Acoustic rhinometry in dog and cat compared with a fluid-displacement method and magnetic resonance imaging

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Straszek, Sune P., Finn Taagehøj, Søren Graff, and Ole F. Pedersen. Acoustic rhinometry in dog and cat compared with a fluid-displacement method and magnetic resonance imaging. J Appl Physiol 95: 635–642, 2003. First published April 18, 2003; 10.1152/japplphysiol.01105.2002.—An increasing number of studies have used acoustic rhinometry (AR) for study of pharmacological interventions on nasal cavity dimensions in dogs and cats, but there have been no attempts to validate AR in these species. This is done in the present study. We compared area-distance relationships of nasal cavities from five decapitated dogs (3.5–41 kg) and cats (3.8–6 kg). AR was compared with magnetic resonance (MR) imaging and a fluid-displacement method (FDM) using perfluorocarbon. AR measured 88% (98–79%) (mean and 95% confidence interval) of nasal cavity volume in dogs determined by FDM and 71% (83–59%) in cats. AR markedly underestimated nasal cavity dimensions when minimum areas were below 0.1 cm2 in dogs and 0.05 cm2 in cats. AR underestimation increased with the severity of the constriction and with distance. Cross-sectional areas in the deeper parts of the cavity measured 76% (99–54%) of FDM in dogs and 52% (66–39%) in cats. AR agreed well with MR, especially in the deeper part of the cavity. MR images showed that the nasal cavities had a very complex structure not expected to be reproduced by AR. MR could not be considered a “gold standard” because definition of the cross-sectional area of the lumen depended critically on subjective choices. FDM produced repeatable measurements and possibly offers the most adequate reference in future evaluation of AR. AR underestimated what we believed were the most correct cross-sectional areas determined by FDM, especially in the deeper part of the dog and cat nasal cavities. Despite these difficulties, AR has been shown to be useful to describe qualitative changes in cross-sectional area.

nasal airway volume; nasal pharmacology; laboratory animals; rhinology

ACOUSTIC RHINOMETRY (AR) is a method that uses sound reflection to measure the nasal cavity geometry in terms of a curve displaying cross-sectional area as a function of distance. The method was first used in laboratory animals (guinea pigs) in 1994 (23). Since then, a small number of studies have used AR to describe the effect of pharmacological interventions on the nasal mucosa in guinea pigs (11, 12, 19–21, 24).

Recent studies (4, 13, 14, 16–18) have used dogs and cats for drug evaluation. Terheyden et al. (26) summarize several attempts to validate how precisely AR measures the nasal cavity in humans, including validation of AR by magnetic resonance (MR) imaging (2, 8) and by a fluid-displacement method (FDM) (7). By comparing volumes determined by AR with volumes of nasal cavity casts, Kaise et al. (12) found AR in guinea pigs to measure only 75% of nasal cavity volume. However, there have been no attempts so far to validate AR in dogs or cats. The importance to address this becomes evident, because there is a growing interest in using AR in larger animals to study pharmacological effects.

We conducted AR, MR, and FDM measurements postmortem on dogs and cats to answer the following questions. 1) How accurately does AR measure both volume and cross-sectional areas of the nasal passageways of dog and cat? 2) What geometric conditions affect AR measurements? 3) Are FDM and MR useful in validation of AR?

METHODS

General Methods

AR. We used a system slightly modified from the one described by Pedersen et al. (23). A sound pulse propagates through a tube, passes a microphone, and enters the nasal cavity through a tightly fitting funnel-shaped nosepiece that is adapted to the nostril. The reflected waves travel back through the tube, are recorded by the microphone connected to an amplifier (GJ Elektronik, Skanderborg, Denmark), and, via an analog-to-digital converter (PC-Card Das16/12, Computer Boards), sampled by a computer (700-MHz Armada, Compaq) at 100,000 Hz. We chose a sound tube with a cross-sectional area of 0.5 cm2 for dogs and 0.126 cm2 for cats.

