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Hog Mandibular Lymph Node Abnormalities and Bacteriological Contamination at Slaughter in Canada

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Abstract

In the context of meat inspection modernization, the current mandatory incision and visual inspection of all hog mandibular lymph nodes (MLNs) to detect signs of infection by *Mycobacterium bovis*, a zoonotic infection is examined. Canadian data of MLN pathology and microbial contamination are scarce and the performance of the current regulatory inspection of MLNs in actually detecting lesions and contamination by *Mycobacterium bovis* has not been documented. A survey of MLN condition in market hogs according to various inspection schemes was undertaken to fill the data gap. One MLN of a representative sample of 149 483 hogs were submitted to standard pathological and bacteriological analyses. Actual abnormal MLNs actually were rare (below 8%). They are often under detected by the current inspection because of limited sensitivity (18%). Such abnormalities, even undetected, have very limited, if any, impact on human health since the pathological and microbiological analyses failed to provide evidence of *Mycobacterium bovis* infection. On the other hand, MLNs can be contaminated with several bacteria potentially pathogenic to humans, raising the risk of cross-contamination during the inspection with incision. Finally, the current incision and visual inspection of all hog MLNs and the proposed visual-only inspection perform similarly in identifying abnormal MLNs.

Keywords: Campylobacter, Meat inspection, *Mycobacterium bovis*, Public health, Salmonella, Slaughter, *Staphylococcus aureus, Streptococcus suis*, Swine

1. Introduction

The main purpose of meat inspection is to detect and prevent public health hazards such as food-borne pathogens and chemical contaminants in meat. In Canada in 1907 the federal Department of Agriculture inaugurated the Canadian Meat and Canned Food Act which led on to the formation of the Canadian federal meat hygiene services (Derbyshire, 2006). The inspections conducted at this time, and since, have been based on a traditional inspection approach. This is based on organoleptic techniques, whereby hazards in the meat are detected using the senses of sight, touch and smell.

However, there have been significant changes in production animal health, husbandry and their slaughter during the last century since meat inspection processes were established. Hazards to health have changed, but meat inspection has largely followed the same traditional approach. For example, hazards such as tuberculosis and brucellosis have been greatly reduced through control measures. In contrast, current hazards, such as *Salmonella*, *Campylobacter, Streptococcus* and *Yersinia*, are not being adequately addressed. These hazards cannot be detected macroscopically during a traditional meat inspection, resulting in public illness. It is necessary that meat inspection evolves to match the hazards of the current age.

In Canada, the degree of positive impact on food safety by conducting traditional meat inspection procedures of palpation and incision of mandibular lymph nodes (MLN) in slaughter pigs must be examined. Under the current regulation, the Canadian Food Inspection Agency (CFIA) veterinary staff palpates, incises and visually inspects

all MLNs to detect abnormalities. Such abnormalities are referred to as grossly detectable abnormalities (GDA). The specific lesion sought is granulomatous lymphadenitis, which can be a signed of infection by *Mycobacterium bovis*, a zoonotic infection. Such *M. bovis* infections are very rare in Canada (see http://inspection.gc.ca/animals/terrestrial-animals/diseases/reportable/tuberculosis/eng/1330205978967/1330206128556).

There has been a shift in some countries towards modernizing meat inspection and optimizing efficiency and resources based on risk-based approaches (Alban et al., 2008). In the context of this modernization, several countries such as Denmark, the Netherlands and the United Kingdom, have recently modified their pig inspection at slaughter, including the move away from the palpate-and-incise practices of the past to a visual-only inspection of the carcass and incising the lymph nodes only where necessary. Prior to this change, risk-assessments based on domestic data were conducted. In Canada, domestic data of MLN pathology and microbial contamination are scarce and the performance (sensitivity and specificity) of the current regulatory inspection of MLNs in actually detecting lesions and more precisely, signs of *M. bovis* infection, has not been documented over the last 40 years.

To fill this data gap, the CFIA undertook a survey of market hog MLNs with the following objectives: 1) To determine the true prevalence of grossly detectable abnormalities (GDA) in MLNs; 2) To establish the pathological and microbiological profile of normal (with no GDA) vs. abnormal (with GDA) MLNs; 3) To determine the abattoir non-detection rate of GDA in MLNs further to visual examination; and 4) To determine the detection rate of GDA further to visual examination and incision.

2. Materials and Methods

The survey was a Canada-wide cross-sectional study of MLNs in market hogs slaughtered in Canada. The survey took place between January 17th, 2005 and May 17th, 2005, in eight (8) hog abattoirs operating under the CFIA regulations. These abattoirs were selected by the CFIA's red meat specialists to be representative of the Western and the Eastern regions of Canada, after obtaining the voluntary consent of the plant operators and the CFIA veterinary staff in those establishments.

