from relevant sources.

Node 2 of the scenario tree determines the FMD viral infectivity based upon whether the cheese was made from pasteurized or un-pasteurized milk. A high-temperature-short-time (HTST = 72ºC for 15s) pasteurization of the milk for cheese production will produce a 10^4 to 10^5 ID_{50} reduction of infectivity. Although substantially reduced but not completely eliminated, the concentration of FMD virus after HTST pasteurization will then be at $10^{1.9}$ to $10^{2.9}$ ID_{50}/litre (Donaldson, 1997; Sellers, 1969). Depending upon the amount of imported cheese from the FMD-endemic countries and the ratio of cheese made from pasteurized milk to cheese made from un-pasteurized milk, the FMD viral infectivity varies. Node 2 and 2a determines the FMD viral infectivity contributed by the imported cheese made, respectively, from un-pasteurized and inadequately pasteurized milk. In the current @Risk model the total FMD infectivity at node 2 and 2a is 5.06 x10^{10} ID_{50} (Table 1), a significant part of which is contributed by the cheese made from un-pasteurized milk.

During cheese production process pH plays a vital role in reducing/removing the FMD viral infectivity. For example at pH 7.6 it takes 30 minutes to inactivate FMD virus compared to only 6 minutes at pH 6.7 (Heng and Wilson, 1993). Node 3 of the scenario tree determines the effect of pH 6.0 or below. If the cheese is matured to pH 6.0 or below, based upon empirical observations it is assumed that the FMD virus is significantly reduced. Minimum, most likely and maximum FMD viral infectivity that may survive during the cheese production process at various pH levels were determined from the literature (Donaldson, 1997; Heng and Wilson, 1993; Hagan and Brunre, 1988). Due to various pH ranges the cheese is subjected during manufacturing stages it is determined that there is a 63.8% drop in FMD viral infectivity, reducing to 1.22 x 10^{09} ID_{50} from 3.37x10^{09} ID_{50} for cheese made from pasteurized milk; and 2.44 x 10^{10} ID_{50} from 6.74 x 10^{10} ID_{50} for cheese made from un-pasteurized milk.

Node 4, the final node of the scenario tree, deals with the ageing process of the cheese. If the cheese that is made from un-pasteurized milk is aged to 120 days or more the FMD virus may not survive (Blackwell, 1976), otherwise it is likely to introduce into the United States depending upon the length of the ageing. If the cheese, made from pasteurized milk, is aged to 35 days or more the FMD virus may not survive (Blackwell, 1976), otherwise it may be introduced into the United States depending on the length of ageing.

There has not been enough empirical data available on FMD virus survivability at various ages of cheese maturation to plot a meaningful curve between the numbers of days cheese is aged and virus infectivity. Blackwell, 1976 reported qualitative data on FMD virus persistence during curing of Cheddar, Mozzarella and Camembert cheese prepared from milk of FMD virus infected cows. In Cheddar cheese samples prepared from raw milk, FMD virus persisted at 2ºC through 60 days but not through 120 days. In Mozzarella and Camembert cheese samples prepared from pasteurized milk (72^0C for 16s), FMD virus persisted at 4ºC through 21 days but not through 35 days. He further reported the FMD viral infectivity in raw or inadequately pasteurized (63^0C for 6s, 67^0C for 6s, 10s and 1 min) milk from experimentally infected cows at day 1, 2, 3, 4, and 5 post inoculation. Using these data we developed prediction equations and a virus infectivity demise curve both at linear and log scale to simulate the cheese ageing scenarios at 15, 30, 60, 75, 90, and 120 days (Table 4, 5, 6).

Using @Risk software the corresponding cheese infectivity distribution for the total FMD infectivity from cheese made from pasteurized, un-pasteurized and both pasteurized and un-pasteurized milk for six ageing scenarios were generated. Ten thousand iterations were performed for the nine outputs and six simulations, and are given in Figures 4 and 5 along with the statistics in Table 7.

Using the predicted values of FMD virus ID_{50} at 15, 30, 60, 75, 90, and 120 days of cheese curing (ageing), the corresponding total FMD infectivity in imported cheese into United States per year are thus calculated. The values for cheese made from pasteurized milk and aged for 15 and 30 days are 363.13 and 8.12 ID_{50} respectively and for 60, 75, 90, and 120 days the amount is 0 ID_{50}. For cheese made from un-pasteurized milk the values are 2.13 x 10^5, 1.41 x 10^4, 9.25 x 10^2, 3.85 x 10^2, 1.88 x 10^2, and 61 ID_{50} respectively. For the six scenarios of ageing, the expected FMD infectivity in terms of ID_{50} from all cheese imported from FMD endemic countries are 2.14 x 10^5, 1.41 x 10^4,

9.25×10^2, 3.85×10^2, 1.88×10^2, and 61 ID_{50} respectively.

