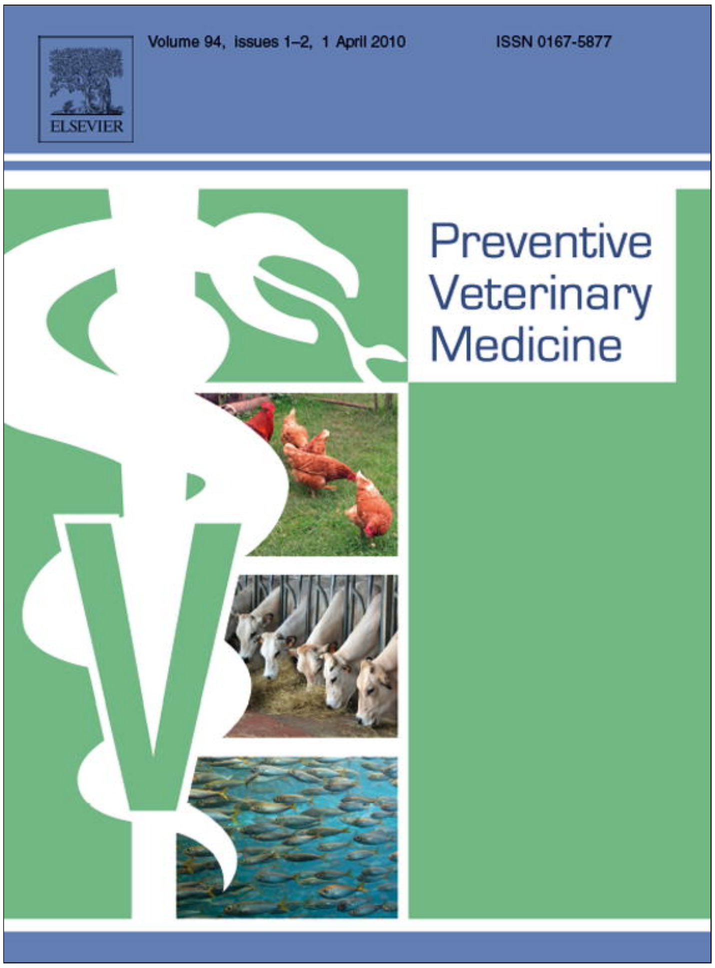
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Interventions to reduce verocytotoxigenic *Escherichia coli* in ground beef in Argentina: A simulation study

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ABSTRACT

A stochastic simulation model was used to assess the effects of measures implemented in the agri-food-chain to reduce the contamination of ground beef with verotoxigenic *Escherichia coli* (VTEC). A published risk assessment model developed in Argentina was used as baseline scenario. Control measures assessed were based on either a reduction in herd prevalence of infection due to vaccination, reduction in opportunity for cross-contamination in the slaughterhouses by the introduction of an on-line hide-wash cabinet, and control of storage temperature in slaughterhouses, retail and home. Additionally, the increase of feedlot production was modelled. Simulations suggested that the greatest potential impact was associated with hide-wash cabinet and vaccination, measures aimed to reduce the VTEC prevalence and concentration in the cattle hides at the beginning of the food-chain. Control of storage temperature was not effective if the carcasses cross-contamination with the pathogen was not prevented or reduced. An increase production (fattening) of cattle in feedlots may raise the risk of VTEC infection and its sequelae. This information can be used as a basis for measures of risk management.

1. Introduction

Verocytotoxigenic *Escherichia coli* (VTEC), is an important zoonotic pathogen, causing uncomplicated or bloody diarrhoea (BD) and in a small percentage of cases, haemolytic uremic syndrome (HUS) (Paton and Paton, 2000). *E. coli* O157:H7 was first identified as a human pathogen in 1982 and currently it is the most prevalent VTEC serotype. However, other VTEC serotypes have been reported to cause outbreaks or sporadic cases of BD and HUS in many countries (Oteiza et al., 2006). A variety of foods have been implicated in those outbreaks, but raw or undercooked meals of bovine origin are primarily responsible. VTEC is present in the faeces and intestines of healthy bovines and can contaminate meat during slaughter (Meichtri et al., 2004; Omisakin et al., 2003; Padola et al., 2002).

