

Validation of an Ultrasound Imaging Technique of the Tympanic Bullae for the Diagnosis of Otitis Media in Calves

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Background: Otitis media is a common disease in calves that can be subclinical, making antemortem on-farm diagnosis challenging.

Objectives: To evaluate the sensitivity and specificity of ultrasonography of tympanic bullae for the diagnosis of clinical and subclinical otitis media and to evaluate the reproducibility of the technique.

Animals: Forty calves 19–50 days of age were selected from a veal calf farm.

Methods: Prospective study. Ultrasonography was first performed on the farm by ultrasonographer A (US A). Ultrasonography was repeated by ultrasonographer A (US A') and another ultrasonographer (US B) at the Centre Hospitalier Universitaire Vétérinaire. Images were later reread by both examiners and a diagnosis was recorded. The calves were euthanized and submitted for necropsy, and histopathologic diagnosis was used as the gold standard.

Results: Forty-five bullae were affected by otitis media and 35 bullae were normal. Sensitivity and specificity of the ultrasound technique ranged from 32 to 63% and 84 to 100%, respectively, depending on the examiner and classification of suspicious ultrasonography results. Kappa analysis to evaluate interobserver agreement between A' and B yielded a κ value of 0.53. Agreement within the same examiner (A versus A') yielded a κ value of 0.48, and real-time ultrasound versus rereading of recorded images for A' and B yielded κ values of 0.58 and 0.75, respectively.

Conclusions: Sensitivity and specificity of the ultrasound imaging technique are, respectively, low and high for diagnosis of clinical and subclinical otitis media in calves, with moderate reproducibility.

Key words: Cattle; Middle ear; Prevalence; Subclinical; Ultrasonography.

Otitis media is a common disease affecting 1- to 2-month-old calves.^{1,2} Morbidity ranges from less than 10–80% during outbreaks, but prevalence of diseased animals can be underestimated as otitis can remain subclinical.^{1,3} Common clinical signs include a drooping ear, ptosis, and head tilt.^{1,4,5} However, when the diagnosis of otitis is based on findings from neurologic examination (NE), using computed tomography (CT) as the gold standard, only 61.1% of cases are detected.³ Furthermore, most cases are chronic by the time neurologic deficits become obvious.^{2,4,5} Chronic cases usually require extended treatment or are refractory to treatment.^{1,2} Consequently, to prevent economic losses associated with treatment costs and decreased growth, there is a need for detection of otitis media in early or subclinical stages of the disease.^{6,7}

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The study was conducted at the Centre Hospitalier Universitaire Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec

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Abbreviations:

CHUV	Centre Hospitalier Universitaire Vétérinaire
CT	computed tomography
NE	neurologic examination
TB	tympanic bullae
US	ultrasonography

Medical imaging techniques have the potential to accurately identify clinical and subclinical otitis.⁸ Computed tomography is considered the gold standard on live animals. It can detect otitis media in calves showing no neurologic signs, consistent with early stages of infection.³ However, CT is limited to referral centers. Ultrasonography (US) is an imaging technique available to most bovine practitioners. Ultrasound evaluation of tympanic bullae (TB) has been described in dogs, cats, and rabbits.^{9–11} When compared with CT for the diagnosis of otitis media in dogs, US has a sensitivity of 37–74% and a specificity of 55–74%, depending on the observer.¹² An approach for ultrasound evaluation of TB has been described in neonatal bovine cadavers and healthy calves.¹³ Abnormalities occur in some clinical cases of otitis media, including anechoic to hyperechoic heterogeneous content, lysis of trabeculae, mucosal edema, as well as irregularities, thinning, deformation, and rupture of the bulla wall.⁵ Internal structures of the bulla can be observed in calves up to 10 weeks of age, supporting the potential use of US to detect early stages of otitis.¹³

The purpose of the current study was to validate the use of US as a diagnostic tool to detect otitis media in calves. The first objective was to describe abnormalities on clinical and subclinical cases. The second objective was to estimate the sensitivity of the technique, among clinical and subclinical cases, and its specificity using

histology as the gold standard, and to evaluate the reproducibility of results among and within ultrasonographers.

Materials and Methods

This study was approved by the Committee for ethical use of animals of the Faculté de médecine vétérinaire of the Université de Montréal.