Dividing these values by the corresponding total kilograms of imported cheese from FMD-endemic countries the results were also calculated in terms of ID_{50} per kilogram of the imported cheese. For the six ageing scenarios of 15, 30, 60, 75, 90, and 120 days, the FMD infectivity in terms of ID_{50} /kg of imported cheese made from un-pasteurized milk is: 435.72, 28.7, 1.89, 7.9×10^{-1}, 3.9×10^{-1}, and 1.2×10^{-1} ID_{50} /kg respectively; from pasteurized milk is: 5.21×10^{-6}, 1.16×10^{-7} ID_{50} /kg and 0 ID_{50} /kg for ageing at 60, 75, 90, and 120 days (Table 7).

The amount of imported cheese (in kg) that an animal must consume by oral route to get infected are calculated by dividing the amount of FMD virus infective doses capable of initiating an infection in cattle and pig by oral route ($10^{6.0}$ ID_{50} and $10^{5.0}$ ID_{50} respectively, Donaldson, 1976) by the total number of infective doses per kg of imported cheese per year. For the six scenarios of ageing (30, 45,60,75,90,120 days) the amount of imported cheese that must be consumed by cattle to get infected, if the contaminated imported cheese is made from un-pasteurized milk, are: 1.08×10^4, 1.64×10^5, 2.5×10^6, 5.99×10^6, 1.23×10^7 and 3.79×10^7 kg respectively; and from pasteurized milk are: 4.85×10^{11}, 2.17×10^{13} kg respectively for 30 and 45 days of ageing. Cheese made from pasteurized milk and aged beyond 45 days has no potential of infecting cattle even consumed at a hypothetically large quantity. For the six scenarios of ageing the amount of imported cheese that must be consumed by a pig to get infected if the contaminated imported cheese is made from: un-pasteurized milk are 1.08×10^3, 1.64×10^4, 2.5×10^5, 5.99×10^5, 1.23×10^6 and 3.79×10^6 kg respectively; from pasteurized milk are 4.85×10^{10}, 2.17×10^{12} kg respectively for 30 and 45 days of ageing. Beyond 45 days of maturing, the cheese made from pasteurized milk pose no threat of FMD infection to pigs at any level of large consumption.

Sensitivity Analysis: The sensitivity analysis of the imported cheese- FMD infectivity is given in Figure 6. This figure represents the effect of various inputs on the output of the FMD viral infectivity. It is a standardized regression (or beta) coefficients from a multiple regression. @Risk creates this multiple regression from the data of the 10,000 iterations using FMD viral infectivity as the dependent variable and the values of the various inputs for each iteration as the independent variables. A beta coefficient for an independent variable indicates the number of standard deviations by which the dependent variable (FMD viral infectivity) increases if the independent variable increases by one standard deviation (assuming all independent variables are held constant). FMDV Infectivity in cheese made from un-pasteurized milk is the most vital input and has significant impact, 52% upon the output. Infectivity reduction due to pH maturation is the next most vital input and has 47.7% impact upon the output. Decreasing the value of pH 6.0 or below by one standard deviation will reduce the virus infectivity by 47.7% standard deviation.

Table 1 Cheese – FMD Risk Assessment Model in @Risk settings with six ageing selections

		A	B	C	D	E	F	G	H	I
	1	Parameters at each node	Minimum	Most likely	Maximum	Expected Values	Proportion	Amount (kg and ID_{50})	Distribution used	Unit
NODE 1	2	Is the cheese imported from FMD country?	61,269,100	75,785,280	97,821,200	77,038,570		77,038,570 kg	RiskPert ( )	kg
NODE 1	3	Amount of Cheese made from un-pasteurized milk and imported from FMD countries	0	190	1300	497		497kg	RiskPert ( )	kg
NODE 1	4	Amount of Cheese made from pasteurized milk and imported from FMD countries				77,038,073		77,038,073 kg	G2-G3	Kg
NODE 2	5	FMDV Infectivity in cheese made from pasteurized milk/liter	7.94E+00		7.94E+01	4.37E+01		3.37E+09 ID_{50}	RiskUniform()	$(ID_{50}/kg) * kg = ID_{50}$
NODE 2	6	FMDV Infectivity in cheese made from un-pasteurized milk/liter	8.00E+05	8.00E+06	4.00E+08	1.00E+08		6.74E+10 ID_{50}	RiskPert( )	$(ID_{50}/kg) * kg = ID_{50}$
NODE 2	7	FMDV Infectivity in cheese made from pasteurized and un-pasteurized milk						7.07E+10 ID_{50}	H5+H6	$ID_{50} + ID_{50} = ID_{50}$
NODE 3	8	Is the cheese matured at desired pH to effectively inactivate FMDV? {=<6.0?}	3.98E+06	3.08E+07	4.42E+07	2.63E+07	63.8%		RiskPert( )	ID_{50}
NODE 3	9	Cheese made from pasteurized milk	Initial FMDV * (1 - % Reduction by pH)					1.22E+09 ID_{50}	H5*(1 – F8)	ID_{50}
NODE 3	10	Cheese made from un-pasteurized milk						2.44E+10 ID_{50}	H6*(1 – F8)	ID_{50}
NODE 4	11	Is the cheese aged >120 days?	= RiskSimtable(Effect of ageing (15,30,60,75,90,120))							
NODE 4	12	Cheese made from pasteurized milk	=VLOOKUP (for the selection of age and get the appropriate ageing effect on cheese made from pasteurized milk)						G9*(1-E12)	ID_{50}
NODE 4	13	Cheese made from un-pasteurized milk	=VLOOKUP (for the selection of age and get the appropriate ageing effect on cheese made from un-pasteurized milk)						G10*(1-E13)	ID_{50}
12		Total FMDV infectivity in ID_{50} in all cheese made from pasteurized milk imported per year		363.13		8.12	0	0	0	0
13		Total FMDV infectivity in ID_{50} in all cheese made from un-pasteurized milk imported per year		2.13E+05		1.41E+04	925.65	385.61	188.54	60.97
14		Total FMDV infectivity in ID_{50} in all cheese imports per year		2.14E+05		1.41E+04	925.65	385.61	188.54	60.97
15a		FMD infectivity per kg of imported cheese made from pasteurized milk		5.2E-06		1.2E-07	0	0	0	0
15b		FMD infectivity per kg of imported cheese made from un-pasteurized milk		435.72		28.7	1.89	0.79	0.39	0.12

