Quantification and characterization of pleural fluid in healthy dogs with thoracostomy tubes

Germaine C. Hung DVM
M. Casey Gaunt DVM, MVETSc
Joseph E. Rubin DVM, PhD
Gregory S. Starrak DVM
Sherisse A. Sakals DVM

Received January 26, 2016.
Accepted March 28, 2016.

From the Departments of Small Animal Clinical Sciences (Hung, Gaunt, Starrak, Sakals) and Veterinary Microbiology (Rubin), Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK S7N 5B4, Canada.

Address correspondence to Dr. Sakals (sherissesakals@gmail.com).

OBJECTIVE
To quantify and characterize pleural fluid collected from healthy dogs after placement of a thoracostomy tube (TT).

ANIMALS
8 healthy Coonhound-cross dogs (mean ± SD weight, 27.2 ± 1.6 kg).

PROCEDURES
Thoracic CT of each dog was performed before placement of a TT and daily thereafter for 7 days. Thoracic fluid volume was calculated from CT images. Effusion was aspirated when detected; volume was recorded, and cytologic analysis and bacterial culture were performed.

RESULTS
Mean ± SD volume of pleural effusion detected by CT was 1.43 ± 0.59 mL/kg (range, 0.12 to 3.32 mL/kg). Mean volume collected via aspiration was 0.48 ± 0.84 mL/kg (range, 0 to 2.16 mL/kg). Cytologic analysis yielded results consistent with an exudate, characterized by septic suppurative inflammation in 6 dogs and mixed inflammation in 1 dog; there was insufficient volume for analysis in 1 dog. Sufficient volume was obtained for bacterial culture for 6 dogs, which yielded pure growths of *Staphylococcus pseudintermedius* (n = 3) and *Streptococcus equi* subspecies *zooepidemicus* (2) and mixed growth of both of these species (1). The TT was removed before day 7 in 4 dogs because of pyothorax (n = 3) and irreversible damage to the TT (1).

CONCLUSIONS AND CLINICAL RELEVANCE
Presence of a TT induced a minimal volume of pleural effusion in healthy dogs. Pyothorax developed in most dogs between 4 and 6 days after TT placement. On the basis of these findings, a TT should be removed by the fourth day after placement, unless complications are detected sooner. (*Am J Vet Res* 2016;77:1387–1391)

Thoracostomy tubes are commonly used to withdraw pleural air or fluid from dogs for diagnostic testing or therapeutic purposes. Some indications include pneumothorax, hemothorax, pyothorax, pleural transudate, and chylothorax as well as postoperative monitoring after thoracic surgery.[1–3] However, a TT can act as a foreign body and stimulate an inflammatory response or act as a portal for pleural contamination and infection.[3] It has been presumed that the presence of a TT results in the production of approximately 2 mL of fluid/kg/d.[2,4] However, to the authors' knowledge, there is no evidence in the veterinary or human literature to support this value. Similarly, although it has been suggested that a TT will induce a serosanguineous pleural effusion containing macrophages and nondegenerate neutrophils,[3,5,6] the authors are unaware of any studies conducted to characterize the cellular component of pleural effusion that is attributable solely to the presence of a TT.

ABBREVIATIONS
TT Thoracostomy tube

This lack of data makes it difficult for clinicians to accurately discern changes in pleural fluid caused by disease.

Additionally, there is little information available regarding the optimal amount of time a TT should remain in place (ie, dwell time).[5] Concerns in human and veterinary medicine associated with increases in dwell times include increases in the duration of hospitalization and the need for nursing care as well as the potential for ascending infections.[7–10]

In theory, the thoracic cavity should remain sterile while a TT is in place. However, a TT may provide a portal for entry of bacteria and the potential for development of pyothorax.[7] To the authors' knowledge, the most common bacterial species and timing of infection have not been investigated.

The purpose of the study reported here was to evaluate the volume and characteristics of pleural fluid and development of infection over time after placement of a TT. Our hypotheses were that the presence of a TT would induce 2 mL of pleural fluid/kg/24 h, that it would be serosanguineous fluid containing

macrophages and nondegenerated neutrophils,[3,5,6] and that the thoracic cavity would remain sterile for the duration of the study period.

Materials and Methods

Animals

Eight healthy Coonhound-cross dogs (3 spayed females, 1 sexually intact female, 3 neutered males, and 1 sexually intact male) were used in the study. Mean ± SD body weight was 27.2 ± 1.6 kg. Dogs were housed separately under routine husbandry conditions. Dogs were deemed healthy on the basis of results for a complete physical examination, CBC, and serum biochemical analysis performed on each dog prior to the study. The study was approved by the University of Saskatchewan Animal Research Ethics Board.

