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ABSTRACT

Using the isolation of *Mycobacterium bovis* as the reference standard, this study evaluated the sensitivity, specificity and kappa statistic of gross pathology (abattoir post-mortem inspection), histopathology, and parallel or series combinations of the two for the diagnosis of tuberculosis in 430 elk and red deer. Two histopathology interpretations were evaluated: histopathology I, where the presence of lesions compatible with tuberculosis was considered positive, and histopathology II, where lesions compatible with tuberculosis or a select group of additional possible diagnoses were considered positive. In the 73 animals from which *M. bovis* was isolated, gross lesions of tuberculosis were most often in the lung (48), the retropharyngeal lymph nodes (36), the mesenteric lymph nodes (35), and the mediastinal lymph nodes (16). Other mycobacterial isolates included: 11 *M. paratuberculosis*, 11 *M. avium*, and 28 rapidly growing species or *M. terrae complex*. The sensitivity estimates of gross pathology and histopathology I were 93% (95% confidence limits [CL] 84,97%) and 88% [CL 77,94%], respectively, and the specificity of both was 89% [CL 85,92%]). The sensitivity and specificity of histopathology II were 89% (CL 79,95%) and 77% (CL 72,81%), respectively. The highest sensitivity estimates (93–95% [CL 84,98%]) were obtained by interpreting gross pathology and histopathology in parallel (where an animal had to be positive on at least one of the two, to be classified as combination positive). The highest specificity estimates (94–95% [CL 91–97%]) were generated when the two tests were interpreted in series (an animal had to be positive on both tests to be classified as combination positive). The presence of gross or microscopic lesions showed moderate to good agreement with the isolation of *M. bovis* (Kappa = 65–69%). The results show that post-mortem inspection, histopathology and culture do not necessarily recognize the same infected animals and that the spectra of animals identified by the tests overlaps.

RÉSUMÉ

En se servant de l'isolement de *Mycobacterium bovis* comme point de référence, nous avons évalué la sensibilité, la spécificité et la statistique kappa des pathologies macroscopiques (examen post-mortem à l'abattoir), de l'histopathologie, et des combinaisons en parallèle ou en série de ces deux examens pour diagnostiquer la tuberculose chez 430 élans et cerfs rouges. Deux interprétations histopathologiques ont été évaluées; histopathologie I, où la présence de lésions compatibles avec la tuberculose était considérée positive, et histopathologie II, où des lésions compatibles avec la tuberculose ou un ensemble restreint de diagnostics additionnels possibles étaient considérés positifs. Chez les 73 animaux à partir desquels *M. bovis* a été isolé, des lésions macroscopiques de tuberculose étaient le plus souvent observées au niveau du poumon (48), des ganglions lymphatiques rétropharyngés (36), des ganglions lymphatiques mésentériques (35), et des ganglions lymphatiques médiastinaux (16). D'autres espèces de mycobactéries ont également été isolées; 11 *M. paratuberculosis*, 11 *M. avium*, et 28 autres espèces à croissance rapide ou appartenant au complexe *M. terrae*. La sensibilité de la pathologie macroscopique et de l'histopathologie I étaient respectivement de 93 % (intervalle de confiance [IC] 94,97 % avec un seuil de confiance de 95 %) et de 88 % (IC 77,94 %), alors que leur spécificité était de 89 % (IC 85,92 %). La sensibilité et la spécificité de l'histopathologie II étaient respectivement, de 89 % (IC 79,95 %) et 77 % (IC 72,81 %). La sensibilité la plus élevée (93–95 % [IC 84,98 %]) était obtenue en interprétant en parallèle la pathologie macroscopique et l'histopathologie, un animal étant considéré positif lorsqu'il était positif à l'un ou l'autre des tests de la combinaison. La plus grande spécificité (94–95 % [IC 91,97]) était obtenue quand les deux tests étaient interprétés en série, un animal étant considéré positif lorsque trouvé positif aux deux tests de la combinaison. La présence de lésions macroscopiques ou microscopiques montrait une relation modérée à bonne avec l'isolement de *M. bovis* (Kappa = 65–69 %). Les résultats démontrent que l'examen post-mortem, l'examen histopathologique et la culture bactérienne ne reconnaissent pas nécessairement le même animal infecté comme positif et qu'un éventail superposé d'animaux infectés sont identifiés par les différentes méthodes de diagnostic.