FDM. The nasal cavity was filled vertically from the nostril with fluid by a Harvard pump delivering constant flow. The pressure at the inlet was measured with a manometer (SCXL004, Parnell Electronics Components, Leeds, UK) connected via an analog-to-digital converter (CIO-DAS08/jy, Computer Boards) to a personal computer using Labtech software. Data were recorded at 2 Hz. The pressure is a measure for the height of the fluid, i.e., the distance into the nasal cavity. Thus the speed of the rising surface is proportional to a change in pressure divided by the change in time.

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The cross-sectional area of the cavity is then
\[ A \times \frac{dV}{dt} = \frac{dP}{dt} \]
where \( A \) is the cross-sectional area, \( dV/dt \) is the flow (i.e., change in volume over change in time), and \( dP/dt \) is the change in pressure over the change in time.

When the cross-sectional area of the cavity increases, the speed of the rising surface slows down, and vice versa. A moving-average algorithm was applied to reduce noise. This involved calculation of a new data set of mean values from 1 to \( n \) sampled points, 2 to \( n + 1 \), etc. This is referred to as a filter. Our algorithm consisted of two consecutive filters, each spanning over \( n = 15 \) samples.

Previous attempts to use FDM were based on water (7). However, from preliminary experiments, we found a significant error because of air bubbles and capillary effect. We decided instead to use a perfluorocarbon because of its favorable physical properties. Perfluorocarbons, consisting of carbon and fluorine, are inactive, nontoxic, colorless, and odorless fluids. In addition, they are not soluble in water or fat, which gives a minimal interaction with biological tissue. They have a low surface tension compared with water and a high density that diminishes the capillary effect otherwise contributing to errors, especially when dimensions are small. Capillarity will cause the surface to rise, thus influencing pressure measurements at the bottom of the tube. A goal in FDM is, therefore, to reduce capillarity. The increase in height (\( \Delta H \)) due to capillarity of the ideal circular surface in a tube is given as
\[ \Delta H = \frac{2 \times \gamma \times \cos \theta}{g \times \rho \times r} \]
where \( \gamma \) is the surface tension, \( \theta \) is the contact angle between fluid and air, \( \rho \) is the density, \( g \) is gravitational acceleration, and \( r \) is the radius of the tube (22). Density, surface tension, and contact angle are the only variables that can be modified by choice of fluid.

To validate perfluorocarbon in FDM, we compared measurements with ethanol and perfluorocarbon on rigid plastic nose models with known dimensions. One of the models is the standard model described by Hilberg and Pedersen (9) for testing equipment used in adult humans, which has dimensions of the same order of magnitude as those of a medium-sized dog. We chose a perfluorocarbon with the formula \( C_{13}F_{25} \) (FP10), a surface tension of 19.7 mN/m, and density of 1.98 kg/l. For comparison, the respective values for water are 72 mN/m and 1 kg/l, and for ethanol are 22.8 mN/m and 0.79 kg/l. Additional technical information on FP10 can be obtained from the manufacturer (F2 Chemicals Lea Town, Preston, Lancashire, UK).

**MR scanning and image processing.** T1-weighted MR images were obtained with a 1.5 T GE scanner (TwinSpeed, General Electric, Milwaukee, WI). Consecutive T1-weighted three-dimensional, fast spoiled gradient MR images with a matrix of 256 \( \times \) 256 zipped to 512 \( \times \) 512, field of view of 12 \( \times \) 12 cm for dogs and 10 \( \times \) 10 cm for cats, and a number of excitations of 2 for dogs and 3 for cats to get a better signal-to-noise ratio for the smaller individuals. The slice thickness was 2 mm overlap zipped to 1 mm, and the number of reconstructed slices was 50–70 depending on the nasal cavity dimensions of the subject. The scan plane was approximated coronal, with scans starting just before the tip of the nose. The coils used were headcoil for the dogs and extremity coil (knee coil) for the cats. Both were quadrature radio frequency coils.