2.1 Sample Collection

In each abattoir, MLNs were systematically collected each Monday and Tuesday morning over a 4-week period. More precisely, each study day (i.e. Monday or Tuesday morning), sample collection was divided into three half-hour periods. During each collection period, 45 MLNs were systematically collected and visually inspected by a CFIA veterinarian for GDA. In case of GDA, the MLN was individually wrapped and set aside for further pathological and microbiological analyses. A feasibility test previously undertaken in one abattoir determined that 45 MLNs by collection period for a total of 135 by morning was the maximum number of MLNs the inspectors and the operations could additionally process for the purposes of the survey without disturbing the normal operations.

In addition to the 45 MLNs, 4 other MLNs were systematically collected during the collection period, individually wrapped and set aside for further pathological and microbiological analyses. These MLNs were collected without inspection and regardless of any abnormalities. Finally, all other MLNs were inspected according to the regulatory veterinary inspection (visual examination and incision) and any inspected MLN that was found abnormal (with GDA) during any collection period was individually wrapped and set aside for further pathological examination.

Because hemorrhagic MLNs are common as the result of pig slaughter, the veterinary staff involved in the MLN inspection and collection under the survey was instructed not to consider hemorrhage as GDA unless the MLN was larger than usual or other lesions were present.

Each day the following MLNs were set aside separately and their total number compiled: MLNs visually inspected (with and without GDA), MLNs not inspected, MLNs with GDA detected by the regulatory veterinary inspection. The number of hogs slaughtered during the three collection periods of the day was also recorded.

2.2 Sample Shipment and Analyses

All the MLNs collected on one morning were identified, refrigerated and shipped overnight to the closest of the two CFIA participating laboratories: in Saint-Hyacinthe, Quebec, for the East and in Calgary, Alberta, for the West. Specimens were kept in a fresh state below 10°C during transportation.

At their arrival at the lab, all MLNs collected were submitted to standard visual examination to detect macroscopic lesions. All MLNs detected with a lesion were further submitted to a standard gross pathological

and histopathological examination by a single team at the Saint-Hyacinthe CFIA laboratory. Finally, some MLNs with granulomatous lesions or other lesions that could be due to a *Mycobacterium* spp. infection were submitted to the Animal Disease Research Institute to confirm the presence of *Mycobacterium* spp. by Gram and Ziehl-Neelsen coloration or by culture.

The MLNs set aside for microbiological analyses were tested for the *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, and *Streptococcus suis*. In addition to those MLNs specifically set aside, all MLNs visually inspected and found with GDA were also tested microbiologically for the same bacteria.

At the laboratory, the MLNs were trimmed of fat, sterilized in surface through boiling 3-5 seconds, weighed, diluted 1/10 in buffered peptone water and homogenized 2 minutes in a stomacher. The homogenate was used for the following analyses. The presence of *Escherichia coli* was detected according to Health Canada MFHPB-35 method (Health Canada, 2001). One mL in duplicate was dispensed onto *E. coli* Petrifilm (3M Canada, London, ON). Plates were incubated at 35°C for 48 ± 2h. The selected and enumerated colonies were the blue ones with gas bubbles. The presence and number of *Staphylococcus aureus* was detected following Health Canada MFHPB-21 method (Health Canada, 2005). Duplicate of 200 µL was spread on the surface of Baird-Parker agar (Fisher Scientific Company, Ottawa, Ontario) and incubated at 35°C over 48 ± 2h. Typical colonies were enumerated and confirmed with the coagulase test. The presence of *Salmonella enterica* was detected following Health Canada MFLP-75 method (Health Canada, 2004). The homogenate was incubated at 35°C over 18-24h. An aliquot of 100 µL enriched broth was dispersed onto a MSRV agar and incubated 24-72 h at 42°C. Sample was suspect when the migration zone was greater than 20mm. Confirmation was done through the TSI, LIA and urea tests, whereas identification was undertaken using GN VITEK®2 (bioMérieux, Ville Saint-Laurent, Quebec, Canada). For *Yersinia*, the Health Canada MFLP-48 method was followed (Health Canada, 1986). A mix of 10 mL homogenate and 10 mL sorbitol broth was incubated at 22-25°C for 5 days. An alkaline treatment was achieved by mixing 0.5 mL of enriched homogenate with 0.5 mL of alkali solution. Immediately afterwards, the broth was spread with a loop on a MacConkey agar and a CIN agar. Typical colonies were colorless on the MacConkey agar and dark red with a colorless edge on the CIN agar. Confirmation was based on the biochemical profile on TSI, LIA and urea medium, the oxydase test and the mobility test at 22°C and 35°C. Identification was undertaken using GN VITEK®2 (bioMérieux, Ville Saint-Laurent, Quebec, Canada). The detection of *Streptococcus suis* was done as follows (Euzéby, 2001). 10µL homogenate was dispersed onto a Columbia agar with sheep blood agar and incubated at 35°C over 24 ± 2h with 5% of CO_2. Small, gray, alpha-haemolytic colonies were considered suspected. Gram coloration, amylase test and Voges-Proskauer test were used for confirmation whereas the identification was undertaken using the API STREP kit (bioMérieux, Ville Saint-Laurent, Quebec, Canada). The detection of *Campylobacter* spp. followed the U.S. Food and Drug Administration guideline (Hunt, Abeyta, & Tran, 2001). A mix of 5 mL homogenate and 5 mL Bolton broth was incubated at 35°C over 4h and then at 42°C over 44h. The enriched broth was spread with a loop on a CCDA agar and on a Preston agar. Suspected colonies were gray on CCDA agar and beige on Preston agar. Suspected colonies were confirmed based on the catalase and mobility tests and identified using the API CAMPY kit (bioMérieux, Ville Saint-Laurent, Quebec, Canada).