(Traduit par docteur Serge Messier)

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INTRODUCTION

It is often difficult to compare the results of one trial that evaluates tests for the diagnosis of tuberculosis to those of another, or to directly apply the conclusions to control and eradication programs. One reason for this is because researchers have not consistently used the same reference standard to estimate the sensitivity and specificity of diagnostic tests for *Mycobacterium bovis* infection. The presence of gross lesions consistent with *M. bovis* infection has been used by some investigators to judge the performance of diagnostic tests (1,2,3), while others chose the isolation of *M. bovis* as their reference standard (4,5). As well, various combinations of gross pathology, histopathology and *M. bovis* isolation have been used to establish the disease status of animals in tuberculosis studies (6,7,8,9).

The Canadian Captive Ungulate Program (CCUP) for the eradication of brucellosis and tuberculosis requires the mandatory testing of captive ungulates on premises involved in the commercial production of Cervidae (10). Cervidae on these premises are tested with the mid-cervical intradermal tuberculin test at 3-year intervals and ungulate carcasses are monitored in abattoirs for lesions compatible with mycobacterial infection. In 1989, a British Columbian fallow deer (*Dama dama*) herd became the first herd infected with *M. bovis* to be detected under the CCUP. During the following 3 years, two separate outbreaks of tuberculosis occurred in Ontario and Alberta which eventually led to the detection and depopulation of 23 cervid and mixed species herds. Because of the large number of animals scheduled for slaughter, the opportunity was taken to compare abattoir post-mortem inspection and histopathology with the isolation of *M. bovis* for the diagnosis of tuberculosis in elk and red deer. Our objective was to investigate the relationship between these tests and determine how that relationship might affect the use of the tests as reference standards.

MATERIALS AND METHODS

ANIMALS

A total of 502 elk and red deer (*Cervus elaphus*) were selected from 5 herds from which *Mycobacterium bovis* had previously been isolated from at least one animal as a result of a CCUP investigation. The animals were individually identified on the quarantined farm of origin with tamper-proof, metal eartags. Most of the subjects were female since most of the testing occurred during the rut and the males were too difficult to handle. The elk were tuberculin tested and bled for tests of cell-mediated and humoral immunity. The results of the antemortem diagnostic tests are reported separately (11).

SAMPLE COLLECTION AND DISTRIBUTION

All of the animals in the study were subject to a post-mortem examination conforming to Canadian guidelines for the inspection of cattle (12). In addition to the lymph node examination specified in the guidelines, the carcass lymph nodes of each animal were incised and examined. The following tissues were collected from every elk in the study: tonsils and the submandibular, parotid, and retropharyngeal lymph nodes (Group 1); lung and the bronchial and mediastinal lymph nodes (Group 2); the mesenteric, ileocecal, and hepatic lymph nodes (Group 3); and the inguinal, iliac, prefemoral, and prescapular lymph nodes (Group 4). If multiple lesions were found, representative samples of each were collected and pooled (Group 5). Each tissue and lesion was divided into 2 parts: 1 was placed in 10% buffered formalin for histopathology and the other frozen at −70°C for subsequent mycobacterial culture. All tissues were excised after the hide was removed and, between animals, table surfaces were disinfected with 1-Stroke Environ (Sanofi, Victoriaville) and/or with hot water rinse (90°C), and knives were immersed in thermal sterilizers at 85–90°C.

When the National Veterinary Services Laboratories, Ames, Iowa (NVSL) joined the study, cultures of 104 elk were in progress at the Animal Diseases Research Institute, Nepean, Ontario (ADRI). All the remaining tissues from one farm (29 elk) were sent to NVSL and specimens from 300 elk, from 4 premises, were randomly allocated to the 2 laboratories. Similarly, ADRI had completed the histopathology on 83 animals, and the remaining specimens (406) were randomly divided between ADRI and NVSL. In total, NVSL processed tissues of 179 elk for culture and 203 elk for histopathology. ADRI processed the tissues of 254 elk for culture and 286 for histopathology. Specimens for histopathology and culture, from any given animal, did not necessarily go to the same laboratory.