			15 days	**30days**	**60 days**	**75 days**	**90 days**	**120 days**
16a	Number of infective doses capable of initiating an infection in cattle and pig by oral route, which is $10^{6.0}$ ID_{50} and $10^{5.0}$ ID_{50} respectively, in cheese made from pasteurized milk.	Total number of infective doses for cattle	3.6E-04	8.1E-06	0	0	0	0
		Total number of infective doses for pig	3.6E-03	8.1E-05	0	0	0	0
16b	Number of infective doses capable of initiating an infection in cattle and pig by oral route, which is $10^{6.0}$ ID_{50} and $10^{5.0}$ ID_{50} respectively, in cheese made from un-pasteurized milk.	Total number of infective doses for cattle	2.18E-01	1.43E-02	9.45E-04	3.9E-04	1.9E-04	6.22E-05
		Total number of infective doses for pig	2.18	1.43E-01	9.45E-03	3.9E-03	1.9E-03	6.22E-04
16c	Number of infective doses capable of initiating an infection in cattle and pig by oral route, which are $10^{6.0}$ ID_{50} and $10^{5.0}$ ID_{50} respectively, for all types of cheese (i.e. cheese made from both pasteurized and un-pasteurized milk).	Total number of infective doses for cattle	2.18E-1	1.4E-02	9.5E-04	3.9E-04	1.9E-04	6.22E-05
		Total number of infective doses for pig	2.18	1.44E-01	9.45E-03	3.9E-03	1.9E-03	6.22E-04

Table 2 Time to inactivate FMD virus to a survival of 0.00001 at 4ºC at various pH (Heng and Wilson, 1993)

PH	Time
2.0	Within 1 min
4.0	Within 2 min
5.5	Within 30min
5.8	Within 18 hours
11.0	Within 2 hours
12.0	Within 2.5 min
13.0	Within 2.5 min

Table 3 Inactivation of FMD virus at various temperatures and pH combinations Heng and Wilson, 1993)

Temperature	Time taken to inactivate FMD virus	
	pH 6.7	pH 7.6
56ºC	6 min	30min
63ºC	1 min	2 min
72ºC	17 sec	55 sec
80ºC	<5 sec	<5 sec
85ºC	<5 sec	<5 sec

Table 4 Effect of ageing on cheese made from pasteurized and un-pasteurized milk

	ID_{50} in cheese made from un-pasteurized milk.		**ID_{50} in cheese made from pasteurized milk.**	
Scenario	Log	Linear	Log	Linear
Scenario 1- 15 Days	8.79E-06	3.97E-02	3.28E-07	2.44E-05
Scenario 2 - 30 Days	5.79E-07	3.71E-03	7.33E-09	2.53E-08
Scenario 3 - 60 Days	3.81E-08	3.25E-05	0.00E+00	0.00E+00
Scenario 4 - 75 Days	1.59E-08	3.04E-06	0.00E+00	0.00E+00
Scenario 5 - 90 Days	7.77E-09	2.84E-07	0.00E+00	0.00E+00
Scenario 6 - 120 Days	2.51E-09	2.49E-09	0.00E+00	0.00E+00

Table 5 Predicted FMD viral infectivity in cheese made from pasteurized milk at various days of post cheese processing.