Calf Selection

Forty dairy-breed calves were purchased from a veal calf producer. To obtain sensitivity and specificity estimates with a 95% confidence interval and precision of 10%, 40 of each otitis-affected and unaffected bullae were determined to be necessary, based on expected estimates of approximately 88%, as obtained in other studies.^{10,14} From May to June 2012, the farm was visited 4 times to select calves from the same cohort at different ages (19–22, 26–29, 40–43, and 47–50 days). The cohort was divided into 19 pens, with 4 to 6 calves per pen. During each visit, 4 to 5 pens were randomly selected and US was performed on all calves from each selected pen. Selected pens were then removed from the randomization process, so that pens were only selected once. During each visit, 10 calves were selected to obtain a similar number of otitis-positive and -negative bullae based on US as described below. All calves with positive bullae were selected, and remaining calves were randomly selected using a random number table, among calves with negative bullae to make up a final sample size of 10 calves per visit.

Upon arrival at the farm at 3–5 days of age, the calves were treated with enrofloxacin in their milk replacer for 7 days.^a Farm records were surveyed for individual antimicrobial treatments received by selected calves.

Ultrasound Examination

On Day 0, an initial US was performed on the farm by ultrasonographer A (US A). After the farm visit, selected calves were brought to the Centre Hospitalier Universitaire Vétérinaire (CHUV) of the Université de Montréal. On Day 1, US was performed a second time by ultrasonographer A (US A') and by another ultrasonographer (US B). Both ultrasonographers were blinded to each other's findings and to NE findings. Both ultrasonographers had been involved in a previous research project for developing an approach for US of TB on calf cadavers. In addition, ultrasonographer B has many years of experience in bovine US at a referral center. Calves were restrained in a standing position and coupling gel was applied ventral to the base of the ear without clipping on Day 0 and after clipping on Day 1. A portable ultrasound unit^b was used with a linear probe^c at a fixed focus and frequency of 7.5 MHz. Ultrasonography was performed on both TB with three probe positions as previously described.¹³ Briefly, the probe was positioned ventral to the base of the ear and caudal to the mandible, in a dorso-ventral axis for positions 1 and 2, and rostro-caudal axis for position 3. One image from each position was recorded by both ultrasonographers, including when possible the landmarks respective to each of the three probe positions.¹³

Ultrasonographic diagnosis of disease was recorded as positive, suspicious, or negative. A disease-positive bulla on US was defined as a reproducible observation, in more than 25% of the visible portion of the bulla, of one abnormality in more than 1 position, or more than one abnormality in 1 or more positions. A bulla was considered suspicious when 1 abnormality was observed in only 1 position and in less than 25% of the visible

portion of the bulla. Evaluated abnormalities were divided based on wall abnormalities, such as thinning, thickening, irregular thickness, irregular contour, deformation and rupture, and content abnormalities, such as anechoic content (mucosal edema, serous exudate), hyperechoic, heterogeneous content (fibrino-suppurative exudate), and partial or complete osteolysis of trabeculae. Recorded images were later reread by both ultrasonographers, blinded to their previous diagnosis, and ultrasonographic diagnosis was recorded as positive, suspicious, or negative.

Clinical Status

General examination and NE were performed by ultrasonographer A and diagnosis was recorded for each bulla as positive, suspicious, or negative. Facial symmetry, ptosis, ear droop, palpebral reflex, menace response, palpebral closure, and response to stimulation of the internal aspect of the pinna were evaluated. A positive NE diagnosis was defined based on evidence of facial nerve paresis or paralysis. A suspicious NE diagnosis was defined by abnormal response (weak or delayed) to one of the tests used to evaluate the facial nerve, while other test responses were normal.

A tympanic bulla was considered clinically affected, ie, otitis associated with clinical signs, when positive on histology and positive or suspicious on NE. A tympanic bulla was considered subclinically affected when positive on histology and negative on NE.

Necropsy

On Day 2, the calves were euthanized and a postmortem examination of the TB was performed within 2 hours. The bullae were cut in transverse sections approximately 1 cm thick using a bandsaw and examined macroscopically by a board-certified pathologist (MJG) blinded to the US and NE results. A positive diagnosis, by gross examination, was defined as TB containing an exudate (serous or purulent), thickening of the mucosa, lysis of the bony trabeculae, or both. The TB sections were fixed for 2 days in 10% neutral buffered formalin, decalcified^d and then trimmed to approximately 3 mm thickness and embedded in paraffin, submitted for routine histologic processing, and stained with hematoxylin, phloxine, and saffron stain. The slides were examined to evaluate the following changes: inflammatory exudate and necrotic debris in the bulla; epithelial erosion, hyperplasia, or both; inflammatory cells in the submucosa; submucosal edema; granulation tissue; and lysis of the bony trabeculae. All slides were blindly examined 3 times by a board-certified pathologist (MJG). A semiquantitative histologic grading system was developed for this study using the following criteria: 0, normal bulla; 1, up to 25% of the bulla had one or more of the changes described above; 2, 25–50% of the bulla had multiple changes as described above (Fig 1); 3, 50–75% of the bulla had multiple changes as described above; 4, greater than 75% of the bulla had multiple changes as described above (Fig 2). A positive diagnosis of otitis by histologic examination was made if grade ≥ 2 .