GROSS PATHOLOGY

Elk with lesions varying from firm or hard white, grey, or yellow nodule with a yellow, caseous, necrotic centre that was dry and solid to thin-walled suppurative abscesses were classified as post-mortem positive (12). Animals without these lesions were classified as post-mortem negative.

HISTOPATHOLOGY

The pathologists trimmed all submitted tissues, and therefore, were aware of the gross pathological status of the animal, but were not aware of other diagnostic test results. Microscopic criteria for diagnoses were defined and agreed upon by both laboratories. Each piece of submitted tissue from every elk was transected every 5 mm and examined carefully for gross lesions. A section from each piece (with or without gross lesions) was embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin and eosin (H&E) using conventional methods (13). Mineralized tissues were treated with Decalcifer II (Surgipath Medical Industries, Richmond, Illinois) or 5% trichloroacetic acid prior to the processing. Sections with lesions were stained by a new fuchsin-methylene blue (NF) (14), a modified Ziehl-Neelsen stain (13), and/or an acridine orange phenylauramine O (AOAO) technique (15). These sections were examined for the presence of acid-fast bacilli or AOAO fluorescent-stained bacilli. If the lesions were not positive for mycobacteriosis, as defined below, additional sections were stained with Giemsa, Gram, Glynn, or Grocott's methenamine silver, as required, to exclude other bacterial, fungal or parasitic agents (13).

If caseous necrosis, mineralization, epithelioid cells, multinucleated giant cells, macrophages, and acid-fast or fluorescent bacilli were present, the

lesion was considered positive for mycobacteriosis (16,17). Lesions consistent with mycobacterial infection in Cervidae, but without demonstrable organisms, were classified as mycobacteriosis suggestive. All other lesions were classified morphologically, or etiologically when an agent could be demonstrated using the histochemical techniques mentioned above. Changes in lymph nodes such as follicular or paracortical hyperplasia or regression, and nonspecific alterations associated with drainage from areas of inflammation were also noted.

Two schemes were used to classify the animals as positive or negative for the statistical analysis. In the first (histopathology I), animals were test positive if lesions compatible with tuberculosis (mycobacteriosis or mycobacteriosis suggestive) were seen. In the second scheme (histopathology II), animals were test positive if they had diagnoses of mycobacteriosis, mycobacteriosis suggestive, or one of: granulomatous lymphadenitis, lymphadenitis with mineralization, focal granulomatous tonsillitis, or tonsillitis with mineralization. These diagnoses include lesions that could have been caused either by mycobacteria or by other agents such as *Actinobacillus* sp., fungi or parasites.

TABLE I. Comparison of gross pathology and histopathology I to mycobacterial culture

Post-mortem[a]/ histopathology I[b]	Interpretation[c]		Culture status		
	In parallel	In series	*M. bovis* Isolated	*M. bovis* Not isolated	Total
Negative/Negative	−	−	5	292	297
Negative/Positive	+	−	0	24	24
Positive/Negative	+	−	4	23	27
Positive/Positive	+	+	64	16	80
Total			73	355	428

[a] Positive: gross lesions compatible with tuberculosis
[b] Positive: histopathological lesions of tuberculosis consistent with or suggestive of mycobacterial infection
[c] + = positive, − = negative

TABLE II. Distribution of lesions at post-mortem

Tissue	Number (%) of animals found at post-mortem to have gross lesions compatible with tuberculosis in the tissue or lymph node listed		
	Total (430 animals)	*M. bovis* not isolated (357 animals)	*M. bovis* isolated (73 animals)
Lung	48/430 (11)	12/357 (3)	36/73 (49)
Retropharyngeal	36 (8)	7 (2)	29 (40)
Mesenteric	35 (8)	12 (3)	23 (32)
Mediastinal	16 (4)	7 (2)	9 (12)
Bronchial	8 (2)	3 (1)	5 (7)
Parotid	4 (1)	1 (0)	3 (4)
Tonsil	14 (3)	11 (3)	3 (4)
Prescapular	4 (1)	2 (1)	2 (3)
Ileocecal	3 (1)	2 (1)	1 (1)
Prefemoral	1 (<1)	0 (0)	1 (1)
Submandibular	1 (<1)	1 (<1)	0 (0)
Inguinal	0 (0)	0 (0)	0 (0)
Iliac	0 (0)	0 (0)	0 (0)