Linear and Log Curve Fitting for ID_{50} Concentration vs. Ageing in pasteurized and reduction ratio						
	y = -0.0425*Day+ 1.4874		y = -0.4064*LN(Day) + 1.4449		Reduction Ratio (Linear)	Reduction Ratio (LN)
Day	Linear ($logID_{50}$)	ID_{50}	Log ($logID_{50}$)	ID_{50}	ID_{50} (Day)/ID_{50} (Initial)	
3	6.3755	2.4E+06	5.947223863	8.9E+05	5.96E-03	2.22E-03
10	4.9825	9.6E+04	3.080805411	1.2E+03	2.41E-04	3.03E-06
15	3.9875	9.7E+03	2.115474081	1.3E+02	2.44E-05	3.28E-07
20	2.9925	9.8E+02	1.430560603	2.7E+01	2.47E-06	6.77E-08
21	2.7935	6.2E+02	1.31440098	2.1E+01	1.56E-06	5.18E-08
30	1.0025	1.0E+01	0.465229274	2.9E+00	2.53E-08	7.33E-09
35	0.0075	1.0E+00	0.098227335	1.3E+00	2.56E-09	3.15E-09
60	0	1.0E+00	-1.185015534	6.5E-02	2.51E-09	1.64E-10

Table 6 Predicted FMD viral infectivity in cheese made from un-pasteurized milk at various days of post cheese processing.

Linear and Log Curve Fitting for ID_{50} Concentration vs. Ageing in un-pasteurized and reduction ratio						
	y = -0.0686*Days+ 8.2278		y = -1.7043*LN(Day)+ 8.1593		Reduction Ratio (Linear)	Reduction Ratio (LN)
Day	Linear ($logID_{50}$)	ID_{50}	Log ($logID_{50}$)	ID_{50}	ID_{50} (Day)/ID_{50} (Initial)	
1	8.16	1.4E+08	8.159	1.4E+08	3.62E-01	3.62E-01
5	7.88	7.7E+07	5.416	2.6E+05	1.93E-01	6.55E-04
15	7.20	1.6E+07	3.544	3.5E+03	3.97E-02	8.79E-06
30	6.17	1.5E+06	2.363	2.3E+02	3.71E-03	5.79E-07
45	5.14	1.4E+05	1.672	4.7E+01	3.47E-04	1.18E-07
60	4.11	1.3E+04	1.181	1.5E+01	3.25E-05	3.81E-08
75	3.08	1.2E+03	0.801	6.3E+00	3.04E-06	1.59E-08
90	2.05	1.1E+02	0.490	3.1E+00	2.84E-07	7.77E-09
105	1.02	1.1E+01	0.228	1.7E+00	2.66E-08	4.24E-09
120	0.00	9.9E-01	0.000	1.0E+00	2.49E-09	2.51E-09

Table 7 Total FMDV infectivity in terms of ID_{50} in all cheese imports per a given year

Ageing in days	15	30	60	75	90	120	
Minimum	254	15	1	0	0	0	
Maximum	2700281	177800	11709	4878	2385	771	
Mean	213722	14061	926	386	189	61	
Std Deviation	281640	18546	1221	509	249	80	
Variance	8005	519	34	14	7	2	
Mode	769174	50609	3332	1388	679	219	
5% Perc	254	15	1	0	0	0	
95% Perc	2700281	177800	11709	4878	2385	771	
FMD infectivity in terms of ID_{50}/kg of the imported cheese made from un-pasteurized milk							
Ageing in days	15	30	60	75	90	120	
Minimum	1.17	7.69E-02	5.06E-03	2.11E-03	1.03E-03	3.33E-04	
Maximum	3068.44	202.11	1.33E+01	5.55E+00	2.71E+00	8.77E-01	
Mean	435.72	28.70	1.89	7.87E-01	3.85E-01	1.25E-01	
Std Deviation	442.72	29.16	1.92	8.00E-01	3.91E-01	1.27E-01	
Variance	24.62	1.62	0.11	4.45E-02	2.18E-02	7.03E-03	
Mode	1358.48	89.48	5.89	2.46	1.20	3.88E-01	
5% Perc	1.17	7.69E-02	5.06E-03	2.11E-03	1.03E-03	3.33E-04	
95% Perc	3068.44	202.11	1.33E+01	5.55E+00	2.71E+00	8.77E-01	
FMD infectivity in terms of ID_{50}/kg of the imported cheese made from un-pasteurized milk							
	Minimum	Maximum	Mean	Std Deviation	Variance	Mode	5% Perc	95% Perc
15	5.43E-08	2.37E-05	5.20E-06	4.35E-06	5.99E-07	1.42E-05	5.43E-08	2.37E-05
30	1.21E-09	5.31E-07	1.16E-07	9.72E-08	1.34E-08	3.19E-07	1.21E-09	5.31E-07