In Argentina, HUS is endemic and approximately 400 new cases are reported each year by hospital nephrology units. The majority (95%) of HUS cases are identified in children less than 5 years old, and in 2002, the annual incidence rate reached 12.2/100,000. In children, HUS is the leading cause of acute renal failure and the second leading cause of chronic renal failure. Approximately 30% of children receiving kidney transplants suffered from HUS (Ministerio de Salud y Ambiente de la Nación, 2005).

Many risk factors were identified in epidemiologic studies (Hussein and Bollinger, 2005; Hancock et al., 2001; Elder et al., 2000). Intensification of farming (including modifications of husbandry practices, increased herd sizes and number of housed animals), has resulted in increased quantities of livestock waste, increased animal contact and stress, factors that have creating a niche in which VTEC can survive (Garber et al., 1995). The principal sources of

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contamination of carcasses following slaughter are associated with hide removal, including faeces and hide contact (Koohmaraie et al., 2007; Bosilevac et al., 2005). In a large number of the reported outbreaks the infection was attributed to consumption of undercooked ground beef or other beef products (Hussein and Bollinger, 2005; Rivas et al., 2003; López et al., 1997).

Understanding the transmission and survival process of food-borne pathogens in a complex system requires a framework to cover all aspects. Moreover, evaluating the interventions interrupts the routine practices and is often costly. Thus, before control measures can be incorporated, research (simulation model) is needed to identify which practices are likely effective, assuming that the model is valid and will identify and capture the most important aspects of the process (Vosough-Ahmadi et al., 2007). The scenario analysis considers the effect of changing parameters for specific factors (model inputs) and examines their effect on intermediate model outputs (occurrence and extent of VTEC contamination, probability of infection, HUS and mortality).

The objective of this study was to develop a stochastic model to investigate the likely benefit of some strategies for controlling the contamination of ground beef with VTEC with the aim to improve the basis for deciding on policy and research objectives for reducing the human health hazard due to VTEC in beef products. Additionally, the impact of an increased production of cattle in intensive systems (feedlot) on the outcomes variables was modelled.

2. Materials and methods

2.1. Model background

The base model used in this study has been previously described (Signorini and Tarabla, 2009). Briefly, the prevalence and counts of VTEC were modelled at various stages along the agri-food beef chain. The start point for the exposure model was the prevalence and concentration of VTEC in cattle faeces. The data used to describe prevalence and distribution of the pathogen in cattle faeces were based on a number of studies carried out in Argentina and ranged from 28.9% to 62.7%. Data on the concentration of VTEC in cattle hide was based on the information reported by O'Brien et al. (2005) and ranged from undetectable to 6.0 log UFC/100 cm^2. Processing was defined as the operations covered between the arrivals of live cattle to carcass storage in the refrigerator. Post-processing was defined as the elapsed time between processing plant departure of fresh ground beef/hamburger and the time of consumption. In the final preparation stage cooking practices were based on consumer survey data in Argentina indicating approximately 90% of consumers prepared well-done hamburgers. The amount of hamburgers ingested in a single serving was assumed to be PERT distributed with a "most-likely" value of 83 g and minimum and maximum values of 60 g and 105 g, respectively. The amounts of hamburger ingested by adults and children were modelled using Argentinean survey data.

The exposure model calculated that the mean pathogen doses ingested by adults and children were 30.96 CFU/kg hamburgers and 81.30 CFU/kg hamburgers, respectively. The model predicted the prevalence of hamburgers contaminated with the pathogen to be 1.82% (95% CI; 2.55×10^{-8}% to 14.9%).

The dose response model was a Beta-Poisson reported by Strachan et al. (2005), which uses *E. coli* O157 outbreak data and data from published studies; it assumes a non-threshold level of illness.

The model was created in Microsoft Excel 2007 with the add-on package @Risk (version 4.0, Palisade Corporation, New York, USA). The model was developed using inputs derived from Argentinean data, survey information and expert opinion, whenever possible. When Argentinean-specific data were not available, international data and scientific literature were consulted to improve the basis for the model.

In this base model, the risk of VTEC infection and its subsequent outcomes was sensitive to type of meat storage at home ($r = -0.416$), slaughterhouse cooling conditions ($r = 0.240$), bacterial concentration in the cattle hide ($r = 0.239$) and retail storage conditions ($r = -0.110$). Because this risk assessment involves complicated relationships among model inputs, scenario analysis illustrates the effect of changing model inputs on model outputs along the farm-to-table. The analysis is conducted by developing different scenarios where some model inputs (cattle prevalence, hide-wash interventions, storage temperature) were intentionally changed and the resultant outputs compared with the baseline model outputs.