MYCOBACTERIAL CULTURE

At ADRI, all tissue from each elk were thinly sliced and examined for lesions. A pool of tissue with lesions or without lesions, (3–10 g) was homogenized in 5 mL 0.67 M phosphate buffer (pH 7.0) using Ten Broek tissue grinders. Ten mL of 2% NaOH solution with 0.2% phenol red was added to 4 mL of the resulting tissue homogenate and neutralized with 2 N HCl after 12 min at room temperature. Sterile distilled water was added to bring the total sample volume to 35 mL. The sample was then centrifuged at 2200 × g for 30 min, the supernatant decanted, and the pellet inoculated onto the isolation media using sterile cotton swabs.

At NVSL, the groups of tissues (4–5 bags per elk) were separately trimmed of excess fat and homogenized for two min in a 480 mL blender jar containing 50 mL of tryptose broth with 0.04% phenol red. Using a wide bore pipette, 10.0 mL of the blended tissue suspension was transferred to a conical centrifuge tube. An equal volume of a 1:1 (V/V) solution of 0.3% Zephiran (Winthrop, New York, New York) and 1:500 sodium hypochlorite was added, and the mixture was centrifuged for 30 min at 2640 × g. The supernatant was discarded, and 2 mL of 10% egg yolk solution was added. The sediment was resuspended and inoculated onto the isolation media.

The isolation media used by both laboratories included: Lowenstein Jensen medium with and without glycerol (18), Herrold egg yolk medium with mycobactin J (18), Herrold egg yolk medium without glycerol, mycobactin J, and malachite green (18), Stonebrink medium (18), and Modified Middlebrook 7H11 medium (19). In addition NVSL used BACTEC 12B medium (Becton Dickinson, Towson, Maryland) and ADRI used Middlebrook's 7H9 broth + OADC (Difco Laboratories, Detroit, Missouri) with and without 4.1 mg/L Na pyruvate. The media were incubated at 37°C for 12 wk and examined every 2 wk for colony formation. All acid-fast isolates were classified using standard growth and biochemical characteristics to determine mycobacterial species (18,20).

STATISTICAL ANALYSES

Sixty-nine elk were excluded from the final statistical analysis since their tissues were either delayed in transit or not clearly identified, thus were unfit for mycobacterial culture. The rates of *M. bovis* isolation from the animals divided between the two laboratories were compared using a Fisher's exact 2-sided test (21). For gross pathology and the two histopathology interpretations, 4-fold tables were generated comparing their diagnostic test results with those of mycobacterial culture. Elk were classified as disease positive if *M. bovis* was isolated, and as disease negative if mycobacteria other than *M. bovis* were isolated or if no mycobacteria were isolated. Using the 4-fold tables, the sensitivity, specificity, positive and negative predictive values and kappa statistic were calculated (21,22). The 95% confidence limits were calculated for the estimates of

TABLE III. Distribution of diagnoses by group of tissues collected at post-mortem (One animal can have more than one diagnosis for each group)

