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Title: Incidence of *Mycobacterium avium paratuberculosis* IS900 DNA on beef carcasses at three packing plants.

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Introduction.
Mycobacterium avium subsp. parartuberculosis (Map) is the cause of Johne's disease in cattle and other ruminants. The disease is characterized by a chronic inflammation of the gut tissue leading to clinical symptoms of persistent diarrhoea and weight loss (Buergelt et al., 1978). Crohn's disease in humans has many of the same clinical symptoms of cattle Johne's disease thus leading to speculation that Map may also be the causative agent; although, large numbers of Map bacteria are not recoverable from human patient's with Crohn's disease (Bull et al., 2003). It is now widely believed that exposure of humans to Map should be minimized as a precautionary measure.

Map is very slow growing, requiring 4 to 8 months before culture identification; therefore, the presence of Map is determined using PCR methods. The infective genes in Map that cause Johne's disease have not been fully identified yet. The insertion sequence `IS900' is the most consistently found DNA element associated with Map infections of Johne's disease (Grant et al., 2000). We have used a PCR test for the IS900 DNA element to estimate the prevalence rate of Map on the surface of beef and dairy cattle carcasses in the processing procedure.

Materials and Methods.
The tail region from beef (n = 200) and diary cattle (n = 100) carcasses were wiped with cotton swabs after skinning or after carcass washing from 3 separate plants in North America. The area swabbed per animal was 1000 cm^2. The swabs were frozen with liquid nitrogen and stored at -80C in 50ml polypropylene tubes until DNA extraction. DNA was recovered from the swabs using a DNA extraction solution containing EDTA, lysozyme, proteinase K, SDS, NaCl and CTAB detergents and 20ng of an Internal amplification control (IAC) DNA template. The buffer solution was cleaned by organic extraction with phenol, chloroform and ethanol precipitation. The total recovered DNA from the swab was stored in 50ul of 10mM Tris-HCl, 0.5mM EDTA pH 8.0 buffer.

The Map IS900 sequence was detected using a nested PCR procedure. In the first round of PCR, swab DNA (2 ul) was incubated with multiplexed unlabelled primers (250nM/ea) designed to amplify a 297 nucleotide region of the IS900 gene and a FAM labelled Taqman primer set for the IAC, in a Qiagen multiplex PCR mix, for a total volume of 25uls. PCR was performed on a Stratagene Mx4000 real-time PCR machine for 40 cycles. The second round of PCR was performed with 2ul of the first round PCR solution, mixed with nested IS900 primers (250nM) designed to amplify a 97 nucleotide region of the IS900 gene and including a CY5 labelled Taqman IS900 probe, in a 25ul reaction. PCR was performed on a Stratagene Mx4000 real-time PCR machine for another 40 cycles. Samples were considered `positive' after the Taqman CY5 probe signal achieved a minimum preset signal threshold.

Results and Discussion.
IS900 elements are present at between 14 to 20 copies per Map genome and are generally regarded as specific for Map. The IS900 element was detected by nested PCR on the skinned carcasses in the three Plants at a frequency of: 44% for plant A, 30% for plant B and 54% for plant C which contained the culled dairy cows. The number of positive samples dropped approximately 18% after washing. Generally a positive IS900 test required 50 cycles of PCR before detection, equivalent to $1.13 \times 10^{+15}$ amplification, and was therefore considered presumptive. The swab DNA samples that tested positive for the IS900 nested PCR reaction were then repeated using the IS900 Taqman PCR primer set on 2ul of swab DNA

solution, without pre-amplification to get a quantitative estimate. Only 2 samples were positive using the direct quantitative PCR test for IS900 in the 176 presumptive positive cattle.

Conclusions.
The high frequency of IS900 positive cattle carcasses in the North American herd would indicate that the prevalence of Map bacterial DNA is common. However considering that the level of IS900 DNA was at very low levels requiring nested PCR detection and not by direct quantitative PCR; the number of viable Map bacteria on cattle carcasses after skinning is likely to be very low.

References.
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Table 1.
Sequences and expected PCR product sizes from the oligonucleotide primers used in the quantitative PCR detection M. paratuberculosis DNA.

	Gene	*Primer Sequence (5' à 3)'*	*Product (bp)*	*Ref.*
Mix A				
MAP primary	IS900	forward ATGTGGTTGCTGTGTTGGATGG reverse CCGCCGCAATCAACTCCAG	297 bp	Bull et al., 2003
Internal Amplification Control	LxR	Forward TTCCACTACAACGTGCTGAG Reverse AGGCGGATCTGTTCTTCTG Probe FAM-ATTCTTCCGTCGCAGTGTCATCAAA-BHQ1	205 bp	GenBank# AY170462
Mix B				
MAP nested	IS900	Forward TCGACCGCTAATTGAGAGATGC Reverse CCTCCGTAACCGTCATTGTCC Probe CY5-CCAGCAGACGACCACGCCGACG-BHQ2	95 bp	

Table 2.
The detection of the IS900 element by nested PCR in DNA samples from skinned and washed beef carcasses at three beef packing plants.

Plant	Carcass Type	Processing Stage	Number tested	IS900 nested positive	Nested PCR cycle ave*
A	Culled Beef	Skinned	47	21	14.6abc
		Washed	49	18	13.4bc
	Fed Beef	Skinned	48	21	11.8bc
		Washed	50	20	11.1bc
B	Culled Beef	Skinned	50	19	17.3a
		Washed	46	23	10.9b
	Fed Beef	Skinned	50	11	9.6c
		Washed	50	3	9.2c
C	Culled Dairy	Skinned	46	25	10.0c
		Washed	45	15	10.9b

* The average number of cycles of nested PCR required for positive identification using real-time detection of dual labelled Taqman probe for the IS900 element. Mean values with same letter are not significantly different.