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Tympanic bullae ultrasonography is feasible in nonsedated healthy rabbits (*Oryctolagus cuniculus*)

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OBJECTIVE
To assess the feasibility of ultrasonography of the tympanic bullae (TB) in live, nonsedated rabbits (*Oryctolagus cuniculus*).

ANIMALS
40 healthy rabbits undergoing TB ultrasonography without sedation between September 2021 and May 2022.

PROCEDURES
For each rabbit, fur was clipped over an area (3 X 3 cm) at the level of the angular process of each mandible, then 3 ultrasonographic planes of each TB were imaged via ventral approach, with measurement of the time taken to complete the examination. Three items were assessed for each plane: TB depth, wall integrity, and contents (present or absent). Results were compared for rabbits grouped as standard-sized breed type versus dwarf-sized breed type.

RESULTS
The examination could be carried out successfully in 36 of 40 (90%) of rabbits with clipping. The restraint and examination were relatively well tolerated by the animals, except for the transverse sections. Obtaining oblique and longitudinal sections, carried out on 33 of 40 (83%) rabbits in our study, allowed for evaluation of the TB. The examination was feasible with all rabbit sizes. The depth of the TB was found to be linked to the size of the rabbit and especially to the size of its jaw. Visualization of the distal bulla wall was observed in 2 of the 40 (5%) subjects, consistent with abnormal fluid contents or bulla osteitis.

CLINICAL RELEVANCE
Ultrasonography of the TB was easy to learn and rapid to perform, with a mean examination time of < 10 minutes (mean of 8.71 minutes) without any sedation.

The rabbit is a species prone to otitis media, generally due to either an infectious agent spreading through the auditory tube or extension of an otitis externa.[1,2] The tympanic bulla (TB) is a bone cavity containing air, and it is the largest part of the middle ear. The inflammation in otitis media causes accumulation of fluid in the TB cavity and other changes to the structure of the TB such as thickening or bone lysis of its wall.[3,4]

Because the signs of otitis media are often not very specific or even absent, access to the TB would greatly assist in the diagnosis of otitis media. However, palpation of the TB is not very specific, and visualization of the TB content by video-otoscopy is not possible without injury to the eardrum.[5,6] Radiography can be used, but CT or MRI is the gold standard to assess the integrity of the TB,[7–10] and those procedures require heavy sedation (for CT) or general anesthesia (for MRI) when used for rabbits (*Oryctolagus cuniculus*). This species presents a high anesthetic risk (1.39% on average vs 0.17% and 0.24% in dogs and cats, respectively[11]). Therefore, a diagnostic imaging method for assessment of the TB in awake patients would be valuable. Ultrasonography has been used successfully to image the TB in several species: dogs,[12,13] cats,[14] calves,[15,16] and donkeys.[17] To our knowledge, 2 studies[9,18] of rabbits in this regard have been carried out to date, both of which were exclusively on rabbit cadavers. Chronologically, the first study[18] provided information regarding the anatomy and ultrasonographic presentation of this region and determined that 8 to 16 MHz and 12-MHz linear probes are the most appropriate equipment for performing ultrasonography of the TB by ventral approach in rabbits. The second study[9] showed that ultrasonography is better than radiography but inferior to CT at detecting fluid in the TB.

The aim of our study was to determine the feasibility of ultrasonography of the TB in live, nonsedated rabbits and the optimal examination modalities (optimal ultrasonographic imaging planes, restraint technique, rabbit's position, and clipping).

Materials and Methods

Pilot study

Three diagnostic imaging assistants (DIAs) and the head of the Oniris Transverse Imaging Service (MSF; 20 years of experience in imaging, including ultrasonography) performed test ultrasounds to define section planes and standardize practices. The ultrasonographic examinations were carried out randomly by 3 DIAs under supervision by the head of the unit.