2.3 Data Management and Analysis

Data were compiled into an Access database and analyzed using the SAS version 9.3 software (SAS Institute, Cary, NC, USA). After usual raw data checking, the MLNs were cross-tabulated by GDA (detected during the inspection at the abattoir) and the actual presence of lesion as confirmed by the laboratory. The cross-tabulation was made separately by region and by the type of MLNs collected (i.e. visually inspected, not inspected and regulatory inspected). The Mantel-Haenszel test was used to test the difference related to the regions in the cross-tabulation of the MLNs visually inspected. The lesions confirmed by the laboratory being considered as the gold standard, the usual apparent and true prevalences of macroscopic lesion were computed with their exact confidence intervals. The epidemiological sensitivity and specificity of the detection of GDA in MLNs at the veterinary inspection point as actual lesion were computed with their exact confidence intervals using the visually inspected MLN data. Some non- or false detection rates were also estimated: the proportion of false negatives under the visual-only inspection (MLNs without GDA after the visual inspection that actually have a laboratory confirmed lesion); the proportion of true positives under the regulatory inspection (MLNs with a laboratory confirmed lesion among those detected with GDA following the regulatory inspection) and the proportion of false positives under the regulatory inspection (MLNs without laboratory confirmed lesion among those detected with GDA following the regulatory inspection). Relative frequencies and their exact confidence intervals were computed by region for each specific lesion as diagnosed after the gross pathological examination,

the number of different lesions, the contamination by each targeted bacterial species and the number of various bacterial species. Finally, the contamination frequencies and exact confidence intervals were computed for the MLNs with and without actual macroscopic lesion to explore the association between anatomo-pathological defect and bacterial contamination. Unless specified otherwise, all differences were claimed statistically significant based on the confidence intervals.

3. Results

Over all collection periods, 149,483 hog carcasses were processed in the eight participating abattoirs. In total 8,700 MLNs were visually inspected for GDA and collected for laboratory analyses, 527 MLNs were collected without any inspection for laboratory analyses, whereas the other MLNs underwent the regulatory inspection (visual examination and incision) resulting in 508 MLNs being considered as having GDA and set aside for laboratory analyses. Actually, 16 MLNs were not sent to laboratory, another 12 arrived at the laboratory at a temperature above 10°C and were not further analyzed. In addition, inconsistencies within the dataset regarding the lesions were detected for 10 other MLNs (the total number of lesions in the record did not match with the sum of the specific lesions recorded). All those 38 MLNs were removed prior to the data analysis. As a result, data were available for 8,689 visually inspected MLNs, 508 not inspected MLNs and 500 MLNs found with GDA after the regulatory inspection. Overall, the MLNs included in the survey were equally distributed between the two regions for the total number of MLNs regulatory inspected and the number of visually inspected ones (Table 1). More than twice the number of non-inspected MLNs were collected in the West region compared to the East (350 vs. 154), whereas less MLNs were found with GDA in regulatory inspected MLNs (189 vs. 311).