Statistical Analysis

Sensitivity and specificity of ultrasound technique for A, A', and B were estimated (95% confidence interval (CI)) against the gold standard histologic result. Sampling weights were used in the analysis to take into account the sampling design. Sampling weights were calculated for each calf as the inverse of the probability of calf selection following US results on the farm at each visit. Estimation of sensitivity and specificity was performed for three classifications of suspicious bullae upon ultrasound examination: excluded, grouped with positive bullae, or grouped with

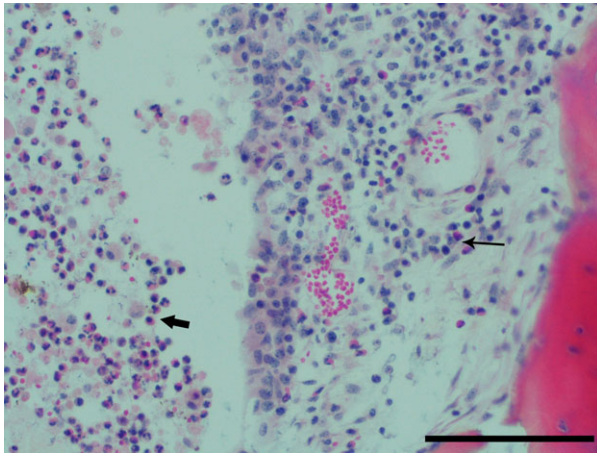


Fig 1. Photomicrograph of the lining of the tympanic bulla from a calf. An inflammatory infiltrate of neutrophils and macrophages with edema is expanding the submucosa (small arrow) and forming an exudate in the lumen of the bulla (large arrow). This bulla was considered to be a grade 2 lesion. Hematoxylin, phloxine, and saffron stain, bar = 150 μ m.

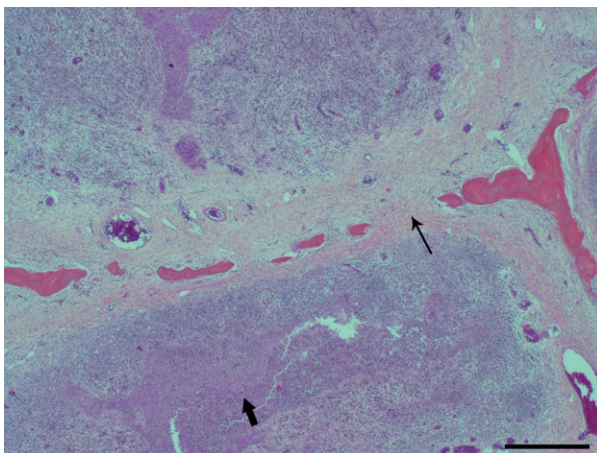


Fig 2. Photomicrograph of the tympanic bulla from a calf. The architecture of the bulla has been effaced by an intense inflammatory exudate filling the lumen of the bulla (large arrow) and mature granulation tissue replacing part of the bony trabeculae in the bulla (small arrow). This bulla was considered to be a grade 4 lesion. Hematoxylin, phloxine, and saffron stain, bar = 300 μ m.

negative bullae. Sensitivities were also estimated separately for clinically and subclinically affected bullae as defined above, for the three classifications of suspicious bullae upon ultrasound examination. Sensitivity and specificity of combined NE and ultrasound examination were estimated. The final diagnosis was considered positive if at least one of the two tests was positive or if both were suspicious. Sensitivities of US A' and US B were compared using a McNemar's test with a continuity correction. A weighted kappa analysis, with a diagnosis of suspicious being considered at equal distance of positive and negative, was performed without sampling weights to evaluate the agreement among examiners and within the same examiner (A versus A', and real-time ultrasound versus rereading of recorded images, for A' and B). Kappa values were interpreted as previously described.¹⁵ Apparent prevalence for all calves tested on the farm

was estimated for 2 classifications of suspicious bullae: grouped with positive and grouped with negative. True prevalence was estimated using sensitivity and specificity of US A at the calf level for each scenario. Analysis was performed using statistical software.^{e,f}

Results

Ultrasound Examination

Ultrasonography on the farm was performed on a total of 102 calves. Of 204 TB, 27 were positive, 10 were suspicious, and 167 were negative. All calves with at least 1 positive/suspicious bulla ($n = 29$) and 11 calves with negative bullae were selected for the study, to yield a study population of 40 calves.