The TB was examined by ultrasonography using a ventral approach, as follows: the probe was positioned immediately caudally to the angular process of the mandible and then oriented in different directions to obtain 3 views of the TB **(Figure 1)**, according to those described by King et al.[18]

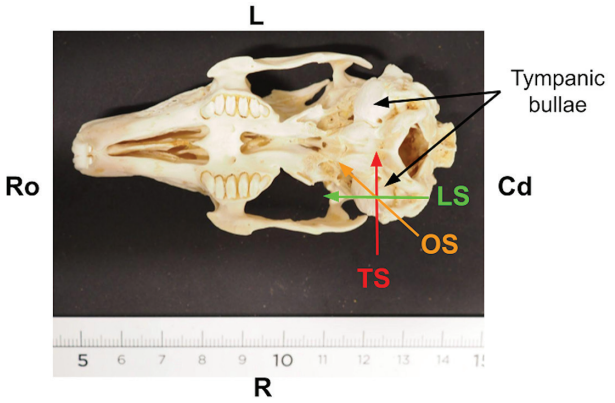


Figure 1—Image of the ventral view of a skull from a clinically normal adult rabbit (*Oryctolagus cuniculus*) displaying the longitudinal section (LS), oblique section (OS), and transverse section (TS), corresponding with the ultrasonographic planes imaged of the right (R) and left (L) tympanic bullae (TB) of 40 client-owned rabbits that were > 6 months of age, had no auricular abnormalities detected on physical examination, and underwent TB ultrasonography without sedation between September 2020 and May 2021. The scale at the bottom of the image is in centimeters. Cd = Caudal. Ro = Rostral.

The sections were referenced under the following acronyms:

- TS R and TS L for the right and left transverse sections, respectively, with the beam oriented in the caudodorsal direction on the transverse axis of the animal.
- OS R and OS L for the right and left oblique sections, respectively (ie, right 45° caudal–left rostral oblique [Rt45Cd–LtRO] plane).
- LS R and LS L for the right and left longitudinal (sagittal) sections, respectively, with the beam oriented in the caudodorsal direction on the longitudinal axis of the animal.

The protocol was implemented on 5 rabbits. Ultrasonography was attempted in these test rabbits without first clipping the fur; subjects were later shaved and reimaged.

Study design and case selection

The TB ultrasonographic examinations took place at the Oniris University Veterinary Hospital between September 2020 and May 2021. Forty adult rabbits older than 6 months were selected for inclusion in the study after approval of the project by the Oniris Veterinary Clinical and Epidemiological Ethics Committee (CERVO-2021-1-V). Emails were sent to students or people working at the school, and client-owned rabbits were solicited when they attended the Oniris University Veterinary Hospital for a consultation. The reason for the consultation was not related to ear damage, and no auricular lesion was present at the time of the examination.

We selected 40 rabbits, 20 of which were specifically chosen because they were a dwarf-sized breed type (< 3 kg) and 20 were specifically chosen because they were a standard-sized breed type (> 3 kg).

Three physical characteristics of the subjects were measured during each examination, as follows:

- Body weight, measured using a digital scale accurate to 0.01 kg.
- Jaw width, as the distance between the 2 angular processes of the mandibles, measured with a ruler to the nearest 0.5 cm.
- Jaw length, as the distance between the angular process of the mandible and the more rostral part of the roots of the mandibular incisor teeth, standardized to a ventral approach, measured with a ruler to the nearest 0.5 cm.

Protocol for TB ultrasonography

All TB ultrasonography was performed with a compact linear ultrasound transducer (L15-7io; Koninklijke Philips NV) that had a frequency range of 7 to 15 MHz and measured 3 cm in length by 1 cm in width. Nonsedated rabbits were positioned in sternal recumbency on an upside-down cushion **(Figure 2)**. The cushion provided elevation to increase the space available under the chin of the rabbit and allow contact with the probe.

Rabbit restraint required 2 people. The first person placed a hand on the animal's lower back. The second person placed a hand behind the skull under the ears and a finger under the chin to stretch the rabbit's neck upward (Figure 2).