Diagnosis	Group 1[1]	Group 2[2]	Group 3[3]	Group 4[4]	Group 5[5]
Mycobacteriosis	37	34	18	3	143
Abscess(G+Cocci/Nocardia)	1	0	0	0	0
Actinobacillosis/Actinomycosis	54	0	1	0	10
Amyloid like deposit	1	1	0	36	0
BALT[6] — Prominent	0	12	0	0	0
Emphysema	0	1	0	0	0
Fat necrosis	0	0	0	2	0
Granuloma eosinophilic	0	0	8	0	12
Hepatitis	0	0	0	0	1
Hepatitis abscess	0	0	0	0	2
Hepatitis parasitic	0	0	7	0	14
Hyperplasia follicular	34	11	13	10	2
Hyperplasia paracortical	4	0	1	1	0
Lipidosis focal	0	0	0	0	1
Lymphadenitis granulomatous	20	11	9	4	3
Lymphadenitis mineralization	7	3	11	1	3
Lymphadenitis mycotic	0	0	1	0	0
Lymphadenitis parasitic	9	10	32	8	3
Lymphadenitis suppurative	42	11	5	16	4
Lymphangiectasia	0	1	1	0	0
Mastitis	0	0	0	0	1
Pleural fibrosis	0	1	0	0	1
Pleuritis	0	1	0	0	1
Pneumonia	0	23[7]	0	0	9[8]
Sialoadenitis	2	0	0	0	0
Tonsillitis inflamatory	96[9]	0	0	0	13[10]
Tonsillitis focal granulomatous	12	0	0	0	0
Tonsillitis mineralization	1	0	0	0	0
Tonsillitis noninflamatory	24	0	0	0	5
No significant findings	203	352	388	418	17

[1] Tonsils, submandibular, parotid and retropharyngeal lymph nodes
[2] Lung, bronchial and mediastinal lymph nodes
[3] Mesenteric, ileocecal and hepatic lymph nodes
[4] Inguinal, prefemoral, and prescapular lymph nodes
[5] Gross lesions compatible with mycobacterial infection
[6] Bronchiolar associated lymphoid tissue
[7] Includes 22 interstitial, 1 mycotic
[8] Includes 5 interstitial, 2 abscess, 1 parasitic, 1 mycotic
[9] Includes 43 crypt abscess, 1 eosinophilic, 2 foreign body, 2 mycotic, 48 non-specific inflamatory lesions
[10] Includes 9 crypt abscess, 4 non-specific inflamatory lesions

sensitivity, specificity, and positive and negative predictive values using a formula based on a normal approximation to the binomial distribution (21). Gross pathology plus histopathology I or II was also evaluated in parallel (an animal had to be positive on at least one of the two tests to be classified as combination positive) and in series (an animal had to be positive on both tests to be classified as combination positive). The total number of animals used in each analysis varied slightly as animals were excluded from the calculations if valid data were missing for one or more tests.

RESULTS

In 356 (83%) of the 428 animals, for which there were complete gross pathology, histopathology, and mycobacterial culture results, the 3 tests agreed on the tuberculosis status of an animal: 64 were disease positive and 292 were disease negative by all 3 tests (Table I). Gross pathology did not agree with histopathology and culture results in 5.4% (23) of the animals studied, and histopathology disagreed with the post-mortem and culture results in 5.6% (24) of cases. Post-mortem and histopathology findings did not agree with the mycobacterial culture results in 4.9% (16+5/428) of the animals.

Complete post-mortem and mycobacteriology data were obtained for 430 animals. Of these, 107 had gross lesions compatible with tuberculosis. The distribution of gross lesions consistent with tuberculosis in *M. bovis* positive and *M. bovis* negative elk is listed in Table II; the lung, the retropharyngeal lymph node, and the mesenteric lymph node were the most commonly affected tissues in culture positive animals.

Complete histopathological and mycobacterial culture results were obtained for 429 animals. Table III lists the histopathological diagnoses made from each group of tissues. Note that Group 5 contains all tissues found to have lesions consistent with tuberculosis at the post-mortem examination. Fluke-induced lesions and chronic bacterial (non-mycobacterial) lesions were present in some elk. Lesions containing small, corpora amylacea-like, mineralized foci, a feature frequently seen in tuberculosis in cattle, were observed in some lymph nodes and tonsils. Other mineralized lesions in these organs were suggestive of nonspecific, chronic inflammatory foci.

There was no difference between ADRI and NVSL rates of identification of *M. bovis* positive animals in the randomly divided group ($P = 0.7$ for a 2-sided Fisher's exact test). One hundred and twenty-three mycobacterial isolates were recovered by culture: 73 (59%) *M. bovis*, 11 (9%) *M. paratuberculosis*, 11 (9%) *M. avium*, and 28 (23%) rapidly growing species or *M. terrae complex* (Table IV). Mixed mycobacterial infections were identified in two cases; one *M. avium* plus an undetermined rapid grower and one *M. avium* plus *M. bovis*.