2.2. Mechanisms of control

The risk control measures assessed were: cattle vaccination, hide-wash cabinet in the slaughterhouses, control of storage temperature and a modification in the cattle fattening system used in Argentina. These control strategies were chosen based on the results of sensitivity analysis performed on the basis model (Signorini and Tarabla, 2009) and the research progress. The basis model parameters and the scenarios parameters are shown in Table 1.

2.2.1. Vaccination

Cattle are the primary reservoir for many VTEC serotypes and have been implicated in most disease outbreaks through contamination of food products or the environment. As such, cattle vaccination could be a logical pre-slaughter intervention to reduce the risk of human exposure. Many authors (Cataldi et al., 2008; McNeilly et al., 2008; Dziva et al., 2007; Potter et al., 2004) reported the success of vaccination on reducing the VTEC prevalence among cattle and the log number of CFU bacteria/gram of faeces of calves.

Firstly, we assumed that the proportion of herd managers complying with recommendations on vaccine (Pr_{vac}) usage was 80–95%. Secondly, the cattle prevalence reduction and the VTEC concentration in faeces in the vaccinated animals were obtained using the data reported by Potter et al. (2004), who used an *E. coli* O157:H7 strain for the vaccine production. The present study assumed that the reduction in the VTEC prevalence would be similar.

Table 1
Baseline and scenarios model parameters.

Scenario	Baseline model parameters	Scenario model parameters
Scenario 1: vaccination		
• Probability of vaccination (Pr_{vac})		• Uniform (0.80,0.95)
• Heard vaccinated		• Binomial (1,Pr_{vac})
• VTEC presence in unvaccinated cattle ($VTEC_{unv}$)		• Beta (20 + 1,92 – 20 + 1) (Potter et al., 2004)
• VTEC presence in vaccinated cattle ($VTEC_{vac}$)		• Beta (9 + 1,100 – 9 + 1) (Potter et al., 2004)
• Cattle prevalence reduction rate (P_{red})		• P_{vac}/P_{unv}
• Prevalence in vaccinated cattle (P_{vac})	• Prevalence of *E. coli* VTEC-associated with HUS ($P_{f\text{-}HUS}$)	• $P_{f\text{-}HUS} - (P_{f\text{-}HUS} \times P_{red})$
• Initial levels of bacteria in animal hides (I_h) (log UFC/100 cm^2)	• Cumulative (−3,6,{−0.0473;1;2;3;4;5;6}, {0.247;0.587;0.899;0.9816;0.9908;1;1}) (O'Brien et al., 2005)	• Cumulative (−4,5,{−1.0473;0;1;2;3;4;5}, {0.247;0.587;0.899;0.9816;0.9908;1;1})
Scenario 2: on-line hide-wash cabinet		
• Slaughterhouses that introduced the cabinet (C_{sla})		• Uniform (0.4,0.6)
• VTEC prevalence before the cabinet (P_{bcab})		• Beta (44 + 1,99 – 44 + 1) (Bosilevac et al., 2005)
• VTEC prevalence after the cabinet (P_{acab})		• Beta (15 + 1,92 – 15 + 1) (Bosilevac et al., 2005)
• Decontamination rate (D_{rate})		P_{acab}/P_{bcab}
• Prevalence in hide ($P_{f\text{-}HUS}$)	• Prevalence of *E. coli* VTEC-associated with HUS	• $P_{hwas} = P_{f\text{-}HUS} - (P_{f\text{-}HUS} \times D_{rate})$
• Reduction in VTEC concentration (R_{vtec}) (log UFC/100 cm^2)		• Normal ((uniform (2.1,3.4)),0.1)
Scenario 3: storage temperature		
• Refrigeration retail storage temperature (T_{rs}) (°C)	• Triangular (4,6,10)	• Triangular (4,6,8)
• Refrigeration temperature in house (T_{house}) (°C)	• Triangular (5,8,15)	• Triangular (4,6,8)
Scenario 4: feedlot production		
• Proportion of feedlot (Pr_{fl})	• 0.2 (Rearte, 2007)	• Uniform (0.5,0.6)
• VTEC prevalence in pasture fattening system (P_{pfs})	• Beta (173,442 – 172 + 1)	• Beta (173,442 – 172 + 1) (Meichtri et al., 2004; Notario et al., 2000; Sanz et al., 1998)
• VTEC prevalence in feedlot (P_{fl})	• Beta (37 + 1,59 – 37 + 1)	• Beta (37 + 1,59 – 37 + 1) (Padola et al., 2004)