In the main study, fur was clipped over a 3 X 3-cm area at the level of the angular process of each mandible prior to ultrasonography, and coupling gel was applied to the skin (Figure 2). For each examination, the "feasible" or "unfeasible" nature of the examination was noted, as follows:

- Feasible, if at least 1 of the ultrasonographic sections had been completed on each of the TB.
- Unfeasible, if none of the ultrasonographic sections could be completed; the reasons were then recorded.

The duration of the ultrasonographic examination, including clipping of fur, was evaluated for each examination. When the TB could be visualized, 1 image of each of the preestablished ultrasonographic planes was saved with the use of available software (PACS Synapse V5; FujiFilm Corp), which allowed retrospective analysis of the images, as follows:

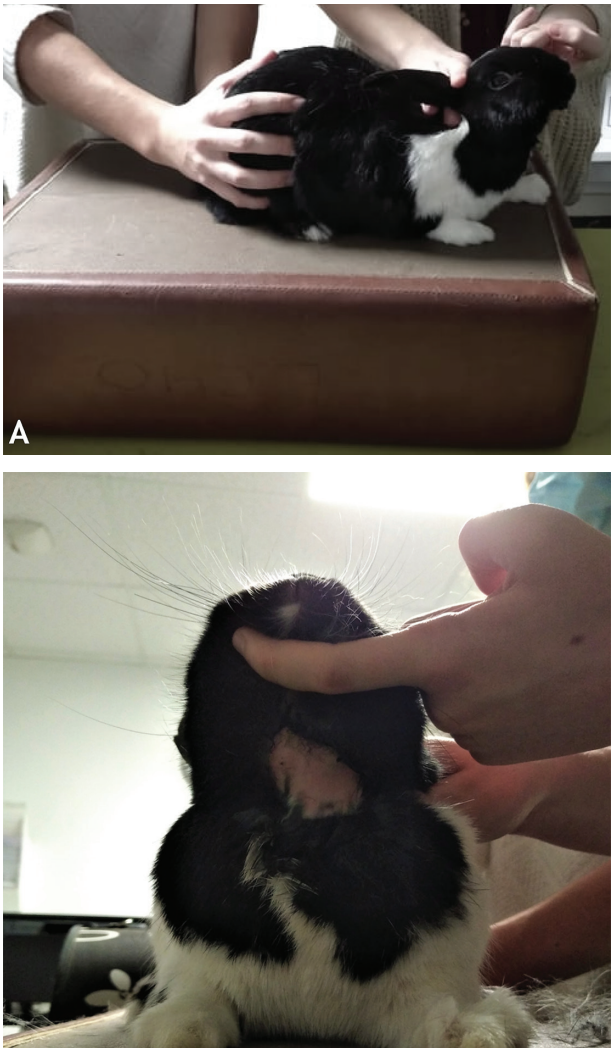


Figure 2—Images depicting manual restraint of a conscious rabbit for ultrasonography of the TBs as described in Figure 1. A—The rabbit is positioned in sternal recumbency on an upside-down cushion, which increased the operator's working space ventral to the TB to allow for proper positioning of the ultrasound probe. B—The rabbit's head is extended upward, revealing where a small patch of fur has been clipped before ultrasonography of the rabbit's left TB.

• Measurement of the depth of the TB, noting the distance between the surface and the ventral wall (the point closest to the surface).

• Determination of whether or not the ventral wall was continuous.

• Recording of the presence or absence of content in the TB and description of its ultrasonographic presentation.[18]

Statistical analysis

The proportion of feasible sections (planes) was calculated on a global and individual basis, and then as a function of the breed type and sonographers. Data were assessed for normality, followed by an unpaired Student *t* test to compare the durations of the examinations on standard-sized breed type versus dwarf-sized breed type rabbits. We performed the same test to compare the size of the jaw depending on the breed type. This test was followed by an ANOVA with a Tukey-Kramer multiple comparison test to compare the durations of the examinations by the 3 sonographers. Bivariable ANOVA was performed to compare the depths of the TB for 4 sections (LS R, LS L, OS R, and OS L) and 2 groups of rabbits (standard-sized breed type and dwarf-sized breed type). The ANOVA analysis on the lines compared the depths according to the sections. As for the ANOVA analysis on the columns associated with a Sidak multiple comparison test, they were carried out to study whether the depths were a function of the breed type (standard-sized or dwarf-sized breed type). For each section, Pearson correlation coefficient (ρ) analysis was performed to determine whether the mean TB depth was associated with the jaw width, and the same analysis was performed for the jaw length.