TT placement

A TT was placed in each dog (day 1). Each dog was sedated by IV administration of dexmedetomidine[a] (0.005 mg/kg) and hydromorphone[b] (0.1 mg/kg). The hemithorax used for TT placement was determined by use of a randomized drawing for each dog: the name of a dog was drawn from one box, and left or right was drawn from another box. Thus, half the tubes were placed into the right hemithorax, and half were placed into the left hemithorax. The side of the thorax for TT placement was shaved and surgically prepared. Intercostal nerve blocks of thoracic nerves 6 through 10 were performed with bupivacaine hydrochloride[c] (2.5 mg/nerve) on the side of TT placement. A 1.5-cm skin incision was made at the level of the 10th intercostal space. Kelly hemostats were advanced through the incision to create a tunnel through the subcutaneous tissues to the eighth intercostal space. Blunt dissection of the intercostal muscles and pleura were used to enter the thorax. A 20F thoracic catheter[d] was passed through the subcutaneous tunnel and into the thorax. The catheter was secured to the skin with a purse string suture and fingertrap pattern by use of nylon suture.[e] A tubing clamp[f] was placed on the TT; in addition, a tube adapter[g] and 3-way stopcock[h] were inserted into the end of the TT to facilitate sample collection. The TT was covered with an occlusive dressing,[i] stockinette,[j] and nonadhesive elastic bandaging material.[k] Sedation was reversed by IM administration of atipamezole[l] (0.05 mg/kg). Analgesia was provided by administration of tramadol hydrochloride[m] (50 mg, PO, q 8 h) until each TT was removed.

Measurement of fluid volume and sample collection

The volume of pleural effusion was measured by 2 methods (volumetric measurement of the region of interest on CT images and direct aspiration of fluid via the TT). Computed tomography was performed by use of a 16-slice helical scanner,[n] with a 3-mm slice thickness, beam pitch of 0.7, 250 mA, 120 kV, and soft tissue and lung algorithms. The first CT was performed after the dogs were sedated and prior to placement of the TT on day 1 to obtain a baseline volume of fluid within the pleural cavity and to rule out thoracic pathological changes. Subsequently, CT was performed daily for 7 days. Dogs were sedated with dexmedetomidine[a] (0.005 to 0.007 mg/kg, IV), and reversal of sedation after CT was achieved with atipamezole[l] (0.05 to 0.07 mg/kg, IM). The CT images were evaluated by a board-certified veterinary radiologist (GSS) for evidence of pleural effusion. When pleural effusion was detected, 3-D regions of interests and volumetric measurements were obtained by use of diagnostic imaging software.[o]

Pleural effusion was aspirated aseptically via the TT by use of a syringe. The volume of aspirated fluid was recorded. When the amount of fluid aspirated was sufficient, samples were submitted for cytologic examination and bacterial culture. Two direct smears were made with the pleural fluid, and 0.5 mL of pleural fluid was placed into an EDTA-containing tube to preserve cellular morphology for cytologic analysis, measurement of protein concentration, and cell count. Cytologic analysis was performed at a veterinary diagnostic laboratory by a board-certified veterinary pathologist.

Two additional 0.5-mL samples were placed in clot tubes and used for bacterial culture. A 500-µL aliquot was frozen at –80°C for subsequent analysis, and 100 µL was used for bacterial culture on blood agar plates. Plates were incubated at 37°C and evaluated for growth at 24, 48, and 72 hours after the start of culture. Bacterial isolates were identified by amplification and sequencing of the *cpn*60 universal target and comparison of sequences with those in the GenBank database.[11] For a sample from which bacteria were cultured, post hoc quantitative cultures were performed by use of the frozen 500-µL aliquot. A 10-fold dilution of the sample was cultured, and the number of CFUs per milliliter was calculated.

Postinsertion monitoring

Dogs were monitored hourly for signs of discomfort and respiratory distress for the study duration. Integrity of the bandages was monitored hourly, and the TT was monitored daily for signs of damage or discharge from the skin incision. Each TT was removed after CT on day 7, except when a dog appeared to be ill, developed pyothorax, or had irreversible damage to the TT prior to day 7. If any of these factors were evident, the TT was removed immediately, and no further data were collected for that dog.

Statistical analysis

Statistical testing was performed with commercially available software.[p] Normality was assessed by use of the Shapiro-Wilk test. A nonparametric Wilcoxon signed rank test was performed to determine whether the volume of pleural effusion detected over 7 days by use of CT or aspiration differed

significantly from the expected value of 2 mL/kg/d. Significance was set at a value of $P \leq 0.05$.