The 95% confidence limits for the sensitivity estimates of all tests and test combinations overlapped (Table V). The sensitivity figures for gross pathology and histopathology I were 93% (CL 84,97%) and 88% (CL 77,94%). The highest sensitivity was obtained by interpreting tests in parallel (93–95% [CL 84,98%]). The highest specificity estimates were generated when the tests were interpreted in series (94–95% [CL 91–97%]). The 95% confidence limits of specificity estimates did not overlap those of the other tests and test combinations (Table V).

At a 17% prevalence of tuberculosis infection, based on the isolation of *M. bovis*, the combination of post-mortem and histopathology I in series gave the highest positive predictive value (80%) (Table VI). All the tests and test combinations had negative predictive values of 97–98%.

There was moderate to excellent (44–80%) agreement (kappa) between the tests evaluated and the isolation of *M. bovis* (Table VII). The highest agreement beyond chance with *M. bovis* isolation was obtained with the series interpretation of post-mortem and histopathology I (80%).

DISCUSSION

Studies using post-mortem examination to determine disease status or to select tissues for further analysis may miss *M. bovis* infection in animals without lesions. As reflected by the high negative predictive value (NPV) (98% Table VI), there were few culture positive, lesion negative animals in this sample (5/323 = 1.5%, Table VII). Of animals from which *M. bovis* was isolated, 7% (5/73) had no gross lesions of tuberculosis at abattoir inspection (Table I). Other studies have found similar rates (3.7–6%) of no gross lesion/culture positive animals in heavily infected herds (23,24).

If post-mortem findings had been used as the reference standard for this analysis, more animals would be classified as positive (107/430 = 25%) than by culture (17%). Of the 107 animals positive for tuberculosis on post-mortem examination, *M. bovis* was isolated from 68 (positive predictive value (PPV) = 64%, Table VI). Twenty-three of the 39 culture negative animals were also negative by histopathology (Table I). The most common histopathology diagnoses for these animals included: actinobacillosis, parasitic hepatitis or lymphadenitis, tonsillar cysts, and pneumonia.

In the Canadian abattoir surveillance program for bovine tuberculosis, tissues with gross lesions typical of tuberculosis are examined by histopathology, and those diagnosed as mycobacteriosis, or possibly being caused by mycobacteriosis, are cultured. The histopathology II interpretation included lesions, that could have been caused by mycobacteria, such as granulomatous lymphadenitis, lymphadenitis with mineralization, and granulomatous tonsillitis, in the positive definition. The sensitivity increased minimally using this scheme, but the specificity decreased by 12% (Table V) and the PPV decreased by 18% (Table IV).

TABLE IV. Mycobacteria isolated compared with the histopathology I diagnosis

Isolate	Histopathology diagnosis: Mycobacteriosis or suggestive of	Other than Mycobacteriosis	No significant findings	Total[a]
M. bovis	64	8	1	73
M. avium	1	9	1	11
M. paratuberculosis	4	5	2	11
M. terrae complex	4	7	4	15
Rapid grower[b]	2	10	1	13
No isolate	29	211	68	308
Total	104	250	77	431

[a] An animal may have more than one isolate
[b] Rapidly growing *Mycobacterium* spp. other than *M. fortuitum* or *M. chelonae*

TABLE V. Sensitivity and specificity estimates of post-mortem, histopathology I, and histopathology II compared to the isolation of *M. bovis*[a]

Diagnostic test	Number of animals	Sensitivity estimate (%)	95% Confidence limits (%)	Specificity estimate (%)	95% Confidence limits (%)
Post-mortem[b]	430	93	84, 97	89	85, 92
Histopathology I[c]	429	88	77, 94	89	85, 92
Histopathology II[d]	429	89	79, 95	77	72, 81
Parallel interpretation[e]					
PM[b]/Histo I[c]	428	93	84, 97	82	78, 86
PM/Histo II[d]	428	95	86, 98	72	67, 77
Series interpretation[f]					
PM[b]/Histo I[c]	428	88	77, 94	95	93, 97
PM/Histo II[d]	428	88	77, 94	94	91, 96