Based on US performed at the CHUV, the number of positive, suspicious, and negative bullae was 19, 18, and 43, respectively, for US A', and 38, 9, and 33, respectively, for US B (Table 1). Ultrasonographic abnormalities included observation of complete trabeculae spread like a fan in anechoic content, similar to that described in newborn calves;¹³ a combination of anechoic content against the wall and hyperechoic content in the middle of the bulla, described in positions 1, 2, and 3, or anechoic content cranially and hyperechoic content caudally observed in position 3; suspicion of abscess against the wall; interrupted and irregular trabeculae, with lysis particularly observed against the wall (Fig 3); and suspected observation of the medial wall of the bulla (Fig 4). Observation of abnormalities was often restricted to a portion of the TB (Fig 5). Differentiation between acoustic shadow and anechoic content was based on observation of trabeculae and a well-defined internal contour of the bulla wall. Among specific wall abnormalities observed in at least one position by US A', US B, or both, 3 bullae had irregular thickness, 5 had irregular contour, 5 had deformation, and no bulla had thinning, thickening, or rupture of the wall. Among content abnormalities, 51 bullae had anechoic content, 13 had hyperechoic content, and 12 had lysis of trabeculae. Among unaffected bullae, anechoic content was noted in 14 bullae, hyperechoic content in 1 bulla, and lysis

Table 1. Distribution of 80 TB according to otitis media histopathologic, ultrasonographic, and clinical status, by ultrasonographer.

Ultrasonographic Results		Histopathologic Results		
		Positive		Negative
		Clinical	Subclinical	
US A'	Positive	9	10	0
	Suspicious	2	9	7
	Negative	5	10	28
US B	Positive	13	22	3
	Suspicious	2	1	6
	Negative	1	6	26

US A' = ultrasonographer A at the CHUV; US B = ultrasonographer B.

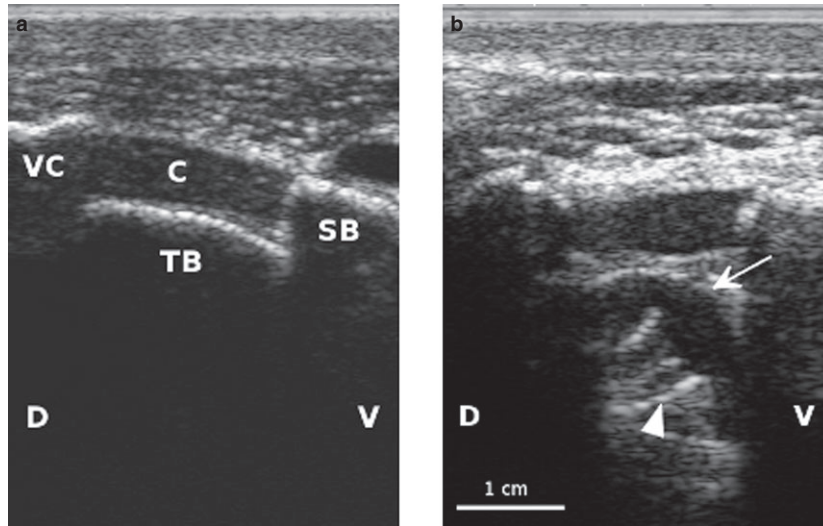


Fig 3. Ultrasound images of TB negative (a) and positive (b) for otitis media in position 2. D = dorsal; V = ventral; VC = vaginal crest; TB = tympanic bulla; C = stylohyoid cartilage; SB = stylohyoid bone. In the affected bulla, content is mostly anechoic against the bulla wall (small arrow). Trabeculae (arrowhead) are interrupted and irregular, and lysis appears concentrated against the wall.