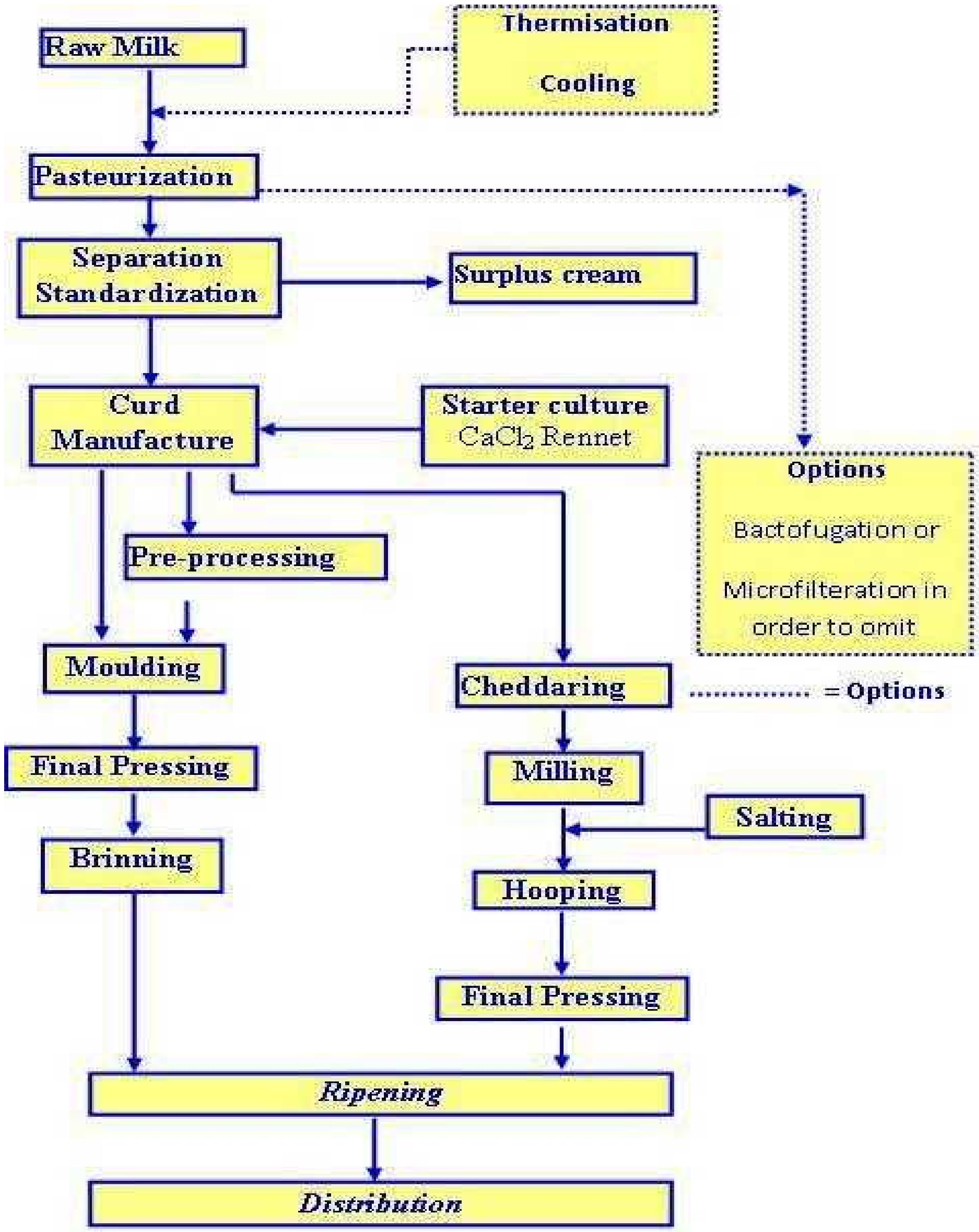


Figure 1 Cheese Production Flow Diagram.
Adopted from: *Dairy Processing Handbook (pp290).* Tetra Pak Processing Systems AB, S-221 86 Lund, Sweden