[a] Reference standard; positive: *M. bovis* isolated; negative: *M. bovis* not isolated
[b] Enhanced abattoir inspection; positive: gross lesions compatible with tuberculosis seen at post-mortem inspection
[c] Positive: histopathological lesions consistent with, or suggestive of, mycobacterial infection
[d] Positive: histopathological lesions consistent with or suggestive of mycobacterial infection, granulomatous lymphadenitis, lymphadenitis with mineralization, focal granulomatous tonsillitis, or tonsillitis with mineralization
[e] Combined screening tests, with test results interpreted in parallel (i.e. an animal is considered positive" if found positive by at least one test)
[f] Combined screening tests, with test results interpreted in series (i.e. an animal is considered positive" only if "positive" on both tests)

The positive predictive value measures the likelihood of an animal, found positive by post-mortem and/or histopathology, being *M. bovis* positive at culture. The likelihood that an animal found negative on post-mortem examination and/or histopathology, being *M. bovis* negative at culture, is the negative predictive value. As the PPV and NPV of a test vary with changes in the disease prevalence, it is not appropriate to use predictive values for "best" test selection. The predictive values were calculated here as another method of examining the effect of various interpretations on test performance in the field.

Tests are interpreted in parallel with the general aim of increasing sensitivity. But, the specificity and the PPV of such a combination decreases. Post-mortem/histopathology I, interpreted in parallel, had 1.6 times the number (63) of false positives than either test alone (39 & 40; Table VII). This indicates that post-mortem and histopathology did not identify the same animals as positive (otherwise the number of false positives with respect to culture would remain the same).

Agreement between biologically independent tests can provide a reasonable summary of overall test performance at determining infection (25). As opposed to sensitivity and specificity, kappa measures the agreement in the positive as well as the negative categories. It has been suggested that a kappa of 40–75% indicates a fair to good level of agreement between tests and over 75% indicates excellent agreement between tests

TABLE VI. Positive and negative predictive values of post-mortem, histopathology I, and histopathology II at a prevalence of 17%, based on *M. bovis* isolation[a]

Diagnostic test	Number of elk	Positive predictive value (%)	95% Confidence limits	Negative predictive value (%)	95% Confidence limits
Post-mortem	430	64	54, 72	98	96, 99
Histopathology I	429	62	51, 71	97	95, 99
Histopathology II	429	44	36, 53	97	94, 99
Parallel interpretation					
PM/Histopathology I	428	52	43, 61	98	96, 99
PM/Histopathology II	428	41	34, 49	98	96, 100
Series interpretation					
PM/Histopathology I	428	80	69, 88	97	95, 99
PM/Histopathology II	428	74	64, 83	97	95, 99

[a] Definitions and abbreviations as in Table V

TABLE VII. Kappa statistic of post-mortem, histopathology I, and histopathology II against mycobacterial culture[a]

Test #1	Test #1/Test #2[b] +/+	+/−	−/+	−/−	Kappa %[c]
Post-mortem	68	39	5	318	69
Histopathology I	64	40	9	316	65
Histopathology II	65	82	8	274	47
Parallel interpretation					
PM/Histopathology I	68	63	5	292	57
PM/Histopathology II	69	98	4	257	44
Series interpretation					
PM/Histopathology I	64	16	9	339	80
PM/Histopathology II	64	22	9	333	76

[a] Definitions and abbreviations as in Table V

[b] Test #1 as listed; Test #2 = mycobacterial culture, where + = *M. bovis* isolated, − = *M. bovis* not isolated

[c] Kappa statistic (percent) of agreement beyond chance beteen test #1 and test #2

(21). The presence of gross lesions typical of mycobacteriosis at post-mortem inspection showed good agreement beyond chance with the isolation of *M. bovis* (kappa = 69%; Table VII). Reasons for lack of a higher kappa may include: lesions caused by agents other than *M. bovis* (e.g. actinobacillosis), lesions caused by mycobacteria other than *M. bovis*, early *M. bovis* infections where lesions are not yet grossly visible, resolving lesions of tuberculosis where *M. bovis* is no longer viable, or failure of mycobacterial culture to isolate the organism.