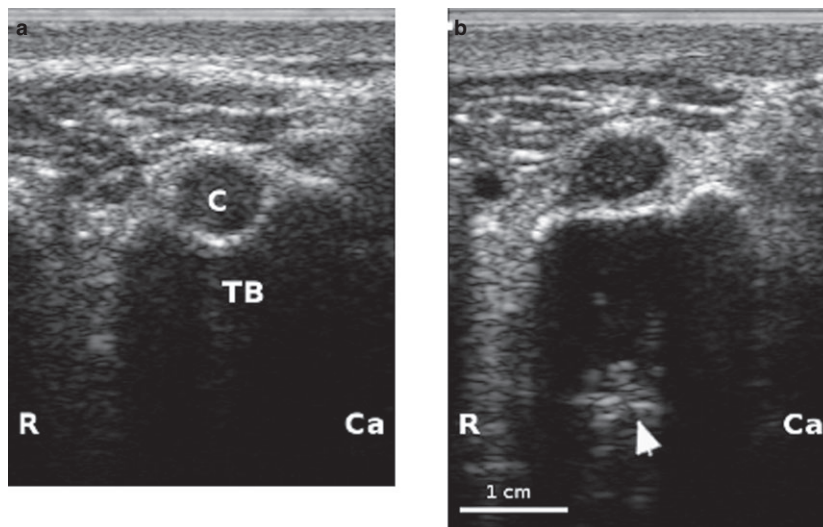


Fig 4. Ultrasound images of TB negative (a) and positive (b) for otitis media in position 3. R = rostral; Ca = caudal; TB = tympanic bulla; C = stylohyoid cartilage. In the affected bulla, content is anechoic to echogenic, trabeculae have disappeared in most of the bulla, and a hyperechoic line is visible at the bottom (white arrow head), suspected to be the medial wall of the bulla.

of trabeculae was not observed. Among bullae in which edema ($n = 42$), exudate ($n = 39$), and lysis ($n = 26$) were observed on histology, 33, 32, and 20 bullae, respectively, were classified as positive by at least 1 ultrasonographer.

Clinical Status and Necropsy

All 80 bullae from the 40 calves were evaluated macroscopically and histologically. Based on necropsy findings, 13 calves were affected with left ($n = 6$) or right ($n = 7$) otitis media, 16 were affected bilaterally, and 11 were unaffected. Among the 45 affected bullae, according to NE, 16 bullae were considered clinically

affected and 29 bullae were considered subclinical. Among unaffected bullae, 2 were considered positive by NE and 4 were considered suspicious. At the calf level, 12 calves were affected with clinical otitis media (one or both TB) and 17 calves were affected with subclinical otitis media. Upon macroscopic examination, 39 bullae were negative and 41 were positive for otitis media. On histologic examination, 35 bullae were negative, of which 16 were grade 0 and 19 were grade 1, and 45 were positive, of which 8 were grade 2, 9 were grade 3, and 28 were grade 4 (Table 2). Five bullae were negative by macroscopic examination, but grade 2 ($n = 4$) or 3 ($n = 1$) based on histology. One bulla was positive on macroscopic examination, but negative

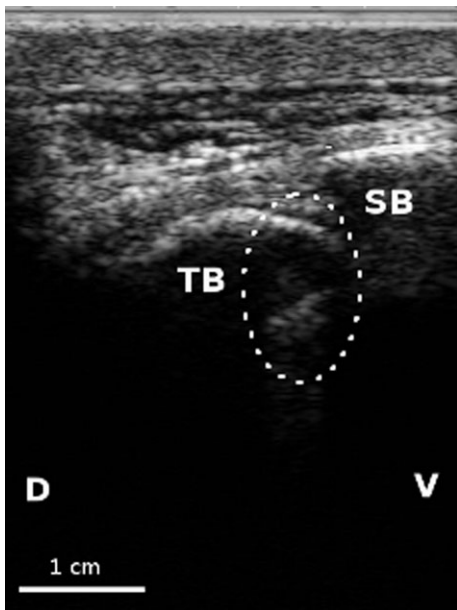


Fig 5. Ultrasound image of a tympanic bulla affected with otitis media in position 1. D = dorsal; V = ventral; TB = tympanic bulla; SB = stylohyoid bone. The most ventral part is obscured by the stylohyoid bone, while the bulla content in the most dorsal part is obscured by an acoustic shadow. The visible portion (circled) shows anechoic to echogenic content and 1 trabecula.

(grade 1) on histology. Among specific changes observed on histology, 42 bullae had edema, 39 had exudate (of which 2 had focal exudate), and 26 had lysis (of which 7 had focal lysis).