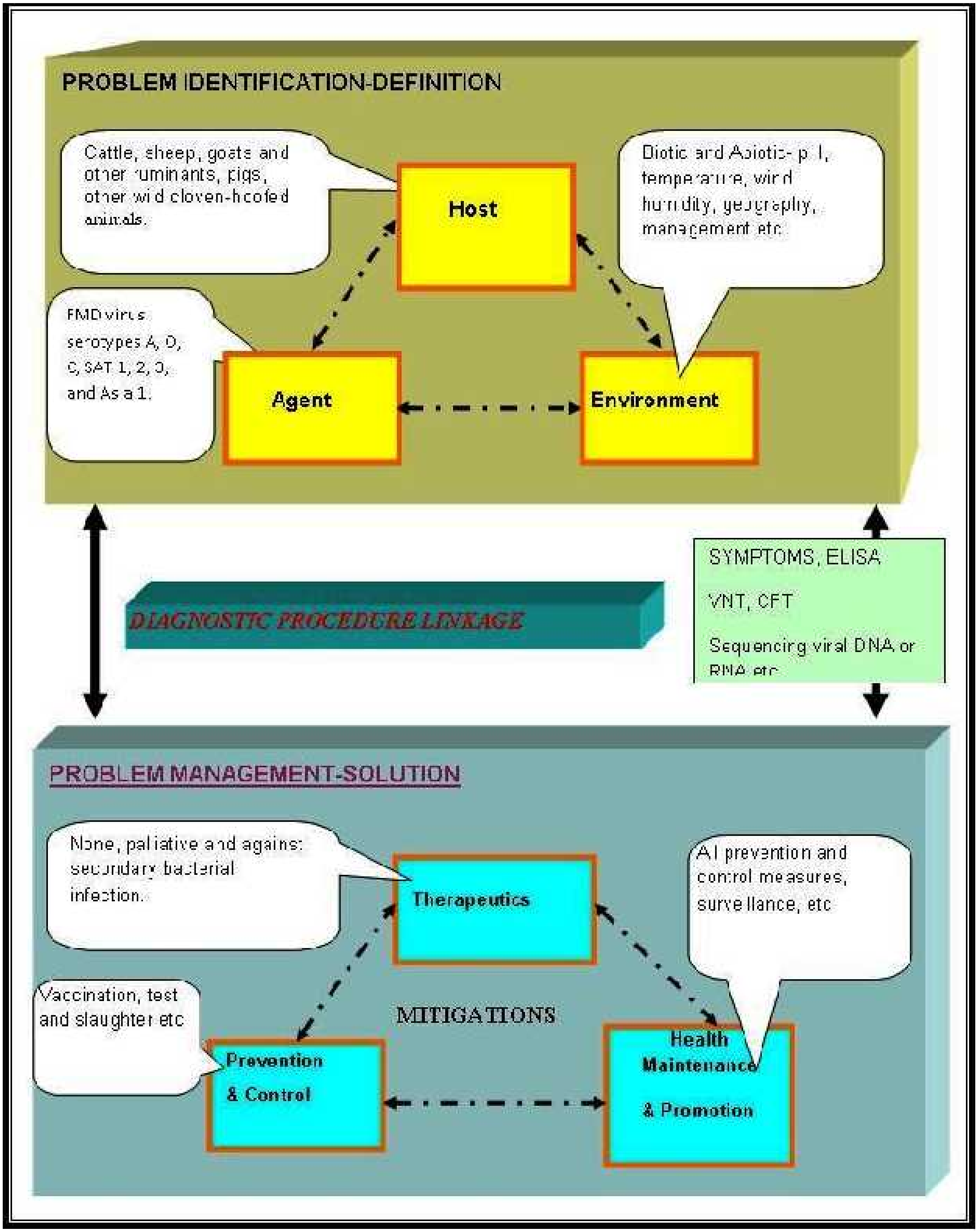


Figure 2 The Epidemiological Problem Oriented Approach

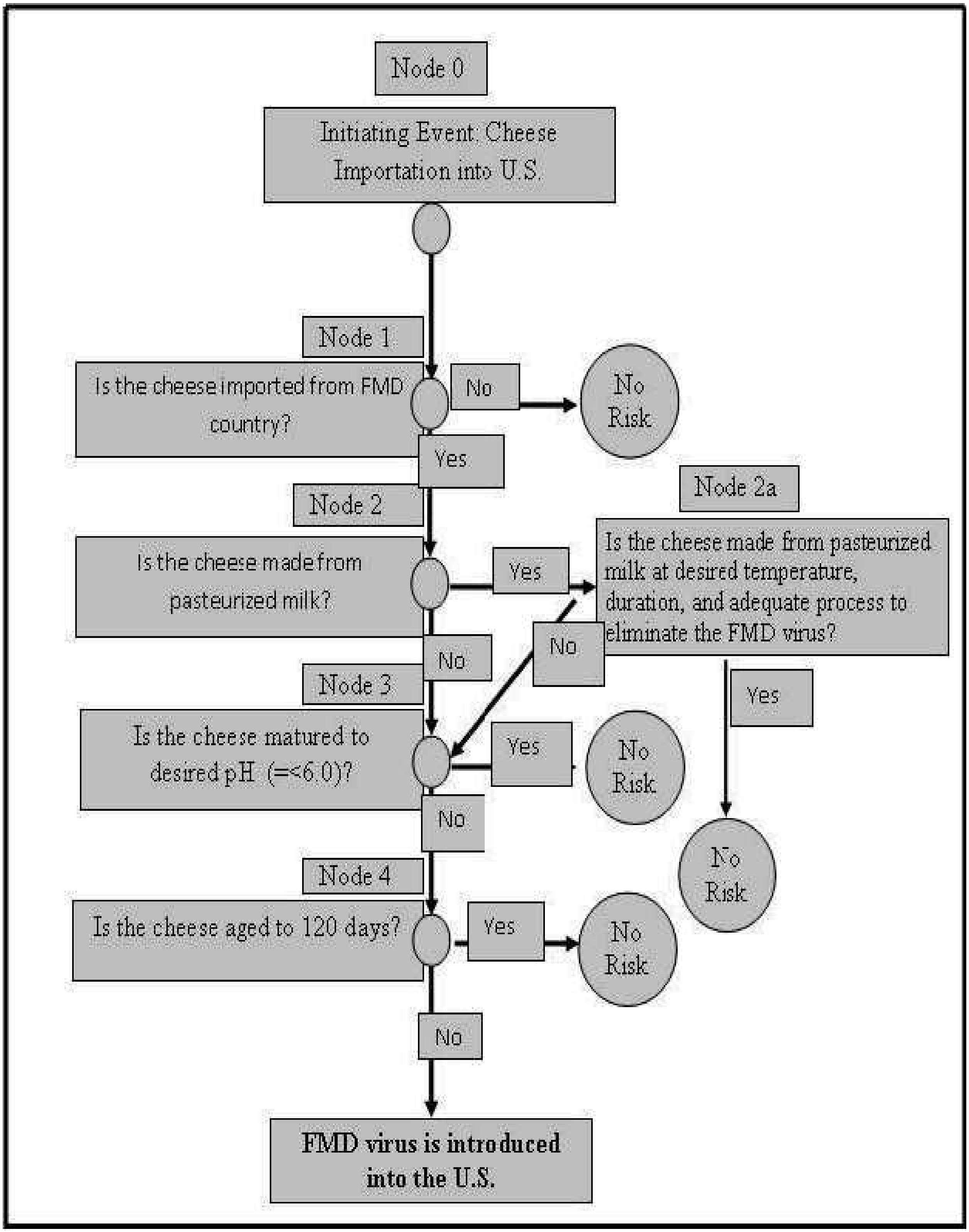


Figure 3 Scenario Tree for FMD virus introduction into the USA via cheese imports.