Table 1. Distribution of MLNs by the presence or absence of GDA and by the presence or absence of laboratory-confirmed lesion according to the MLN sampling

a. Among visually inspected MLNs

	West			East			Both regions		
GDA	Lab confirmed lesion	No lesion	Total*	Lab confirmed lesion	No lesion	Total*	Lab confirmed lesion	No lesion	Total*
Yes	2	2	4	36	7	43	38	9	47
No	11	4308	4319	164	4159	4323	175	8467	8642
Total	13	4310	4323	200	4166	4366	213	8476	8689

b. Among not inspected MLNs

	West			East			Both regions		
	Lab confirmed lesion	No lesion	Total	Lab confirmed lesion	No lesion	Total	Lab confirmed lesion	No lesion	Total
	4	346	350	5	149	154	9	495	504

c. Among the MLNs with grossly detectable abnormalities found after routine inspection (systematic visual inspection and incision)

	West			East			Both regions		
GDA	Lab confirmed lesion	No lesion	Total	Lab confirmed lesion	No lesion	Total	Lab confirmed lesion	No lesion	Total
Yes	178	11	189	296	15	311	474	26	500
Total	NA	NA	147,147	NA	NA	142,626	NA	NA	289,773

* Region effect: Mantel-Haenszel Statistics = 692.48; df = 1; p<0.0001

NA: not available

Table 1 displays the distribution of MLNs broken down by the presence or absence of actual macroscopic lesion based on the pathological examination at the laboratory and the detection of GDA at the abattoir according to the region of origin of the samples and to the type of MLN. Table 2 shows the point and interval estimates of the apparent (based on GDA) and true (based on the actual presence of lesion) prevalence that can be derived from Table 1 as well as the estimate for the sensitivity and the specificity of the visual detection of lesion (GDA) on the inspection line. Based on the visually inspected MLNs, the apparent prevalence of lesion was 0.09% in the West and 0.98% in the East, whereas the true prevalence was 0.3% and 4.6%, respectively. The difference between the two regions was statistically significant for both the apparent and true prevalence (Table 2). The specificity of the visual detection of lesion on MLNs was close to 100% in both regions, whereas its sensitivity was 15.4% in the West and 18.0% in the East with no differences between the two regions.

Table 2. Prevalence of lesion on MLNs and performance of their detection

a. Among visually inspected MLNs

	West	95%CI	East	95%CI	Both regions	95%CI
Apparent prevalence (GDA detected following visual-only inspection)	4/4,323 = 0.093%	(0.03 - 0.24%)	43/4,366 = 0.98%	(0.71 - 1.32%)	47/8,689 = 0.54%	(0.40 - 0.72%)
True prevalence (laboratory confirmed lesion)	13/4,323 = 0.30%	(0.16 - 0.51%)	200/4,366 = 4.58%	(3.98 - 5.24%)	213/8,689 = 2.45%	(2.14 - 2.80%)
Sensitivity of GDA detection based on visual-only inspection	2/13 = 15.4%	(19.2 - 45.4%)	36/200 = 18.0%	(12.9 - 24.0%)	38/213 = 17.8%	(13.0 - 23.7%)
Specificity of GDA detection based on visual-only inspection	4,308/4,310 = 99.95%	(99.9 - 100%)	4,159/4,166 = 99.8%	(99.6 - 99.9%)	8,467/8,476 = 99.9%	(99.8 - 100%)
Proportion of MLNs without laboratory confirmed lesions among those detected with GDA following the visual-only inspection (False positive)	2/4 = 50.0%	(0 - 100%)	7/43 = 16.3%	(5.1 - 27.4%)	9/47 =19.1%	(7.8 - 30.5%)
Proportion of MLNs with laboratory confirmed lesion among those without GDA following the visual-only inspection (False negative)	11/4,319 = 0.25%	(0.13 - 0.46%)	164/4,323 = 3.39%	(3.24 - 4.41%)	175/8,642 = 2.02%	(1.74 - 2.34%)

b. Among not inspected MLNs

	West		East		Both regions	
True prevalence (laboratory confirmed lesion)	4/350 = 1.13%	(0.31 - 2.87%)	5/154 = 3.25 %	(1.06 - 7.41%)	9/504 = 1.79%	(0.81 - 3.34%)

c. Among the MLNs with grossly detectable abnormalities found after routine inspection (systematic incision and visual inspection)

	West		East		Both regions	
Apparent prevalence (GDA detected following current regulatory inspection)	189/147,147 = 0.13%	(0.11 - 0.15%)	311/142,626 = 0.22%	(0.19 - 0.24%)	500/289,773 = 0.17%	(0.16 - 0.19%)
Proportion of MLNs without laboratory confirmed lesions among those detected with GDA following the current regulatory inspection (False positive)	11/189 = 5.8%	(2.9 - 10.2%)	15/311 = 4.8%	(2.7 - 7.8%)	26/500 = 5.2%	(3.4 - 7.4%)

The true prevalence estimated from the MLNs not inspected was different from the one derived from the visually inspected MLNs for each region (Table2); however, because the confidence intervals overlapped, the difference within each region was not significant. The apparent prevalence estimated from the regulatory inspected MLNs was similar in the West compared the estimate derived from the visually inspected MLNs (0.13 vs. 0.10%), whereas it was lower in the East (0.22 vs. 0.98%).