Among selected calves, six were treated, on the farm, with florfenicol for suspected bronchopneumonia. All those calves were affected with otitis media based on histology. Five of them had been selected as positive or suspicious upon ultrasonography on the farm. The other one was only declared positive on ultrasonography at the CHUV.

Sensitivity and Specificity of the Ultrasound Examination

Table 3 provides sensitivity and specificity results of the 3 ultrasound examinations alone and in combination with NE, as well as sensitivity among clinically affected and subclinically affected bullae after adjustment for sampling probabilities.

Table 2. Distribution of the 80 TB according to age of calves and histopathologic lesion grade.

Grades	Age in Days			
	19–22	26–29	40–43	47–50
Grade 0–1	10	9	8	8
Grade 2	2	3	1	2
Grade 3	2	1	3	3
Grade 4	6	7	8	7

Reproducibility of the Ultrasound Examination

Interobserver agreement between A' and B yielded a κ value of 0.53, which is considered moderate agreement. Intraobserver agreement ranged from moderate to substantial, with κ value of 0.48 for A versus A', and κ values of 0.58 and 0.75 after rereading images by A' and B, respectively.

Prevalence and Predictive Values of Ultrasound Examination on the Farm

On the farm, apparent prevalence of calves with otitis media based on US was 20.6 or 28.4%, depending on whether a suspicious result was considered negative or positive, respectively. True prevalence was estimated to be 49.7%. For US A at the calf level, when suspicious results were grouped with negative, sensitivity and specificity were 37.5 and 96.1%, respectively. When suspicious results were grouped with positive, sensitivity and specificity were 49.3% and 92.2%, respectively.

Discussion

The purpose of the current study was the evaluation of the ultrasound imaging technique on clinically and subclinically affected calves. An important feature of this study was the low severity of US abnormalities observed in many bullae. It could have contributed, among other factors that will be discussed, to the low sensitivity results, with sensitivity being lower for subclinically affected bullae. On the other hand, the technique has a high specificity and moderate reproducibility. Low severity of otitis was also observed in many bullae on histologic examination, confirming the importance of its use as gold standard.

One objective of the current study was to describe abnormalities observed on US in calves affected by clinical and subclinical otitis media. Three positions of the probe were used, with position 3 being the most helpful for identification of the bulla.¹³ Observation of all landmarks was occasionally difficult from either positions 1 or 2, but abnormalities could still be observed without perfect positioning. Abnormalities previously reported included anechoic to hyperechoic content, lysis of trabeculae, and thinning, deformation, and rupture of the bulla wall.⁵ Abnormalities observed in this study were of low severity. Subjectively, only few bullae had mild wall changes. Abnormal content was more often anechoic than hyperechoic, and lysis of trabeculae was observed in a minority of bullae. Observation of the medial wall of the bulla, which had not been reported in clinical cases but had been reported in bovine fetus cadavers and dogs,^{12,13} was suspected in a few cases in the current study. Taken together, the observed changes suggest a less advanced stage of disease compared with calves from the previous study. Findings from the current study suggest that wall abnormalities are uncommon. In the early stages, content abnormalities, especially observation of

Table 3. Sensitivity and specificity of 3 ultrasound examinations alone and in combination with neurologic examination for the diagnosis of otitis media in calves, using histology as the gold standard and adjusted for sampling probabilities (95% CI).

Test	Sensitivity (95% CI)			Specificity (95% CI) (n = 35)
	All Bullae (n = 45)	Clinical Bullae (n = 16)	Subclinical Bullae (n = 29)	
Ultrasound examination only				
Suspicious by ultrasound examination excluded				
US A	0.36 (0.16–0.56)	0.79 (0.54–1.00)	0.25 (0.06–0.44)	0.98 (0.96–1.00)
US A'	0.39 ^a (0.14–0.63)	0.64 (0.36–0.93)	0.32 (0.02–0.61)	1.00 (0.88–1.00) ^c
US B	0.61 ^b (0.36–0.86)	0.93 (0.74–1.00)	0.54 (0.24–0.84)	0.97 (0.94–1.00)
Suspicious by ultrasound examination with negatives				
US A	0.32 (0.16–0.49)	0.69 (0.43–0.94)	0.23 (0.07–0.39)	0.98 (0.96–1.00)
US A'	0.33 ^a (0.13–0.53)	0.56 (0.29–0.84)	0.27 (0.03–0.51)	1.00 (0.90–1.00) ^c
US B	0.59 ^b (0.35–0.83)	0.81 (0.60–1.00)	0.53 (0.24–0.82)	0.98 (0.95–1.00)
Suspicious by ultrasound examination with positives				
US A	0.42 (0.22–0.63)	0.81 (0.60–1.00)	0.32 (0.12–0.53)	0.97 (0.93–1.00)
US A'	0.47 ^a (0.25–0.69)	0.69 (0.43–0.94)	0.42 (0.15–0.68)	0.84 (0.67–1.00)
US B	0.63 ^b (0.38–0.87)	0.94 (0.80–1.00)	0.55 (0.25–0.84)	0.84 (0.70–0.99)
Ultrasound and neurologic examinations combined ^d				
US A	0.37 (0.19–0.56)	Not applicable		0.98 (0.95–1.00)
US A'	0.40 (0.19–0.60)			0.98 (0.96–1.00)
US B	0.63 (0.38–0.87)			0.96 (0.92–1.00)