Prevalence in cattle vaccinated ($VTEC_{vac}$) and unvaccinated ($VTEC_{unv}$) was modelled using a beta-distribution to obtain a prevalence reduction rate (P_{red}). Finally, we assumed that the concentration of VTEC in the faeces of infected animals in vaccinated herds (I_{hvac}) was 10-fold less than that of unvaccinated herds (I_{hunvac}), according with results reported by Potter et al. (2004) and used by Vosough-Ahmadi et al. (2007). The VTEC reduction in hide was assumed as the same magnitude.

2.2.2. Hide-wash cabinet

Cattle hides are major sources of beef carcass contamination during processing, since the pathogens are transferred from the hide to the carcass and it is a source of cross-contamination (Elder et al., 2000). Processes that effectively clean the hides before hide removal are successful in lowering carcass microbial contamination (Bosilevac et al., 2005). There are many strategies to mitigate microbial contamination on carcasses such as organic acid washes or steam pasteurization that can be implemented during the slaughter process that have proven benefits, but the Argentinean meat regulation only allows the use of potable water (CAA, 1998).

The use of an on-line hide-wash cabinet that used a sodium hydroxide wash and a chlorinated water rinse was modelled using the data provided by Bosilevac et al. (2005). The scenario model considered that approximately 40–60% of slaughterhouses introduced the hide-wash cabinet in the slaughter line (C_{sla}). This hide-wash cabinet reduced the *E. coli* O157 on hides from 44% to 16%. Two beta-distributions were used, assuming that VTEC strains have the same pattern than *E. coli* O157, to model the pathogen prevalence in hide before (P_{bcab}) and after (P_{acab}) passage through the cabinet to obtain the decontamination rate (D_{rate}). The reduction in the pathogen concentration (R_{vtec}) was modelled using a normal distribution with mean of 2.1–3.4 log CFU/100 cm^2 (uniform distribution to model the uncertain parameter) and standard deviation of 0.1 (Bosilevac et al., 2005).

2.2.3. Storage temperature

One of the most important factors correlated with the risk of VTEC infection was the storage temperature during the retail and home storage (Signorini and Tarabla, 2009). A scenario model was developed considering a strictly temperature control (less than 8 °C) during the ground beef and hamburger distribution.

2.2.4. Feedlot production

Feedlot cattle are housed under high density conditions (sometimes without efficient systems for manure removal), providing ample VTEC opportunity to persist within the herd. A study reported by Padola et al. (2004) showed that the VTEC prevalence in feedlot cattle (P_{fl}) was 62.7% whereas in animals fed on pastures the cattle prevalence (P_{pfs}) was 38.9% (Meichtri et al., 2004; Notario et al., 2000;

Sanz et al., 1998). Two beta-distributions were used to incorporate the variability and uncertainty in the VTEC prevalence in both fattening systems.

Traditional beef production in Argentina relies mainly on natural or cultivated pastures, with the usage of concentrates only for short periods of time at the end of fattening. However, this system is being threatened by the progress of agriculture and the displacement of farming into less suitable ecological areas, generating a shift towards more intensive farming systems such as fattening in feedlots. Nowadays in Argentina, about 20% of slaughtered animals come from feedlots (Pr_{fl}), targeted to the domestic market (Rearte, 2007). In the present scenario analysis, the effect of an increase of this phenomenon was modelled, simulating that 50–60% of cattle were fattened in feedlot.

2.3. Scenario analysis

The scenarios models described above were created in Microsoft Excel 2007 with the add-on package @Risk and were each simulated for 5000 iterations and their results were contrasted with the base scenario by comparing the distribution of the infection, HUS and mortality per meal, considered the most important outputs of the model. The number of iterations provided adequate convergence of the simulation statistics (<1%).