The abattoir non-detection rate of actual lesions in MLNs further to visual inspection (false negatives) was 11/4,319 (0.25%) in the West and 164/4,323 (3.8%) in the East, the difference between the two being significant (Table 2). The proportion of MLNs without laboratory confirmed lesion among those detected with GDA following the visual-only inspection was high in the West (50% based on a very small number), in the East (16.5%) and overall (19.1%) (Table 2). The non-detection rate of actual lesions following the regulatory inspection (false positive) was 5.8% in the West and 4.8% in the East, without significant differences between the regions (Table 2).

A majority (53.6%) of MLNs with actual macroscopic lesions as detected at the laboratory had only one specific type of lesion and 35.5% had two different specific types (Table 3). The most frequent specific lesion was abscesses (43.5%) followed by granulomas (34.5%) and enlargement or hypertrophia (34.5%) with haemorrhage at the fourth rank (25.1%) (Table 3). Statistically significant differences existed between the regions for haemorrhage and enlargement, and almost significant differences for abscesses. Twenty-two MLNs were sent for Mycobacterium culture and two were positive. These two MLNs both had abscesses, one had granulomas, and none had calcification, enlargement, fibrosis, haemorrhage, lipidosis or emphysema, or necrosis. They were both Gram negative and Ziehl-Neelsen negative. Actually the 32 MLNs submitted to the Ziehl-Neelsen coloration were all negative.

Table 3. Specific defects detected in MLNs with laboratory confirmed lesion

	West	n=195	East	n=501	Both regions	n=696
	%	95%CI	%	95%CI	%	95%CI
Defect						
Abscesses	50.8	44 - 58	40.3	36.0 - 44.8	43.3	39.5 - 47.0
Granulomas	33.9	27.2 - 41.0	34.7	30.6 - 39.1	34.5	31.0 - 38.2
Enlargement or hypertrophia	26.2	20.1 - 33.0	37.7	33.5 - 42.1	34.5	31.0 - 38.2
Hemorrhage	15.4	10.6 - 21.2	28.9	25.0 - 33.1	25.1	22.0 - 28.6
Fibrosis	14.9	10.1 - 20.7	10.2	7.7 - 13.2	11.5	9.2 - 14.1
Necrosis	4.1	1.8 - 7.9	3.0	1.7 - 4.9	3.3	2.1 - 4.9
Calcification	2.5	0.8 - 5.9	5.2	3.4 - 7.5	4.5	3.1 - 6.3
Lipidosis or emphysema	0.5	0.01 - 2.8	1.6	0.7 - 3.1	1.3	0.6 - 2.4
Other	0.5	0.01 - 2.8	2.4	1.2 - 4.2	1.9	1.0 - 3.2
Number of defects						
1	63.1	56.3 - 69.9	49.9	45.5 - 54.3	53.6	49.9 - 57.3
2	26.2	20.0 - 32.4	39.2	34.9 - 43.5	35.5	31.9 - 39.1
3	9.7	5.5 - 13.9	8.2	5.8 - 10.6	8.6	6.5 - 10.7
4	1	0.0 - 2.4	2.6	1.2 - 4.0	2.2	1.1 - 3.3
5	0		0.2	0.0 - 0.6	0.1	0.0 - 0.3

Among the 508 MLNs specifically collected for bacteriological testing, the most common species was *S. aureus* (present in 80% of MLNs), followed by *E. coli* (22.9%) whereas the four other species were present in less than 10% of MLNs (Table 4). In the West, compared to East, MLN contamination was higher overall (100% of MLN contaminated by at least one species vs. 50%), higher for *S. aureus* (100 vs. 34%), higher for *Yersinia* (9.3 vs. 2.6%) and lower for *S. suis* (0 vs. 1.3%). When the results from the bacteriologically tested MLNs in addition to the 508 MLNs specifically collected for that purpose were added, the results did change a little within each region, whereas it decreased the proportion of MLNs contaminated with at least one type of bacterial species (from 84.8 down to 74.7%) (Table 4).