Results

Pilot study

The protocol was implemented on 5 rabbits: a New Zealand breed type, a farm rabbit, an Aries rabbit, a dwarf rabbit, and a lion-headed dwarf rabbit.

It was not possible to obtain measurements on the dwarf-sized rabbit type. For the farm rabbit and the Aries rabbit, the images obtained were incomplete, of poor quality, or both. The only complete examination producing adequate images was performed on the New Zealand rabbit. In addition, the examination, whether or not complete, lasted 30 minutes on average.

Taking into account the results of these first measurements, a clipping of 3 X 3 cm at the level of the angular process of each mandible was added to the initial protocol.

The rabbits in this prestudy were reexamined with clipping, except for the New Zealand rabbit, for which the review had been completed.

Study population

For the dwarf-sized breed type rabbits, there were 10 females and 10 males. The mean age was 2 years and 9 months. The age of the youngest was 6 months, and the oldest was 7 years and 11 months old. Their mean weight was 1.78 kg. Regarding the size of the jaw, the mean width was 3.1 cm and mean length was 4.5 cm.

For the standard-sized breed type rabbits, there were 16 females and 4 males. The mean age was 1 year and 6 months. The youngest was 6 months old, and the oldest was 3 years and 7 months old. The heaviest was 6.36 kg, and the lightest was 0.9 kg. Their mean weight was 3.92 kg. Regarding the size of the jaw, the mean width was 4.3 cm and the mean length was 7.2 cm. The mean width and length of the jaw were significantly ($P < .001$ for both) greater for the rabbits of standard-sized breed type (4.3 and 7.2 cm, respectively), compared with the dwarf-sized breed type (3.1 and 4.5 cm, respectively).

Ultrasonographic examinations

Ultrasonographic examination was possible (at least 1 section on each ear) for 90% (36/40) of the rabbits **(Table 1)**.

Table 1—The numbers and percentages of successful measurements of the tympanic bulla (TB) depth in 40 client-owned dwarf-sized (n = 20) and standard-sized (20) breed type rabbits (*Oryctolagus cuniculus*) that were > 6 months of age, had no auricular abnormalities detected on physical examination, and underwent TB ultrasonography without sedation between September 2020 and May 2021, stratified by TB ultrasonographic view, as follows: 36 longitudinal sections of the right TB (LS R), 35 longitudinal sections of the left TB (LS L), 35 oblique sections of the right TB (OS R), 34 oblique sections of the left TB (OS L), 5 transverse sections of the right TB (TS R), and 6 transverse sections of the left TB (TS L).

	Ultrasonographic section (plane)					
Rabbits	**LS R**	**LS L**	**OS R**	**OS L**	**TS R**	**TS L**
All (n = 40)	36 (90)	35 (88)	35 (88)	34 (85)	5 (13)	6 (15)
Dwarf-sized breed type (n = 20)	19 (95)	18 (90)	19 (95)	19 (95)	0	0
Standard-sized breed type (n = 20)	17 (85)	17 (85)	16 (80)	15 (75)	5 (25)	6 (30)

At least 4 of the sections could be carried out (2 on each ear) for 83% (33/40) of the rabbits. The examination was complete for 13% (5/40) of the rabbits. It was possible to perform 5 sections (3 on one ear and 2 on the opposite ear) for 3% (1/40) of the rabbits, with TS R not being carried out. For 68% (27/40) of the rabbits, 4 sections could be acquired, with TS R and TS L not being carried out each time.