MR images were converted from DICOM to Tiff format (Osiris 4.09, Geneva University Hospital). Further image processing and manipulation was done in JASC Paint Shop Pro 7.04 (JASC Software). An area was selected that contained the lumen of the cavity, and the magnitude of this area was calculated from the number of pixels. A correction factor, derived from the average light intensity (brightness) of the pixels, allowed calculation of the cross-sectional area of the cavity not occupying tissue. The same procedure was done for computerized tomography (CT) images obtained from a single animal.

**General statistics.** One AR measurement of a nasal cavity is defined as the mean curve of 10 consecutive runs displayed as area-distance curves, where area is a function of distance with 1.72 mm between sampling points. The measurements of one cavity were pooled into one series. Confidence intervals are always found for series, unless otherwise stated. The errors defining the confidence limit (CL) are
\[ CL = t_{a/2} \times \frac{\text{SD}}{\sqrt{n}} \]
where \( n \) is the number of series included and \( t_{a/2} \) is the time value from the time distribution for 95% confidence and \( n - 1 \) degrees of freedom. The 95% confidence interval (mean ± CL) is presented in parenthesis. When comparing differently sized dogs and cats, the relative cross-sectional area in relation to maximum area is a function of relative distance intervals presented as the percentage of distance to the maximum area.

The cross-sectional area of the cavity is then
\[ A \times \frac{dV}{dt} = \frac{dP}{dt} \]

Confidence intervals of fractions are given by Gauss’s formula (3). The errors (CL) of the function \( F = z/y \) can be expressed as
\[ CL(z/y) = \sqrt{\left(\frac{CL_z}{y}\right)^2 + \left(\frac{-z \times CL_y}{y^2}\right)^2} \]
where \( CL_z \) and \( CL_y \) are errors of \( z \) and \( y \), respectively.

Volumes are calculated as integrated area-distance curves between given limits.

**Experimental Protocol**

**AR, FDM, and MR.** Five cats and five dogs were used (10 cavities each). The animals were of different race and sex and were donated postmortem from an animal hospital. The dogs ranged in size between 3.5 and 41 kg, whereas the cats ranged between 3.8 and 6 kg. Animals were killed with 30% pentobarbital sodium injected intravenously for dogs and intraperitoneally for cats and were decapitated between C2 and C3. The heads were subsequently frozen at −18°C and later placed in a refrigerator to slowly defrost 24 h before experiments.

Before measurements, the heads were placed with the axis of the nasal cavities in a vertical position, with the nose tip pointing down. The equipment was checked by measurements on nasal models. A funnel-shaped plastic nosepiece (Mediplast, Söderfors, Sweden) originally intended for otoscopy was used in dogs, and a pipette tip cut short was used for cats. The sound tube was fitted to the nosepiece, and AR was performed for each nostril/cavity. The respective nosepieces were inserted into one nostril at a time. Because of the variation in size of the nasal cavities, we chose nosepieces with the narrowest diameter between 3 and 7 mm.

**DOGS.** One series of two to three AR measurements was conducted on each cavity before FDM. Before each measurement, the nosepiece was removed and inserted again. With the head kept in a vertical position, a nosepiece was inserted
A plastic tube was inserted into the nosenose. The tube was connected to a syringe that contained PP10 that was placed in a Harvard Pump delivering a constant flow of 0.0672 ml/s for big dogs and 0.0169 ml/s for cats and small dogs. Before measurements in each animal, the manometer was carefully calibrated, and a correction factor was determined by relating the height of perfluorocarbon in a vertical tube to the measured pressure. Five consecutive single FDM measurements were obtained on each nasal cavity. Measurements were stopped when the fluid reached the end of the nasal septum and ran out of the opposite nostril. All dogs had AR measurements carried out both before and after FDM. The heads were then frozen and later thawed for MR. One head was furthermore examined by CT scan.