3. Results

The introduction of a vaccine for VTEC applied to the cattle, induced a reduction in the pathogen prevalence in animal faeces, therefore a reduction in microbial load on the animal hide, and subsequently a great decrease on the extent of contamination of carcasses with VTEC before processing compared to the base scenario. The lower bacterial load at the beginning of the food-chain leaded to a lower risk (about 0.5 log over the base model) to suffer VTEC infection, HUS, chronic renal failure and mortality (Table 2).

The risk management strategy that generated better results in the simulation model was the introduction of an on-line hide-wash cabinet. The significant reduction of microbial load in the cattle hide slaughtered, reduced the likelihood of cross-contamination. This lower load at the beginning of the process was maintained along the food-chain, meaning less pathogen exposure by the consumers. Therefore, the risk of VTEC infection, HUS, chronic renal failure and mortality was approximately 1 log lower in the model that simulated the introduction of an on-line hide-wash cabinet over the baseline model (Table 2). This means that, according with the risk model, 100,000,000 hamburger servings consumed in Argentina would generate about 1.7 cases of HUS. If the hide-wash cabinets were introduced in at least 40–60% of the Argentinean slaughterhouses, the number of servings consumed to generate the same number of HUS cases would be 10-times higher (Figs. 1 and 2).

According to the simulation model, if the prevalence of animals shedding VTEC in their faeces and the pathogen concentration on the carcasses cannot be avoided, it would

Table 2
Simulation model predictions (means and confidence interval 95% after 5000 iterations) of the most important output distribution.

Output	Scenarios mean (95% CI)				
	Basic model	Vaccination	Hide-wash cabinet	Storage temperature	Feedlot production
Prevalence of contaminated carcasses	0.28 (0.21 to 0.35)	0.17 (0.04 to 0.31)	0.20 (0.08 to 0.34)	0.28 (0.21 to 0.35)	0.36 (0.22 to 0.51)
VTEC concentration in bovine hide (log CFU/cm^2)	−2.59 (−6.31 to 0.50)	−3.48 (−7.33 to 0.23)	−5.34 (−9.19 to 2.20)	−2.59 (−6.29 to 0.5)	−2.59 (−6.37 to 0.49)
VTEC concentration in carcass before processing (log CFU)	0.15 (−4.15 to 4.11)	−0.72 (−5.22 to 3.44)	−2.59 (−7.20 to 1.45)	0.15 (−4.18 to 4.12)	0.15 (−4.20 to 4.18)
VTEC concentration in carcass before cooking (log CFU/g)	−2.93 (−5.64 to 0.56)	−3.35 (−5.79 to 0.08)	−3.94 (−5.98 to 1.23)	−2.94 (−5.67 to 0.4)	−2.84 (−5.59 to 0.56)
Probability for VTEC infection in adults (log)	−6.1 (−8.99 to 2.38)	−6.52 (−9.09 to 2.94)	−7.11 (−9.38 to 3.73)	−6.11 (−9.05 to 2.46)	−6.01 (−8.94 to 2.31)
Probability for VTEC infection in children (log)	−6.51 (−9.41 to 2.81)	−6.93 (−9.56 to 3.36)	−7.52 (−9.75 to 4.11)	−6.52 (−9.4 to 2.86)	−6.43 (−9.34 to 2.78)
Probability for HUS (log)	−7.75 (−10.68 to 3.96)	−8.18 (−10.79 to 4.6)	−8.75 (−10.95 to 5.41)	−7.76 (−10.70 to 4.08)	−7.67 (−10.61 to 3.96)
Mortality in children (log)	−9.22 (−12.16 to 5.56)	−9.64 (−12.31 to 6.12)	−10.22 (−12.46 to 6.83)	−9.23 (−12.18 to 6.63)	−9.14 (−12.1 to 5.42)
Probability for chronic renal failure (log)	−9.05 (−11.99 to 5.37)	−9.48 (−12.09 to 5.9)	−10.05 (−12.25 to 6.71)	−9.06 (12.00 to 5.38)	−8.97 (−11.91 to 5.26)