Table 4. Bacteriological contamination of MLNs

a. MLNs specifically collected for bacteriological testing

	West	n=354	East	n=154	Both regions	n=508
Bacteriological species	%	95%CI	%	95%CI	%	95%CI
Escherichia coli	23.1	18.9 - 27.9	19.5	13.6 - 26.6	22.1	18.5 - 25.9
Salmonella spp.	4.5	2.6 - 7.2	2.6	0.7 - 6.5	3.9	2.4 - 6.0
Campylobacter spp.	0.28	0.1 - 1.6	3.3	1.1 - 7.4	1.2	0.4 - 2.6
Staphylococcus aureus	100	99.9 - 100	34.4	26.7 - 42.5	80.1	76.4 - 83.5
Streptococcus suis	0.0	0.0 - 0.1	1.3	0.2 - 4.6	0.4	0.05 - 1.4
Yersinia spp.	9.3	6.5 -12.8	2.6	0.7 - 6.5	7.3	5.2 - 9.9
Number of bacteriological species						
0	0		50	41.8 - 58.2	15.2	12.2 - 18.6
1	67.2	62.1 - 72.1	39	31.3 - 46.7	58.7	54. - 63.0
2	28.8	24.1 - 33.5	8.4	4.0 - 12.8	22.6	19.0 - 26.2
3	3.4	1.5 - 5.3	2.6	0.1 - 5.1	3.2	1.7 - 4.7
4	0.6	0.0 - 1.4	0		0.4	0.0 - 0.9

b. All MLNs bacteriologically tested

	West	n=369	East	n=386	Both regions	n=735
Bacteriological species	%	95%CI	%	95%CI	%	95%CI
Escherichia coli	22.8	18.6 - 27.4	23.1	19.0 - 27.6	22.9	20.0 - 26.1
Salmonella spp.	4.3	2.5 - 7.0	4.7	2.8 - 7.3	4.5	3.1 - 6.2
Campylobacter spp.	0.27	0.1 - 1.5	1.8	0.7 - 3.7	1.1	0.5 - 2.1
Staphylococcus aureus	100	99.9 - 100	31.6	27.0 - 36.5	65.0	61.5 - 68.4
Streptococcus suis	0.0	0.0 - 0.1	1.3	0.4 - 3.0	0.7	0.2 - 1.5
Yersinia spp.	8.9	6.2 -12.3	3.1	1.6 - 5.4	6.0	4.4 - 7.9
Number of bacteriological species						
0	0		51.3	46.2 - 56.3	26.3	23.1 - 29.5
1	67.9	63.1 - 72.7	34	29.3 - 38.7	50.5	46.9 - 54.1
2	28.3	23.7 - 32.9	12.7	9.4 - 16.0	20.3	17.4 - 23.2
3	3.3	1.5 - 5.1	2.1	0.7 - 3.5	2.7	1.5 - 3.9
4	0.5	0.0 - 1.2	0		0.3	0.0 - 0.7

Overall, the MLNs with at least one confirmed macroscopic lesion were less frequently contaminated compared to the ones without any lesion (48.1 vs. 83.8%) (Table 5). Actually, the bacterial species-specific contamination rates were similar between the MLNs with and without lesion except for *S. aureus* by which MLNs with lesion were less frequently contaminated (34.9 vs. 76.8%).

Table 5. Association between bacteriological contamination and actual macroscopic lesion in MLNs

	With laboratory confirmed lesion	n=213	Without lesion	n=542
	%	95%CI	%	95%CI
Bacteriological species				
Escherichia coli	21.1	15.9 - 27.2	23.6	20.1 - 27.4
Salmonella spp.	5.2	2.6 - 9.1	4.2	2.7 - 6.3
Campylobacter spp.	0.9	0.1 -3.4	1.1	0.4 - 2.4
Staphylococcus aureus	34.9	28.5 - 41.7	76.8	73.0 - 80.3
Streptococcus suis	0.9	0.1 -3.4	0.6	0.1 -1.6
Yersinia spp.	3.8	1.6 - 7.3	6.8	4.9 - 9.3
Number of bacteriological species				
0	51.9	45.2 - 58.6	16.2	13.1 - 19.3
1	31.1	24.9 - 37.3	58.1	53.9 - 62.3
2	15.1	10.3 - 19.9	22.3	18.8 - 25.8
3	1.9	0.1 - 3.7	3.0	1.6 -4.4
4	0		0.4	0.0 - 0.9

4. Discussion

This study was undertaken because of the lack of data in Canada about the performance of a simplified inspection of market hog MLNs (visual-only inspection) compared to the performance of the current regulatory inspection (mandatory visual examination with incision) on the detection of GDA, as a proxy for the detection of *Mycobacterium bovis* infection, a food safety hazard to humans. In addition to the performance of detection at the slaughterhouse, there was also a lack of data about the prevalence of lesions in MLNs and their microbiological contamination, in particular by *Mycobacterium* species.