**Cats.** Only one AR measurement of 10 runs was conducted on each cavity, and cats were only measured before FDM, because initial tests showed that fluid in the narrow feline nasal cavity made AR unreliable. FDM and MR were conducted as described above.

**Evaluation of nosepieces.** We evaluated two different nosepieces in the dog study. A human nosepiece made of plastic was applied externally and did not enter the nasal cavity (Hood Laboratories, Pembroke, MA). The other is described above. For the evaluation, we used three dogs (6 cavities) ranging in size from 13 to 41 kg.

The external nosepiece covered the nostril, and a gel was used to make sure that there were no leaks between the nosepiece and tissue. Several measurements were done with both nosepieces. We changed back and forth between the nostrils so that we never recorded two measurements in a row without moving the nosepiece first.

**Evaluation of nasal anatomy.** Anatomic descriptions below of the dog and cat nasal cavity are based on studies of color atlases (1, 5). For a more detailed description, we refer to these.

**RESULTS**

**MR**

**Dogs.** In Figs. 1A, 2A, and 3A, typical MR frontal segments from a dog 2, 5.5, and 9 cm from the tip of the nose, respectively, are shown. Figures 1B, 2B, and 3B show additional CT scans from the same dog.

From the nostril, a duct leads into the nasal cavity. The duct is divided into an upper and lower tract by an alar fold, with only narrow medial communication (Fig. 1A). The medial wall at this point is made of vomer and is deeper inside the nasal septum of the ethmoid bone. From the lateral wall, the conchae protrude (Fig. 2A), which include the ventral, dorsal, and ethmoidal conchae, which are made of cartilaginous or slightly ossified scrolls covered with mucosa. Deeper inside the cavity, convoluted structures are no longer apparent, and it is increasingly difficult to define the lumen (Fig. 3A).

From the MR and CT scans of the same head, Fig. 1 shows clearly visible, but minor, discrepancies, which may partly be due to the deformation of the tissue after insertion of the nosepiece under AR measurements and the following freezing. Both parts of Fig. 2 agree well, even though MR has a clearly higher resolution than CT. On the CT scan (Fig. 3B) the ethmoidal conchae, almost invisible on MR, appears well defined as a solid mass of mucosal septa. Also notice the maxillary recesses laterally corresponding to the maxillary sinuses in humans. They communicate openly with the nasal cavity more caudally (not shown) and were only separated from the open cavity by ossified septa that were almost invisible by MR. The transverse lamina divides the nasal cavity into two compartments: an upper compartment containing the ethmoidal conchae, and a lower tract, the choana, leading to the nasopharyngeal meatus.

**Cats.** The anatomy of the cat nasal cavity is much like that of the dog. Figures 4–6 show frontal slices 1, 2, and 3 cm, respectively, from the tip of the nose. Near the tip of the nose, the geometry is simple with a sharply defined mucosa. Then a fine web of convoluted septa or scrolls nearly fills the entire cavity (Fig. 5). One notable difference between dogs and cats is that the maxillary recess is almost nonexistent in cats. As for the dog, the cavity geometry is more difficult to describe by MR deeper inside the cavity. In Fig. 6, it is almost impossible to define the cavity from compartments closed by ossified or cartilaginous septa.

**Evaluation of PP10**

In preliminary tests, we found perfluorocarbon in FDM to be superior to water and ethanol in repeatability and agreement. Area-distance curves on the standard model (9) with PP10 are presented in Fig. 7. An AR measurement of the same model is presented for
comparison. PP10 did not only show area-distance curves with high repeatability, but also better agreement with the model. After having established that PP10 portrays the actual geometry of a model with exceptional precision, we carried out all FDM measurements by using PP10.