^{a-b}For each scenario, sensitivity on all bullae is significantly different between US A' and US B ($P \leq .005$).

^cExact confidence interval.

^dDiagnosis was considered positive if at least one of the 2 tests was positive or if both were suspicious.

trabeculae because of the presence of fluid, would be more indicative of otitis than wall abnormalities. Although observed in fewer bullae, osteolysis of trabeculae was not observed in otitis-negative bullae and would be more specific of a pathologic condition than anechoic content with intact trabeculae. A bulla could have been noted as having anechoic content if an artifact, such as a short hyperechoic line, was misinterpreted as a part of a trabecula. On the other hand, lysis of trabeculae would usually have been noted if more than one trabecula were visible on a longer part and presented irregularity or discontinuation. Despite previous experience using the technique, 11–23% of the bullae were classified as suspicious because of low confidence of the ultrasonographers in the face of subtle changes. In a previous study in dogs, the lack of a previously known correlation between abnormalities detected by imaging techniques and histopathology was hypothesized to affect the confidence in the diagnosis of early changes.¹⁶ Mild histologic changes can result in subtle abnormalities on US, which can be misinterpreted as artifacts, consequently having a negative effect on sensitivity.

Overall sensitivity ranged from low to moderate, which could be partly attributed to the generally low severity cases. Nevertheless, this study was able to demonstrate that a medical imaging technique, such as US, could be used to detect subclinical otitis. Sensitivity for subclinically affected bullae was lower than in clinically affected bullae, but this could be simply because of the difficulty encountered to obtain ideal positioning for evaluation of the bulla and landmarks,

preventing observation of subtle changes. Bones in the area caudal to the mandible restrict the space available for manipulation of the probe, as well as the acoustic window. With a lateral ultrasonographic approach, the ventral portion of the bulla is covered by the stylohyoid bone; therefore, mild changes in this region are obscured.¹³ Moreover, the most dorsal part near the opening of the eustachian tube, and probably the first part to show histologic changes, is not visible. Finally, if changes do not occur against the lateral wall, they can be obscured by the acoustic shadow normally created by the bone-air interface.¹³ This has been previously reported in dogs and supposedly attributed to poorer results in mild cases with partially filled bullae.¹² Positioning the animal in lateral recumbency and performing ultrasound by lateral approach from beneath can overcome this problem,⁹ but this was not specifically tested in the present study. Among calves selected, previous antimicrobial treatment did not prevent detection of affected bullae by ultrasonographers. However, among unselected calves, antimicrobial treatment might have affected their classification as negative on the farm, and therefore their probability to be selected.

Specificity of the ultrasound technique ranged from good to excellent in the present study. A previous study in calves used macroscopic postmortem examination as the gold standard,⁸ which, if used in this study, would have underestimated the specificity of ultrasound examination. In fact, our study suggests that histopathology should be favored as the gold standard. On the other hand, nonpathologic accumulation

of fluid in the bulla, ie, accumulation of fluid without inflammatory changes, could have affected the specificity of the technique by increasing false positive results. Liquid in the bulla has been observed in newborn calves,¹³ and the time period in which fluid is present after birth possibly overlaps with the time period at risk for otitis media. In cats, middle ear effusion can result from auditory tube obstruction.¹⁷ Eustachitis in calves has been described^{7,18,19} and might similarly result in fluid accumulation in the TB of calves.