For 2 of the 40 (5%) rabbits in the study, 3 of the sections could not be carried out: TS R, TS L, and OS L for the first rabbit and TS R, TS L, and LS L for the second rabbit. Ultrasonography of 1 of the 40 (3%) rabbits only allowed 2 of the 6 sections to be obtained: LS R and LS L. All sections that were not carried out were in fact attempted but not tolerated by the rabbits. Ultrasonography was not possible for 10% (4/40) of the rabbits. For these rabbits, it was decided to stop the examination to avoid an excessively high level of stress, excessive agitation of the subjects, or both. Even when a rabbit tolerated ultrasonography, it was often not possible to carry out transverse sections (Table 1).

In terms of the dichotomy between dwarf-sized and standard-sized breed type rabbits, we noticed that performing the TS R and TS L sections was not possible in 100% of the dwarf rabbits and 75% (15/20) and 70% (14/20) of the standard-sized breed type rabbits for TS R and TS L, respectively.

Among the 4 examinations that were initially unfeasible (without clipping), each DIA performed an ultrasonographic examination and DIA 1 performed 1 more than the others. The random distribution of these examinations by DIA confirmed that the unfeasible nature of the examination was not a function of the DIA but only of the rabbit.

Examination duration

The examination duration was quantified for 35 of the 36 rabbits for which the ultrasonographic examination was possible. The time for the 36th rabbit was not taken into account because the examination was performed without clipping. The examination durations were analyzed with different groupings, depending on the breed type or the DIA. There was not a significant (P = .962) difference between the mean examination durations for the standard-sized breed type (8.69 minutes) and the dwarf-sized breed type (8.74 minutes).

The mean duration of the ultrasonographic examination was significantly (P < .001) longer when performed on rabbits without hair clipped (pilot study; 30 minutes; n = 5) versus with hair clipped (larger, main study; 8.71 ± 3.04 minutes; 35). Ultrasonography was performed on 15 rabbits by DIA 1, 15 rabbits by DIA 2, and 10 rabbits by DIA 3. The mean duration of the ultrasonographic examinations for DIA 3 (11.89 minutes) was statistically longer (P < .001) than the mean examination times for DIA 1 and DIA 2 (8.25 and 7.07 minutes, respectively). However, the difference between the mean examination times for DIAs 1 and 2 was not significant. The examination duration was longer for the first ultrasonographic examination for all DIAs (9, 10, and 16 minutes for DIAs 1, 2, and 3, respectively).

Ultrasonographic image analysis

Echogenicity of the TB—The ultrasonographic presentation of the TB on different sections was comparable for the dwarf-sized breed type rabbits and standard-sized breed type rabbits. Healthy TB (filled with gas) appeared in our study as a convex hyperechoic surface. The TB creates acoustic shadowing, appearing as an area of high amplitude specular echoes created by highly attenuating structures (intact cortical surface of the TB).[19] This acoustic shadowing masks most of the deeper structures **(Figure 3)**.