MR and FDM

Dogs. We compared MR with FDM for all dogs (10 cavities) by calculating the fraction of cross-sectional areas (MR/FDM) as a function of the distance \(L_{\text{max}}\), i.e., the distance from the tip of the nose to the point with maximum area \(A_{\text{max}}\) measured by FDM. The cavity was divided in four segments (0–25, 25–50, 50–75, and 75–100%) of \(L_{\text{max}}\), and we calculated the mean fraction of areas MR/FDM for all segments. This is illustrated in Fig. 8. Despite the difficulties with interpretation of MR scans, cross-sectional areas estimated from MR did show acceptable agreement with FDM in some parts of the cavity. The first part (0–25%) is influenced by the nosepiece, which results in a difference in geometry between MR and FDM. In the second part (25–50%), MR and FDM agree well. However, MR apparently underestimates area by FDM in the third part, to finally agree again in the fourth part.

Cats. The cat nasal cavity was divided in segments ranging from 50 to 75 and 75 to 100% of \(L_{\text{max}}\) (not shown). The first two segments (0–25 and 25–50%) were occupied by the nosepiece. Corresponding values of fractional area MR/FDM are 88.0%. There is no significant difference between the two methods, even though MR tends to underestimate cross-sectional area in the segment between 50–75% of \(L_{\text{max}}\).

Validation of AR

Typical AR, FDM, and MR measurements of a dog and cat nasal cavity are presented in Fig. 9. It is seen how both AR and MR measure smaller cross-sectional areas in most of the cavity compared with FDM. MR typically agrees well with FDM in the deepest part of the cavity, whereas AR continually underestimates until the end of the nasal septum, which is the right limit of the curve.

Dogs. We calculated the volume deviations as the fraction of volume by AR to volume by FDM. The volumes were measured over a distance between a
minimum area \( (A_{\text{min}}) \), defined as the lowest point on the AR curve found by the computer, and the \( A_{\text{max}} \) before the end of the nasal septum. We chose the \( A_{\text{min}} \) determined by AR, because it was always well defined. In Fig. 10A, the deviation for dogs is presented as a function of \( A_{\text{min}} \). We differentiated between AR measurements obtained before and after FDM. It looks like there is a lower limit of \( A_{\text{min}} \) at \( \sim0.1 \text{ cm}^2 \), below which the underestimation of AR measurements obtained with a 0.5-cm\(^2\) sound tube is substantial. If measurements with an \( A_{\text{min}} \) of \(<0.1\text{ cm}^2\) are removed, AR before FDM measures 88.3\% (98.1–78.5\%) of total volume by FDM, and AR after measures 74.3\% (85.0–63.5\%).

Cats. Figure 10B presents the fraction of volumes by AR and FDM for cats as a function of \( A_{\text{min}} \), but with different axis compared with Fig. 10A. With a 0.126-cm\(^2\) sound tube, AR measures 70.7\% (82.9–58.6\%) of volume by FDM. No clear lower limit for \( A_{\text{min}} \) was found, but measurements indicated a value of \( \sim0.05\text{ cm}^2\).

To better visualize the underestimation of the cross-sectional area by AR, the fraction of the areas measured by AR and FDM is presented as a function of \( L/L_{\text{max}} \), i.e., the distance to a given point (\( L \)) starting...
from the nose tip, divided by the distance \( L_{\text{max}} \) with \( A_{\text{max}} \) measured by FDM.

**Dogs.** Figure 11A shows the area measured from dogs by AR before FDM since it is theoretically closest to the in vivo state. Measurements with a \( A_{\text{min}} \) of \(<0.1\) cm\(^2\) are excluded. The maximum depth of nosepieces is also marked.

AR before FDM falls from 127 to 77.6% of the area measured by FDM and stabilizes at this level. AR after FDM (not shown) reached a low of 69.2%. There is no significant difference between AR before and after FDM. Nevertheless, it still seems reasonable to use AR before FDM as the measurements closest to portraying the in vivo situation.