Histopathology I had a moderate agreement beyond chance with culture (kappa = 65%; Table VII) and a moderate PPV (62%; Table VI). Infections with mycobacteria other than *M. bovis* and the fact that the two procedures cannot physically test the same portion of tissues may account for the lack of stronger agreement. Also, 29 of 104 tissues classified as positive by histopathology did not yield a mycobacterial isolate (Table IV). This may have been due to false positive histopathology results and/or false negatives by the reference standard (culture). When false negatives occur with a reference standard, any test evaluated against that standard may have an overestimated sensitivity and an underestimated specificity.

The impact of missing true positives by the reference standard would not have had a large impact on the sensitivity and specificity figures in this trial. If, for example, there was a 15% increase in the number of culture positive animals (that is, 10 histopathology positive/culture negative were reclassified as histopathology positive/culture positive and 1 histopathology negative/culture negative animal was reclassified as histopathology negative/culture positive), the sensitivity of histopathology I would remain at 88% and the specificity would increase from 89% to 91%.

Interpreting the tests in series, where only tissues with gross lesions were considered for histopathology and only those which were histopathology positive were compared to culture, detected 87.7% (64/73) of the *M. bovis* infected animals in this sample (Table I). The kappa statistic and the PPV, for this interpretation, were the highest of all analyses. As each animal was required to meet two positive criteria, only the most likely infected animals were considered positive for comparison to culture; therefore, the false positive rate (the greater source of error in this population) was minimized (Table VII).

The need for mycobacterial isolation and identification, or more specific histopathology techniques, in cases where a definitive diagnosis is required is illustrated in Table IV. Fourteen percent of histopathology positive elk yielded mycobacteria other than *M. bovis* (Table IV). Norton et al (26) report the effect of the sample collection techniques on the incidence of environmental mycobacterial contamination; in their study, 63 isolates of mycobacteria other than *M. bovis* were obtained from tissues collected by routine field necropsy, as opposed to none from tissues collected using aseptic technique. The abattoir post-mortem technique we used should have minimized environmental contamination of the tissues. The *M. terrae* and rapidly growing mycobacteria we isolated were probably present within the cultured tissues. Note that 61% (17/28) of saprophytic isolates were from the tissues of animals having histopathology diagnoses other than mycobacteriosis or mycobacteriosis suggestive, whereas only 18% (5/28) were isolated from the tissues of animals having no significant findings at histopathology (Table IV).

The prevalence of *M. paratuberculosis* infection in this population may have been higher than indicated by the 11 isolates recovered. The sampling protocol, decontamination methods, and incubation time used were designed for *M. bovis* isolation and not for the recovery of *M. paratuberculosis*. Histopathology changes due to *M. paratuberculosis* infection in deer may be very similar to those caused by *M. bovis* infection (27). A portion of the histopathology positive/culture negative cases may have been *M. paratuberculosis* infection missed by the culture protocol used. *Mycobacterium paratuberculosis*

infection in farmed Canadian elk will require further study before its prevalence and implications are understood.

Our study supports the concept that gross pathology, histopathology, and culture do not consistently identify the same animals as tuberculosis positive. It follows then, that a parallel interpretation of all three methods together would maximize the detection of *M. bovis* infected animals (recognizing that specificity would decrease). Our results also show that using gross pathology and histopathology alone, or in combination, resulted in only small changes in sensitivity. Specificity, however, can vary greatly depending on the interpretation or combination of the two.

As animal populations, production techniques, tuberculosis prevalence, diagnostic resources, and control efforts are unique to each country or outbreak, it is not in the scope of this report to discuss the application of our findings to tuberculosis surveillance or eradication schemes. The decision to choose gross pathology, histopathology, mycobacterial culture, or combinations thereof, as a reference standard for test evaluation will depend on the needs of the program in which the test(s) will be used.

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