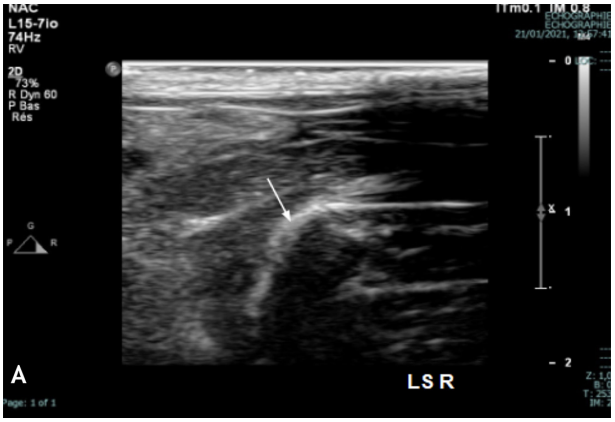
Depth of the TB

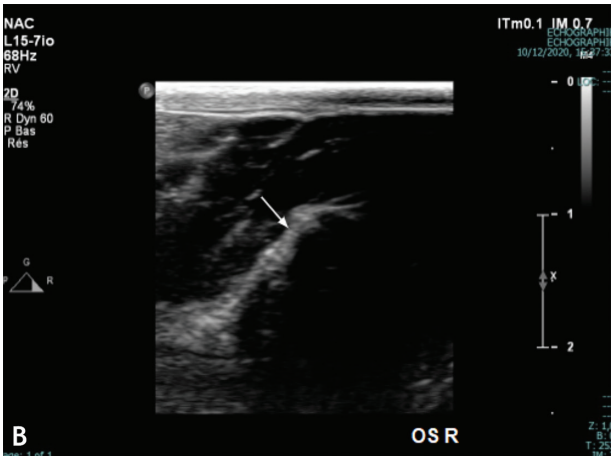
Depending on the breed type—The results for TB depth were normally distributed. The mean TB depth did not differ on the basis of the section (LS R, 1.18 cm; LS L, 1.18 cm; OS R, 1.18 cm; OS L, 1.13 cm; TS R, 1.07 cm; and TS L, 1.2 cm for standard-sized breed type and LS R, 0.88 cm; LS L, 0.84 cm; OS R, 0.84 cm; and OS L, 0.78 cm for dwarf-sized breed type). However, when grouped on the basis of breed type, the mean TB depth for each section was significantly (P < .001) greater for the standard-sized breed type, compared with the dwarf-sized breed type **(Figure 4)**.

Depending on the size of the jaw—The TB depth, irrespective of the section, significantly (P < .05) and positively correlated with jaw length (ρ = 0.6) and jaw width (ρ = 0.4).

Content of TB

Gas is the physiologic content of the TB and was found in 95% (38/40) of cases. In 2 of 40 (5%) rabbits, the dorsal wall was visible on the 2 sections acquired





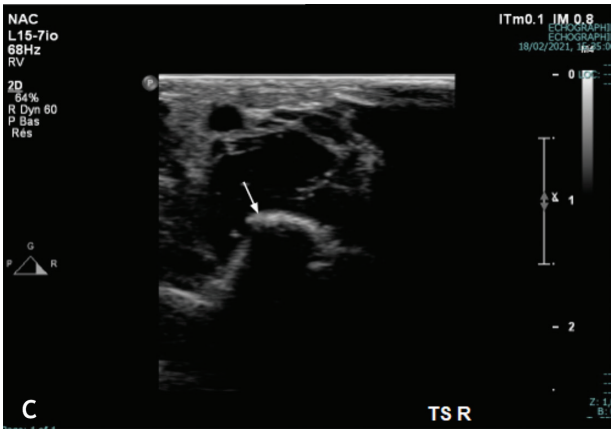


Figure 3—Representative ultrasonographic images of TBs (arrows) of 3 rabbits described in Figure 1, with the scale toward the right in each image in centimeters. A—Longitudinal section of the right (LS R) TB of a dwarf-sized breed type rabbit. B—Oblique section of the right (OS R) TB of a farm rabbit (standard-sized breed type). C—Transverse section of the right (TS R) TB of a New Zealand rabbit (standard-sized breed type).

(LS L and OS L), consistent with unilateral otitis media. Both of these rabbits were a dwarf-sized breed type. One of these rabbits had TB contents that were hypoechoic on LS L and mixed echogenicity (anechoic and of intermediate echogenicity) on OS L. The remaining rabbit had mixed echogenicity (anechoic with areas of intermediate echogenicity) on the 2 sections **(Figure 5)**.