**Cats.** Figure 11B presents the same relations for the cats. The relative deviation between the two methods increases with a distance from \( L_{\text{max}} \) of \(-60\)–\(100\). Area measured by AR falls from 92.6 to 52.3% of the area measured by FDM.

**Evaluation of Nosepieces**

In the dog, we focused on the variability between measurements obtained with the two nosepieces, as well as the reproducibility for the specific nosepiece. We found no significant differences in mean values between the two nosepieces in the areas measured (Fig. 12), but measurements obtained with the external nosepiece did have a higher reproducibility.

**DISCUSSION**

**AR Compared with MR**

As demonstrated, both dogs and cats have highly complex nasal cavities that may influence differently the results of AR and MR.

In this study, we expected that MR could be used as a “gold standard” in validation of AR. Two problems occurred while the cross-sectional area of the images was being determined. First, it was impossible to outline the very complex structure of the nasal cavities by planimetric measurements. Therefore, we instead used an aver-
aging method described in METHODS. Second, the conchae were difficult to distinguish, and it was complicated to determine what part of the lumen was connected with the nasal cavity and what was not.

Possible explanations for the difficulties of MR could be that a freeze-thaw process results in protein denaturation leading to extrusion of water from intracellular compartments and reduced water-holding capacity (6) and that will make it more difficult to detect the air-tissue borderline by MR. Furthermore, it was not possible with the technology available to obtain a satisfactory signal-to-noise ratio, and, therefore, MR seems less suitable for determination of the geometry of dog and cat nasal cavities.

The overall impression was that the estimated cross-sectional areas from the MR scans were very much dependent on subjective choices in the image processing. Also, MR lacks the ability to define airspaces in communication with the nasal cavity. We attempted to use an image analysis program to reconstruct a three-dimensional image of the nasal cavity, but even though it is possible in humans, the complexity of the dog and cat nasal cavity made the result unsatisfying. On the basis of these problems, MR cannot presently be recommended as a gold standard for validation of AR. In a single experiment, CT was able to see structures partly hidden to MR, even though the resolution was not as good, and we think that CT is probably better suited than MR to outline the nasal cavity under the given circumstances, at least with the standard equipment used in the present investigations.

**FDM**

We believed that PP10 would be superior to ethanol and water in FDM studies. This was based on tests and theoretical considerations involving physical and chemical properties such as density, surface tension, and solubility. We found area-distance curves obtained with perfluorocarbon to have less noise and better agreement with models than those obtained with water or ethanol. Perfluorocarbon was shown to accurately portray the structure of models with minimum areas between 0.5 and 0.02 cm². Minimum areas of nasal cavities were often close to 0.1 cm². That would, due to the capillarity effect, increase the height by only 1 mm (estimated by capillarity in a glass pipette). Noise due to differentiation of the pressure-time curve is minimal, due to the application of a moving average algorithm. It is our conviction that, with the application of PP10, we have been able to optimize FDM to unprecedented precision (Fig. 7).

**Validation of AR**

Previous evaluation studies using AR in animal studies have focused primarily on changes in nasal cavity volumes rather than area. Acoustic equipment adjusted to measure the volume of one known model may in fact be inaccurate in other situations where dimensions differ. Temperature differences between sound tube and cavity, affecting the speed of sound, also play a theoretical role but were found to be of no importance in this study. Narrowing of a certain dimension will cause AR to underestimate the actual volume of a nasal cavity on the other side (10). This becomes evident, because an apparently accurate volume could be a result of over- and underestimation of cross-sectional areas over distance. Evaluation studies using AR should therefore include measurements of changes in area as a function of distance and not only a single volume. Our study, therefore, focused on both area and volume determination.

In this study, the $A_{\text{min}}$ was defined as the lowest point on the AR curve and a well-defined point to start area integration for volume.