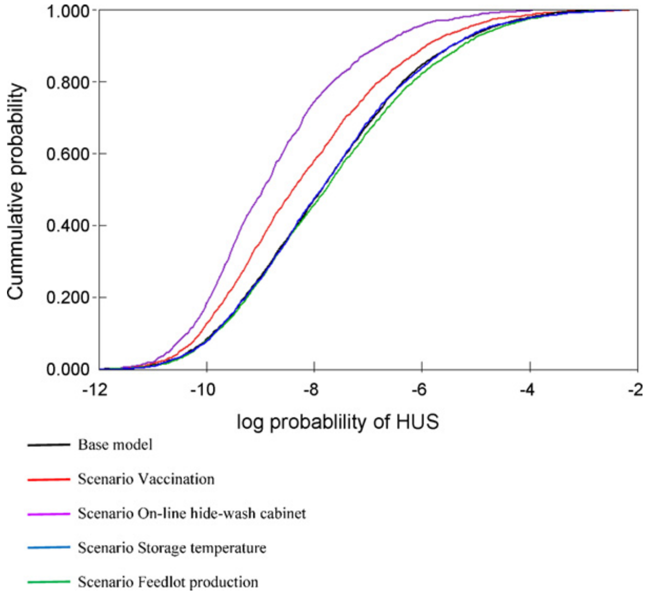

Fig. 1. Cumulative probability distribution for the probabilities of HUS for each scenario.

be difficult to control the microbial growth in the later stages of the process. When the temperature of hamburger processing, storage and sale did not exceed 8 °C, the final exposure of VTEC was similar than in the baseline model (Table 2).

If the international trade of commodities continues with the actual trend, agriculture (especially soybean and corn tillage) will increase its surface and forcing the movement of livestock to less suitable areas and increased fattening of cattle in feedlots. This process would generate an increase in the prevalence of cattle shedding VTEC in their faeces (approximately 30%) at slaughterhouses. Under this assumption, 100,000,000 hamburger servings would generate, on average, 2.1 cases of HUS, which is approximately 25% more cases than those under the current conditions (Table 2).

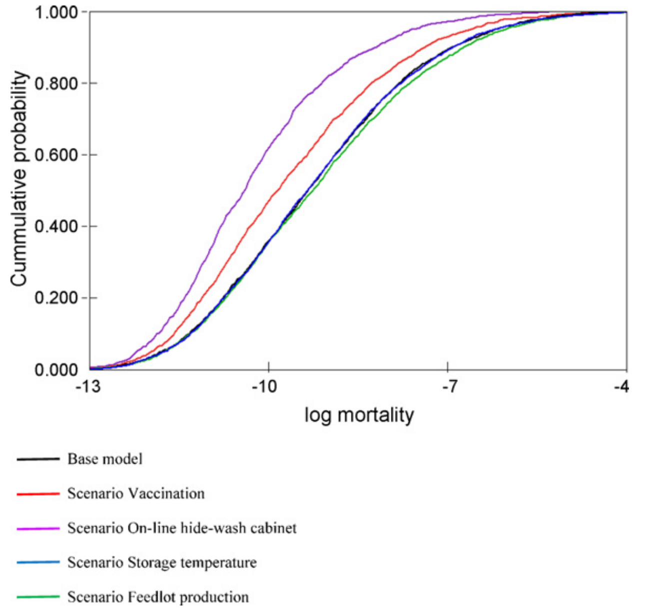

Fig. 2. Cumulative probability distribution for the mortality of HUS for each scenario.

4. Discussion

In this study, we evaluated the effectiveness of three interventions in reducing the prevalence of VTEC in cattle using a stochastic simulation model. Additionally, we assessed a scenario of increasing feedlot production. Simulation models applied in this study aims to generate scenarios to serve the basis for determining which would be the most effective risk management measures to reduce the risk for VTEC infection and which would require further research and technological development. The scenarios were chosen from results reported by a sensitivity analysis applied on the baseline model in an earlier study (Signorini and Tarabla, 2009). Another criterion considered for the choice of the risk management measures was research progress, without any consideration about the feasibility of their implementations or cost-effectiveness advantages.

According to this sensitivity analysis, one of the most important factors on the risk of VTEC infection or its sequelae, was the pathogen concentration on cattle hides. Therefore, it is reasonable that the most significant scenarios were focused on pre-slaughter, reducing both, the prevalence and the VTEC load on cattle hide. Some authors (Arthur et al., 2007; Dewell et al., 2008) have shown that transportation can impact on VTEC hide concentration, but there are many others (Fegan et al., 2009; Schuehle-Pfeiffer et al., 2009; Rice et al., 1997; Cornell, 1998) that did not find this association and the evidence suggests that there is no significant difference in faecal prevalence between the farm and slaughter plant and the duration of transportation was not associated with faecal positive status. Based in these papers and taking into account the discrepancy between authors, transportation effect was not incorporated in the base model.