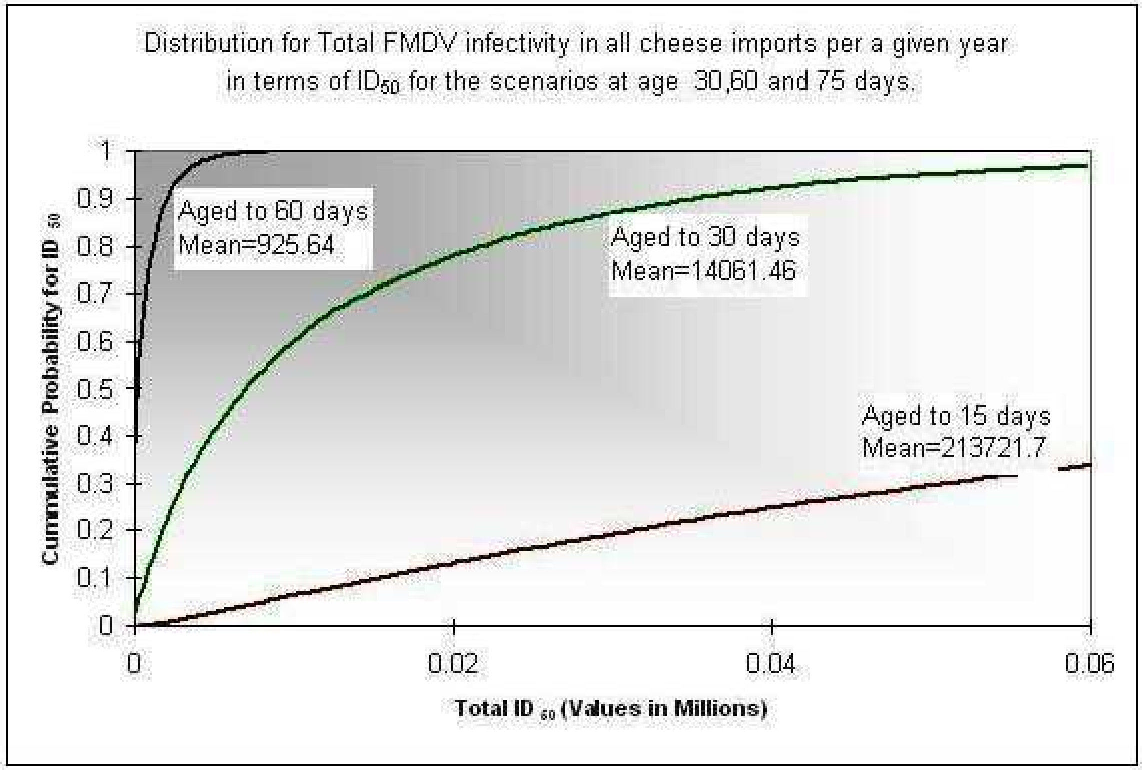


Figure 4 @Risk distribution of FMD total virus infectivity in terms of ID_{50} in all imported cheese: scenario at age 15, 30, and 60 days.