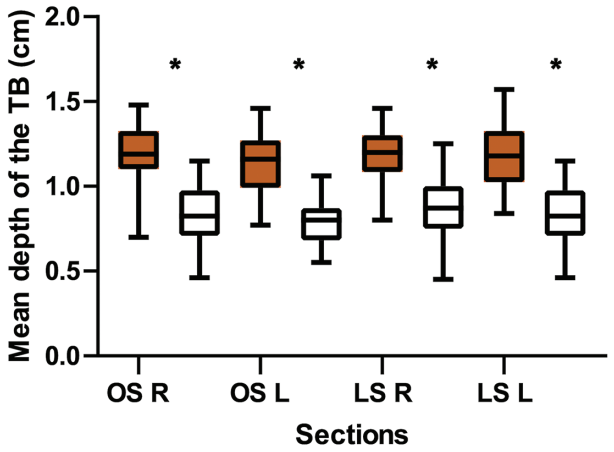


Figure 4—Box-and-whisker plots for Sidak multiple comparisons of the mean TB depth on LS R, OS R, longitudinal section of the left (LS L), and oblique section of the left (OS L) TB ultrasonography in standard-sized (brown) versus dwarf-sized (white) breed types of rabbits described in Figure 1. For each plot, the line in the box represents the mean, the box represents the interquartile (25th to 75th percentile) range, and the whiskers represent the SD. Asterisks indicate results that differed significantly ($P < .001$) between groups.

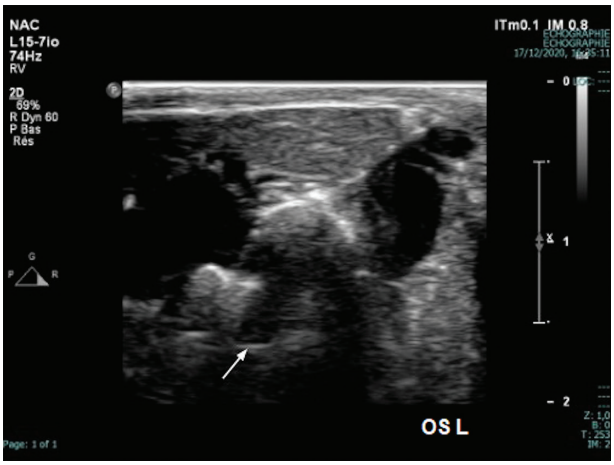


Figure 5—An OS L ultrasonographic image of a dwarf-sized breed type rabbit described in Figure 1, showing mixed echogenicity (anechoic with areas of intermediate echogenicity) ventral to the TB dorsal wall (arrow). In the image, dorsal is toward the bottom and ventral is toward the top. The scale toward the right is in centimeters.

Continuity of the ventral wall of the TB

None of the examinations in the study revealed a discontinuity in the wall of the TB. This observation is consistent with the defined criteria for recruitment.

Discussion

The examinations were very well tolerated by the rabbits thanks to a minimally stressful and restrictive restraint. The absence of clipping prior to the ultrasonographic examination was retained in the first instance. This caused very great difficulty in obtaining the images, explained by major difficulty in

maintaining correct contact between the probe and the skin and consequently a considerably lengthened time of examination. Thus, by achieving a risk-benefit balance, clipping was found to be acceptable using a silent clipper to reduce stress. This clipping subsequently allowed a significant reduction in the examination time, with the completion time being 3 times shorter (30 minutes without clipping vs 8.71 minutes, on average, with clipping). The overall stress level was, therefore, lower for the patients. The examination time was compatible with routine practice, with a mean ± SD duration of ultrasonographic examination of both TBs (including clipping fur) of 8.71 ± 3.04 minutes.

Complete ultrasonography of an organ requires performing 2 sections along 2 different axes.[16] In our study, 2 sections (longitudinal [sagittal] and oblique) were performed on each TB (right and left) on 83% (33/40) of rabbits.

No cost assessment was performed in our study. The average cost of ultrasonography is less than the cost of a CT or MRI and comparable to the cost of the 4 radiographic films that would otherwise be required. Although exotic pets are now increasingly medicalized, the cost of procedures often remains a limiting factor.

The study allowed the identification, or at least suspicion, of otitis media in 2 rabbits. Our findings were consistent with data in the literature, which indicate a high frequency of subclinical otitis media in this species (between 11.5% and 32% of rabbits, depending on the study[1]).