In response to these data gaps, the study results show that the prevalence of macroscopic lesions (based on examination at the laboratory) in market hog MLNs ranges between 0.16 to 7.4% (true prevalence) in Canada. The prevalence for macroscopic lesions detected at the slaughterhouse was lower, between 0.03 and 1.3 % (apparent prevalence) when results from the visual-only and the regulatory inspections are combined. This latter figure is comparable with the 5% prevalence of grossly detectable abnormalities (i.e. apparent prevalence) in cervical/submaxillary lymph nodes reported by an extensive survey in Australia in the late 90's (Pointon, Hamilton, Kolega, & Hathaway, 2000). The two most common abnormalities found in our study were abscesses (in 30 to 60% MLNs with lesions) and granulomatous lesions (in 30 to 40% MLNs with lesions). Multiplied by the apparent prevalence observed in our survey, the apparent prevalence of abscesses in MLNs was between 0.015 and 0.9% and a prevalence of granulomas between 0.015 and 0.5%. The latter figure is comparable to the 0.75% prevalence of granulomatous lesion in MLNs observed in the Netherlands (Komijn et al., 2007). It is greater than the very low prevalence (0.01% to 0.02%) of granulomatous lymphadenitis in Danish finisher pigs reported over the period 1997-2006 (Alban et al., 2008).

With an epidemiological sensitivity around 13-23% and specificity almost perfect, the visual inspection of MLNs of the inspection chain performs moderately well for the detection of macroscopic lesions in MLNs at the slaughterhouse. This explains the lower apparent prevalence of lesions in MLNs compared to its true prevalence. Under the visual-only inspection, about 18% of actual lesions are missed, up to 4.4% of the MLNs classified as without GDA actually have a lesion (false negative) and about 16 to 19% (not counting the results from the West because of a very low number; n=4) of MLNs classified as GDA have no lesion (false positive). The current regulatory inspection (incision and visual examination) yields a similar apparent prevalence of lesion in MLNs but has proportionally less false positives (over both regions 5.2% vs. 19.1%). Consequently, both the current regulatory inspection and the visual-only inspection should yield the same numbers of MLNs with GDA but the number of those MLNs without actual lesion would be 4 times greater under the visual-only inspection, meaning it will be less efficient in term of the use of resources. Considering the low number of such hog carcasses detected with GDA, the relative inefficiency of visual-only inspection may be of minor importance in terms of the operations.

The bacterial contamination of MLNs ranges between 15 and 25% for *Escherichia coli* and below 10% for *Salmonella* spp., *Campylobacter* spp., *Streptococcus suis* and *Yersinia* spp. This is within the range of results observed elsewhere. In Norway, a survey conducted in 2000-2001 found that MLNs were contaminated by *Yersinia* spp. in five slaughtered pigs out of 97 tested (5.9%) and none were contaminated by *Campylobacter* spp. (Nesbakken, Eckner, Hoidal, & Rotterud, 2003). An Australian extensive survey found that 2 out of 97 (2.1%) submaxillary lymph nodes with GDA were contaminated with *Yersinia enterocolitica* whereas it was 7 out of 500 (1.4%) in MLNs without GDA (Pointon et al., 2000). The contamination by *S. aureus* was present in all MLNs in one region but not as present in the other region (this regional difference will be discussed later). As a result, all MLNs were contaminated by at least one of the targeted bacteria, while this was the case in half the MLNs in the East region. Human pathogenic strains exist within each of the bacterial species targeted. This relatively common prevalence of those species in MLNs raises the concern of cross-contamination of other parts of the carcass which may occur because of the incision of all MLNs on the inspection chain. Considering that, in case of doubt about *M. bovis* infection following MLN incision and visual inspection, the mesenteric lymph nodes have to be incised and visually assessed and, following the evidence of potential *M. bovis* infection, five other carcass lymph nodes have to be incised and examined, all these operations further increase the possibility and probability of cross-contamination and the extent of newly contaminated carcass parts.