Our choice of using $A_{\text{max}}$ and corresponding $L_{\text{max}}$ as a reference point in the correction for size is debatable. Another possible point could have been the area at the termination of the nasal septum. But this area was less well defined. Therefore, we believe that $A_{\text{max}}$ marks the best-defined reference point.

As seen in Fig. 11, AR overestimated cross-sectional areas in the first part of the nasal cavity and underestimated area increasingly deeper inside the cavity compared with FDM for both dogs and cats. There may be several reasons for this. It is a simplification to assume that sound waves propagate following a straight line from nostril to epipharynx as the conchae and turbinates change the direction of the sound waves. FDM measures distance along a vertical line through the nasal cavity. Comparison of AR with FDM is only justified if the sound path is identical to the direction of the gravitational axis. The sound axis in humans is probably curved, as shown by FDM with nasal casts in different positions (15) and by comparisons of AR with MR in humans obtained at different angles (7). In four-legged animals, the axis through the nasal cavity is a continuation of the axis through the lower airways and, therefore, follows a straight line. This is not the case in humans, where, due to the upright posture, it is curved by $\sim 90^\circ$. But a potential error still remains if the animal head is not properly oriented with respect to gravity. Given the nasal cavity of the animal is oriented correctly according to gravity before FDM, the difference between axial direction in FDM and AR is of much less importance than in humans. Deviations in the cross-sectional area measured by FDM and AR are more likely explained by 1) differences in the accessibility of sound and PP10 to the maxillary recesses in the dogs, e.g., fluid cannot enter air pockets if the air cannot escape (it is also questionable how well AR can measure these pockets); 2) the fact that the fluid actively forces its way into compartments previously closed by secretions that are inaccessible to sound waves; and 3) the fact that PP10 deforms compliant mucosal structures, resulting in a widening of the nasal passageways.

Therefore, FDM and AR may indeed portray different parts of the nasal cavity. On the basis of the considerations above, it is expected that AR will underestimate nasal cavity geometry in both dogs and cats compared with FDM. This is also supported by other studies in
press (25) where AR on cats in vivo only measured 46 ± 19% of the volume of nasal cavity casts.

In Fig. 11, AR is seen to overestimate areas compared with FDM in the beginning of the cavity but underestimates deeper inside. This is partly explained by the fact that the geometries were indeed different because PP10 was delivered through a tube inserted into the nosepiece, thus not measuring the funnel-shape of the nosepiece, as measured by AR.

Despite the differences in methods stated above, we still find it relevant to compare AR to FDM. Based on perfluorocarbon, FDM is extremely reproducible, easy to conduct, and superior compared with other alternatives like MR, CT, and casts.

**Evaluation of Nosepieces**

AR measurements obtained on three dogs with both internal and external nosepieces did not show any significant difference in relative mean values, although external measurements were more reproducible (Fig. 12). The latter is likely caused by the fact that the external (hood) nosepiece only can be fitted in one way since it follows the geometry of the nose. It was easy to work with but limited to larger dogs, because it required a nose of certain dimensions, thus excluding cats and small dogs. The internal nosepiece can be inserted at changing angles, and the opening of the nosepiece may face the cavity wall or be partly obstructed by secretions. Despite these problems, it was still possible to obtain useable measurements with the internal nosepiece. In future studies, use of an external nosepiece, when possible, is recommended.

In conclusion, we found that AR underestimates what we believe is the most accurate cross-sectional area of the dog and cat nasal cavities, but could still be useful to describe relative changes in cross-sectional area and volume.

The favorable physical properties of perfluorocarbons make FDM very reproducible and simple to perform, and may be the most adequate alternative to validate AR in smaller laboratory animals, e.g., guinea pigs, rats, and possibly mice. MR cannot presently be recommended as a reliable method to validate AR in dogs and cats because of problems in defining the segment of the cavity from which the cross-sectional area is derived. The same problems apply to CT scanning, but CT still may be better to separate air from tissue than MR.

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