The study did not reveal any discontinuity in the wall of the TB. This observation also indicated the absence of bone lysis of the TB for the 2 rabbits in the study that had subclinical otitis media. This is in line with what the literature indicates, as the presence of bone changes corresponds more to high-grade III or IV otitis media, which has been assumed to be clinical.[3,4]

It is important to underscore that our feasibility study focused on rabbits of very different sizes, which was not the case in previous studies.[9,18] Our examinations, comprising at least 4 sections, were carried out for 75% (15/20) of the standard-sized breed type rabbits and 90% (18/20) of the dwarf-sized breed type rabbits. We hypothesized that standard-sized rabbits were less used to being handled than dwarf rabbits.

Our study provides new data not previously described in the literature. Concerning the acquisition of images, distinction of the mastoid process allowed easier identification of the TB.[9] In our study, the sonographers also identified the mandible. The angular process of the mandible appeared to be easier to locate since it was directly palpable at the base of the probe during acquisition of the images.

The depth of the TB on ultrasonography was not dependent on the plane orientation. However, it depended on the breed type of the rabbit being scanned. The TB was indeed deeper in the standard-sized breed type. This finding is in agreement with the fact that the depth of the TB increases as the size of the jaw increases. This could influence image quality and help identify TB in larger rabbits.

While previous studies only examined ultrasonography of the TB in the New Zealand breed type of rabbit, our study compared the performance on different breed types. The breed type did not appear to influence the feasibility of the examination when it comes to LS and OS. By contrast, TS was not possible on dwarf rabbit types.

There were some elements of our study that need to be pointed out as limitations. We did not have the same number of male and female rabbits. Yet male rabbits have slightly larger skulls than females; this was, therefore, a bias of our study.[20]

In most cases, the TS was not possible. The mandible, which is prominent in the rabbit, interfered with optimal contact of the TB with the probe. An ultrasonographic imaging in the TS plane resulted in pressure being applied on the larynx, which was poorly tolerated by the rabbits. This was accentuated in dwarf rabbit types since the length of the probe (3 cm) was close to the width of their jaw (on average, 3.1 cm).

Ten percent (4/40) of the examinations could not be performed (ie, ultrasonography for none of the sections could be carried out). These examinations were ruled out due to the subjects being too agitated. This reason was also noted when < 4 sections (2 on each TB) were carried out on 7.5% (3/40) of the rabbits.

The depth of the TB was not always measured at the same point on the wall on all TBs. The rotation and movement of the probe were often limited. This constituted a bias in the statistical analyses taking into account the depth of the TB.

The jaw size was measured inaccurately using a ruler, which also may have biased the statistical analyses involving jaw width and length. In order for the observations and the images to be more comparable, it would have been better if each rabbit had been scanned by all 3 DIAs. Unfortunately, such a protocol was technically impractical in our clinic.

Ultrasonographic examination of the TB in rabbits required an ultrasound probe of high frequency, for better resolution at shallow depth, and small size, to optimize handling and contact. This equipment is not widely available on a routine basis and may limit the performance of this examination to veterinary centers specializing in imaging of exotic pets.

No confirmatory examination (CT or MRI) was performed to confirm the absence or presence of lesion of the TB. It would have been ethically inappropriate to take the risk of sedating the animals. Nevertheless, another study would be necessary on symptomatic rabbits to validate the use of ultrasonography in the diagnosis of otitis media in rabbits.

In conclusion, results of our study demonstrated the feasibility of using ultrasonography to assess the TB in 40 live, nonsedated, healthy rabbits. Our results also indicated that ultrasonographic techniques used in the present study were rapid and easy to learn and the average examination time was < 10 minutes with clipping. Ninety percent (36/40) of the rabbits could be assessed with ultrasonography. The

remaining 10% (4/40) could not be assessed with ultrasonography without resorting to sedation.

The restraint and ultrasonographic examination, except for transverse plane imaging, were relatively well tolerated by the animals. Obtaining oblique and longitudinal sections on 83% (33/40) of the rabbits in our study allowed proper evaluation of the TB. Our study found that the examination feasibility applied to all types of rabbits.

The depth of the TB is linked to the size of the rabbit and especially to the size of its jaw. This makes it possible to anticipate the depth of the TB and to refine the search area on the screen of the ultrasound machine.

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