Inhibition of intestinal colonization may be a good strategy for the development of human and bovine VTEC vaccines. Although the data used in this simulation model were based on vaccines against *E. coli* O157:H7, the implications of the results could be extended to different strains of VTEC. Many vaccines are being investigated using different VTEC virulence factors (Cataldi et al., 2008; McNeilly et al., 2008; Dziva et al., 2007; Potter et al., 2004) and develop effective vaccines for the reduction of colonization of cattle by VTEC. It is presumed that cattle vaccination would generate both, a reduction in the VTEC prevalence and in the VTEC concentration in faeces. However, it is difficult to apply this type of measures, especially considering that the infection of the gastrointestinal tract of adult cattle, weaned calves and 5-day-old gnotobiotic calves by VTEC serotype O157:H7 is normally asymptomatic (Stevens et al., 2002). Hence, VTEC vaccination would not imply any improvement on cattle production as itself, but only a reduction in the risk of pathogen exposure. Therefore, to impose a cattle vaccination system would require an intense public awareness campaign or free of charge vaccination to all animals. Another risk management measure could be the introduction of probiotic bacteria that compete with or are inhibitory to the target organism (Hancock et al., 2001).

In this study, the process that effectively cleaned the hides before its removal (on-line hide-wash cabinet) was successful in lowering carcass microbial contamination and therefore considerably reduced the risk of VTEC infection and its sequelae. Another advantage of this system is that it operates on all the microorganisms present in the cattle hide. The impact generated on the public health is greater than the costs of installation and maintenance of equipment. The use of a hide-wash cabinet requires large volumes of water and therefore, a recirculation of the wash and rinse compounds are required to be cost effective. This intervention may not be suitable for all beef processing plants to implement, due to cost and space restrictions (Koohmaraie et al., 2007). The usefulness of this system would be based on VTEC prevalence in cattle herds. Under the current conditions of high prevalence it may prove useful to reduce the pathogen exposure. However, its effectiveness should also be assessed under a scenario of low VTEC prevalence.

If any risk management measure to reduce the number of cattle shedding VTEC in their feaces in the slaughterhouses were introduced, the pathogen could still enter into the food-chain and continue to grow. Cross-contamination of whole carcasses with faecal-derived bacteria due to airborne transmission, contaminated equipment or carcasses is considered unavoidable (Jordan et al., 1999; Cassin et al., 1998). Therefore, it would be very difficult to minimize the public health risk through the strictly control of temperature of production, distribution and storage of ground beef without applying pre-slaughter measures. The greatest benefits from pre-slaughter control of VTEC will occur when the post-slaughter controls are highly effective at reducing microbial growth, especially when the storage temperature of ground beef throughout the food-chain (in the slaughterhouses, cutting and deboning, retail and in home) are under control.

Currently, Argentina still has production characteristics that allow cattle to be fattened on pastures, rendering several advantages in terms of beef food safety. However, the increment of agricultural land is a major challenge for the achievement of enough beef production to supply both domestic and international demands, without encouraging an increase in beef production on feedlot (Rearte, 2007). One of the most important characteristics of the latter is the high cattle concentration, generating higher VTEC prevalence than those fattened in the traditional system (Garber et al., 1995; Padola et al., 2004). Therefore, the scenario presented in this study is highly feasible in the comings years in Argentina.

Many mitigation strategies such as organic acid washes and steam pasteurization have been proved beneficial in reducing pathogen contamination on carcasses during slaughter. Therefore, researchers and policy makers need to consider the shortcomings of any model before applying the results within the slaughterhouse.

5. Conclusions

The pre-slaughter interventions such as vaccination and the measures aimed to reduce or suppress the shedding of VTEC in feaces may prove to be useful to reduce the entry of VTEC into the food-chain and hence the risk of VTEC infection in consumers. The most effective management measure to reduce the risk of VTEC infection was the reduction of pathogen load in the cattle hide. Management measures to control the pathogen once present in the meat are inefficient in reducing the risk of zoonoses. The intensification of livestock production could result in greater risk of the public health given the increased exposure to the pathogen generated by these practices.

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