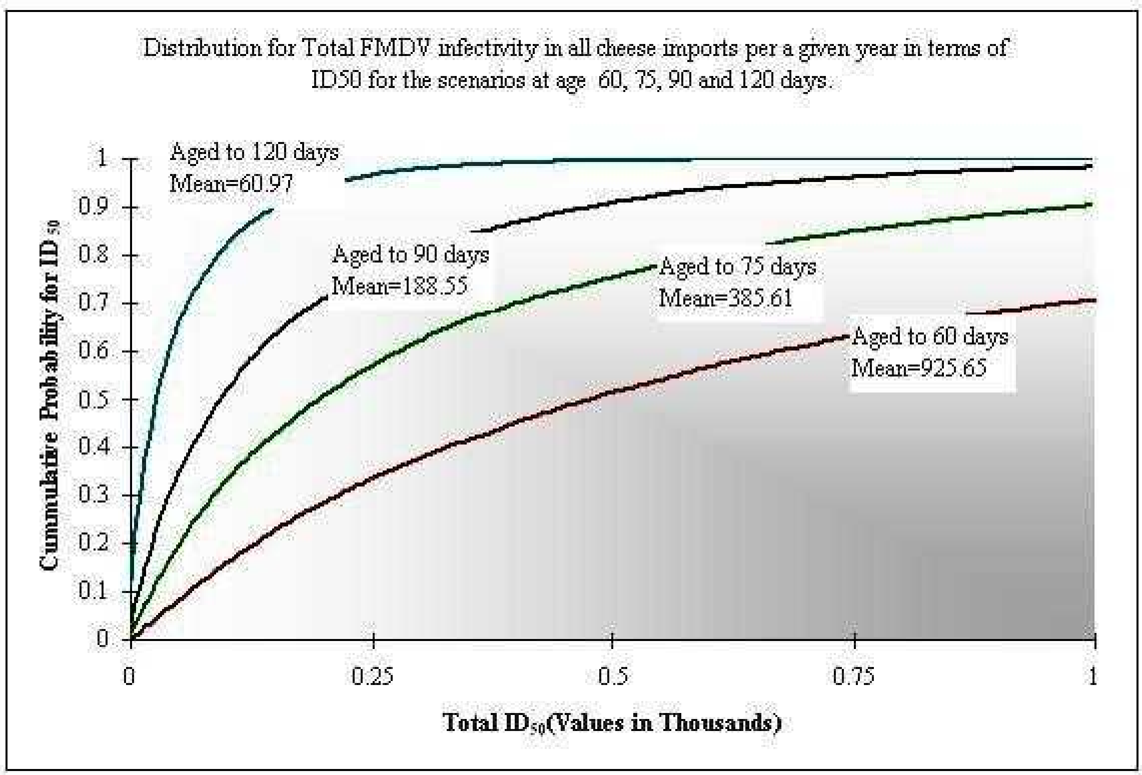
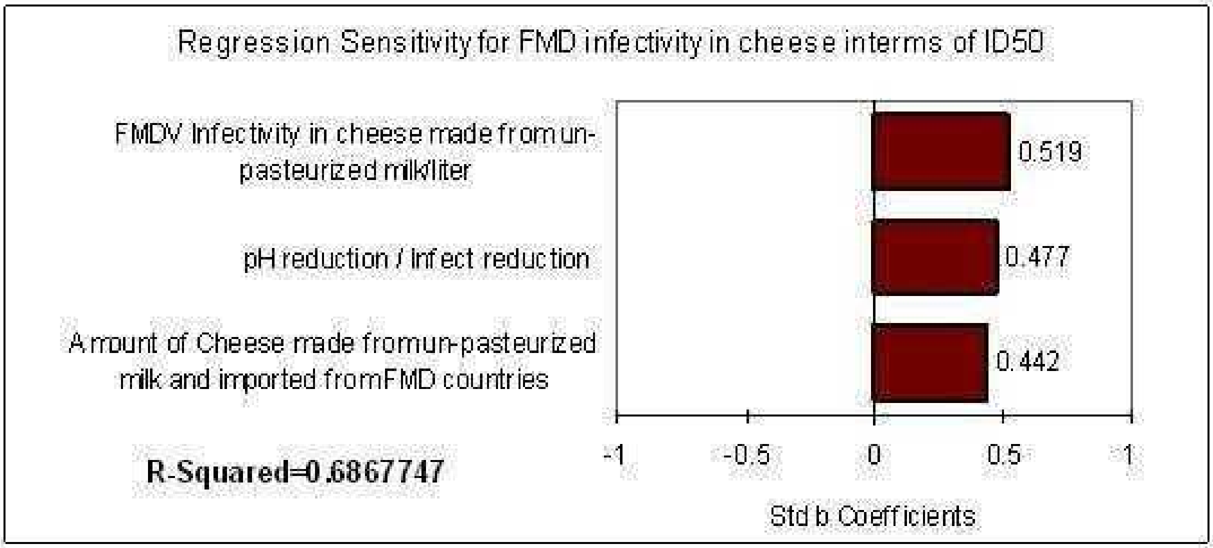


Figure 5 @Risk distribution of FMD total virus infectivity in terms of ID_{50} in all imported cheese: scenario at age 60, 75, 90, and 120 days



Rank for the model Total ID_{50}	Parameter Name	Regression Sensitivity for the model Total ID_{50}
#1	FMDV Infectivity in cheese made from un-pasteurized milk/liter	0.519
#2	pH reduction / Infect reduction	0.477
#3	Amount of Cheese made from un-pasteurized milk	0.442

Figure 6 Sensitivity analysis of FMD infectivity in cheese.

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