Comparison of our results with those of studies assessing the use of ultrasound in other species must be done with caution as case selection differed significantly; three of the previous studies used cadavers,^{10,14,20} and the study on live dogs used CT as a gold standard.¹² Ultrasonography for diagnosis of otitis media in dogs is reported to have a sensitivity ranging from 37 to 74% and a specificity ranging from 55 to 75%.¹² Comparing these results with those of our study, the technique in calves has a similar sensitivity, but a superior specificity. One explanation for this difference may be that in dogs, reverberation artifact could be misinterpreted as the opposite wall, resulting in a false positive.¹⁴ In calves, radially oriented trabeculae are less likely to be confounded by such an artifact.

Based on interobserver and intraobserver agreement, reproducibility of US was moderate. Moderate interobserver agreement could be attributed to the fact that ultrasonographer B was more experienced than ultrasonographer A. Ultrasonographer B obtained a superior sensitivity compared with ultrasonographer A. The moderate agreement between real-time ultrasound versus rereading of US images may be explained by the fact that the second reading was performed on static recorded images. The dynamic evaluation performed for the first reading allowed better observation and identification of subtle changes. Recording of a video of the complete ultrasound examination instead of images of each position may allow better interpretation upon review.

Very few studies have estimated the prevalence of otitis media in veal calves, being reportedly of 1.6%.²¹ In preweaned calf studies, the prevalence greatly varies, ranging from 3 to 78%.^{19,22–26} All reported prevalence estimates were based on clinical signs and do not include subclinical cases. True prevalence estimate on the farm was 49.7%, indicating that approximately half the calves of this cohort were affected by clinical or subclinical otitis. Additionally, this study took place from May to June; therefore, true prevalence estimate could have been higher if the study was conducted during winter months as prevalence of otitis is reportedly higher during that season.^{4–6,18,19,25}

Histology was used as the gold standard in this study. Specifically, the results of the study show that macroscopic examination alone is not sensitive enough and will miss an important number of cases. Also, few mild cases showed only mild multifocal epithelial and submucosal changes without exudate accumulation in the ventral aspect of the TB. Therefore, to avoid false

negative, a section spanning the entire bulla should be evaluated. Mild cases detected by histologic examination raise the question about clinical significance of these cases. Subclinical otitis is associated with more severe lesions.¹⁸ It is unknown if low-grade histologic lesions, if left untreated, develop into higher grade lesions. Mild cases were evenly distributed among groups in our study, which suggests that otitis of increasing severity is not related to age. Clinical significance and the effects of subclinical otitis media on calf growth need to be further studied.

In summary, when performing US for the diagnosis of otitis media in calves, position 3 is critical for identification of the bulla because of the readily recognizable stylohyoid cartilage.¹³ Additional imaging should be performed using either position 1 or 2 for examination of the longer axis of the acoustic window. Abnormalities researched are trabeculae, seen as hyperechoic lines spread like a fan, and hyperechoic, heterogeneous content. Interpretation of mild abnormalities can be challenging and the authors propose that suspicious ultrasound results be repeated later or complemented by NE.

Ultrasonography of the TB is accessible to practitioners, unlike radiography and CT. Most practitioners have access to a portable ultrasound unit and are familiar with ultrasound technique. However, a short linear probe must be available, as low-quality image is obtained with the linear rectal probe.¹³ Furthermore, the ease of mastering the technique for the TB has not been evaluated. Similar sensitivities between US A and US A', which differed mainly by the restricted time allowed for examination on the farm and by calves not being clipped, suggest that US can be performed rapidly under field conditions to screen a group of calves. These attributes make it the best diagnostic tool for practitioners attempting to confirm a clinical suspicion of otitis media or to detect a subclinical infection. The US technique could also be used in further studies to estimate the prevalence of otitis media, including subclinical cases, as well as to determine the impact of subclinical otitis on well-being and growth of the animals. It could also provide a useful aid for understanding the kinetics of the disease in a calf group and determining an ideal time to initiate treatment, reducing the cost associated with chronic and refractory cases.

Footnotes

^a Editors' note: "Information about use of enrofloxacin in calves in this study is included as part of the description of the clinical care of these calves. Extralabel administration of enrofloxacin to food animals is prohibited in some countries, including the United States, but not in Canada. Readers should be aware of local regulations and more general concerns regarding use of fluoroquinolone antimicrobials in food producing animals."

^b A6V, SonoScape, Shenzhen, China

^c L745 (46 mm), SonoScape

^d RDO Rapid Decalcifier, Apex Engineering Products Corporation, Aurora, IL

^e SAS version 9.3 (surveyfreq procedure), SAS Institute Inc, Cary, NC

^f R version 2.15.2

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