Only two MLNs among the 9,697 included in this study were possibly contaminated by a microorganism of the *Mycobacterium* gender, but not *M. bovis*. This very low, if not null, prevalence of *M. bovis* in pigs was expected considering Canada is free of bovine tuberculosis in its cattle, and although *M. bovis* infection occurs from time to time in wildlife, all pigs are raised indoors. The situation is similar to Denmark (Alban et al., 2008) and in Europe more generally with a prevalence of *M. bovis* infection in pigs estimated at 0.0004% (EFSA Panels on Biological Hazards (BIOHAZ), 2011). The very low detection of *Mycobacterium* in lesions suspected of infection by this microorganism has been documented in an extensive study in Great Britain (Bailey, Crawshaw, Smith, & Palgrave, 2013). A total of 874 suspected lesions of tuberculosis in MLNs, lung, thoracic or mesenteric lymph nodes were systematically submitted to *Mycobacterium* culture over the period 2007-2011: *M. bovis* was detected in 12.8% of them, *M. avium* in 11.7%, whereas the culture was negative in 48.2% and inconclusive in 27.3%. Generally, *M. avium* seems the species most often involved in mycobacterial of swine (Alvarez et al., 2011; Eisenberg et al., 2012; Johansen, Agdestein, Lium, Jorgensen, & Djonne, 2014; Lara et al., 2011; Pate, Zdovc, Pirs, Krt, & Ocepek, 2004). In Argentina though, *M. bovis* was the most frequent species observed in pigs (Barandiaran et al., 2015), followed by co-infection with *M. avium*. Swine infection by other *Mycobacterium* species are possible (van Ingen, Wisselink, van Solt-Smits, Boeree, & van Soolingen, 2010). The presence of *Rhodococcus equi* has been reported more frequently in lesions suspected of *Mycobacterium* infection (Alban et al., 2008; EFSA Panels on Biological Hazards (BIOHAZ), 2011; Komijn et al., 1999; Komijn et al., 2007; Pate, Pirs, Zdovc, Krt, & Ocepek, 2004). Although the prevalence of *Rhodococcus equi* has not been documented in Canadian swine, we suspect that this infection is common in MLNs with granulomatous lesions as it is in other countries (Lara et al., 2011; Pate et al., 2004).

Differences between the two regions (West and East) appeared during the study and in the results. The number of MLNs collected without inspection and sent for laboratory pathological and bacteriological analyses were not balanced between the regions; all bacteriologically tested MLNs from the West were contaminated with *S. aureus* and none were positive to *S. suis*. Whether this reflects a natural difference or it results from bias is not an easily answered question. No relevant hypothesis can be formulated to explain this difference. Due to the unbalanced number of MLNs sent for bacteriological testing, the combined results for *S. aureus* and *S. suis* are biased toward the situation in the West, which sent twice as many MLNs. Under the reasonable assumption that both regions are equally important in terms of hogs slaughtered, an equally weighted average of the region-specific bacteriological contamination proportions should provide a better Canadian estimate than the combination of all MLNs. Despite similar volume of hogs slaughtered and inspected over all collection periods, the apparent and true prevalence of lesions in MLNs was greater in the East compared to the West. Again, it is impossible to certify that it outlines natural differences between the two regions. The swine industry is younger in the West compared to the East region. The herds there may be bigger and their management different using more modern facilities. But the effect of such herd features on their sanitary status remains unknown.

Other study limitations relate to the study design and sampling. The study took place in winter and spring, not over a full year. Extrapolation of the results to the other periods of the year cannot be granted; however the swine production is almost entirely indoors and in a controlled environment in Canada due to its continental cold climate, thus no major seasonal variations on the hog MLN pathological and microbiological state are expected. The data collection was made of three bouts of 30 minutes each during the same morning. Because pigs usually

are transferred and slaughtered in batches coming from the same facility, the carcasses examined for the study may belong to one or a few batches, implying that the MLNs examined for the study were not completely independent, potentially resulting in bias of unknown direction and amplitude. Obtaining the complete independence between carcasses is difficult pragmatically. The amplitude of the bias, if present, may be limited in the case of hogs because they usually all have the same age and weight, are all in a good health and pass ante mortem inspection, which is less obvious with reproductive animals (sows and boars). In addition, the difference from one facility to another in terms of sanitary status affecting the marketed hogs is considered low. Overall, this lack of complete independence between carcasses should impact the representativeness to a very limited extent, if any. Finally, the MLNs were inspected in eight slaughterhouses that had been carefully selected. The choice was made on practical, operational basis for feasibility and not based on historical data about lesion or bacterial contamination in MLNs or pigs. Furthermore, since the number of swine abattoirs was limited to 42 in 2005, the eight participating slaughterhouses represent 20% of this number and include a good fraction of all pigs slaughtered in Canada.

5. Conclusion

This survey of the Canadian market hogs showed that abnormal MLNs actually are rare (below 8%). They are often under detected by the current inspection because of its limited epidemiological sensitivity (18%). Such abnormalities, even undetected, have very limited, if any, impact on human health since the pathological and microbiological analyses failed to provide evidences of infection by *Mycobacterium bovis* in MLN. On the other hand, MLNs can be contaminated with several bacteria potentially pathogenic to humans, raising the risk of cross-contamination during the inspection with incision. Finally, the current incision and visual inspection of all hog MLNs and the proposed visual-only inspection perform similarly in identifying abnormal MLNs. Although these results altogether support the proposed change in hog MLN inspection, a formal risk assessment should be undertaken to support any decision about this change.

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