Results

Pneumothorax was detected by use of CT on the day after TT placement in 4 dogs. Mean ± SD volume was 80.8 ± 58.4 mL (range, 20 to 153 mL). No clinical signs of pneumothorax were evident in any of these dogs, and resolution of pneumothorax (as determined by use of CT) was achieved within 48 to 72 hours after TT placement.

Four dogs developed complications during the study period that required premature (ie, before day 7) removal of the TT. Two dogs chewed the thoracic catheter and tube adapter, which disrupted the closed system; these 2 dogs subsequently developed pneumothorax. After each of these events, the adapter was reconnected and air removed from the thorax via aspiration. The TT was removed from 1 of these dogs on day 4 when a high rectal temperature (39.6°C) and mild lethargy were detected. The other dog was lethargic and had a high rectal temperature (39.1°C) on day 5, and the TT was removed following CT and aspiration. Fluid obtained from each of these 2 dogs was turbid. Pyothorax was subsequently diagnosed on the basis of results for culture of pleural fluid, and data for these 2 dogs were excluded from statistical analysis. A third dog became febrile (rectal temperature, 39.8°C) and lethargic on day 6. After CT and aspiration were performed, the TT was removed. Pyothorax was confirmed on the basis of results for both cytologic analysis and bacterial culture. These 3 dogs were treated with amoxicillin-clavulanic acid[q] (22 mg/kg, PO, q 8 h) and enrofloxacin[r] (10 mg/kg, PO, q 24 h). Each responded to treatment, and clinical signs resolved within 24 hours after initiation of antimicrobial treatment. The fourth dog that required premature removal of the TT chewed the TT on the evening of day 6, which caused severe pneumothorax. Resolution of the pneumothorax was achieved by use of needle aspiration, but the TT had to be removed because of irreversible damage.

The TT was removed from each of the remaining 4 dogs after CT was performed on day 7. On day 7, one of the dogs was lethargic and had a rectal temperature of 39.3°C; in addition, that dog had been inappetent for 2 days. Bacterial culture yielded positive results for that dog.

Pleural effusion was first detected by use of CT in all dogs between days 2 and 6. Effusion was detected on day 2 in 1 dog, day 3 in 3 dogs (the dogs with pyothorax), day 4 in 1 dog (the dog that chewed the TT on the evening of day 6), day 5 in 2 dogs, and day 6 in 1 dog. The presence of pleural effusion persisted in each dog until the TT was removed.

Overall, there were minimal amounts of pleural effusion produced, and on many days, not all dogs produced fluid. Therefore, data for the entire 7 days were combined for statistical analysis. Mean ± SD volume of pleural fluid produced over the 7 days in all 6 dogs, as measured by aspiration of fluid via the TT, was 0.48 ± 0.84 mL/kg (range, 0 to 2.16 mL/kg). Mean volume as calculated by use of CT was 1.43 ± 0.59 mL/kg (range, 0.12 to 3.32 mL/kg). The amount of pleural effusion was significantly ($P = 0.03$) less than the expected volume of 2 mL/kg/d as measured by both aspiration and CT.

Cytologic analysis was performed on pleural effusion obtained from 7 of 8 dogs. Pleural effusion from 2 dogs (TT removed on day 7) was composed of mildly degenerate neutrophils, macrophages, and mesothelial cells, which was consistent with suppurative inflammation. There were 22.5×10^9 nucleated cells/L for one of these dogs, but there was an insufficient volume for determining the protein concentration. There was an insufficient volume for cell count or protein concentration in the other dog.

Pleural effusion from 2 dogs that chewed their TT and developed pyothorax contained 98.6×10^9 nucleated cells/L and 50 g of protein/L and 21.8×10^9 nucleated cells/L and 45 g of protein/L. The fluid was characterized by marked degenerate neutrophilic inflammation with macrophages, mesothelial cells, and intracellular and extracelluar cocci, and it was considered an exudate marked by septic suppurative inflammation. Fluid from the dog that was lethargic on day 7 (TT was removed on day 7) contained 161.9×10^9 nucleated cells/L and 46 g of protein/L, whereas fluid from the third dog with pyothorax contained 65.9×10^9 nucleated cells/L and 52 g of protein/L; both of these dogs had septic suppurative inflammation consisting of degenerative neutrophils, chains of extracellular cocci, and intracellular bacteria, and macrophages were also present in the effusion of the dog that was lethargic on day 7.

No samples were collected from the dog that chewed its TT on the evening of day 6, which necessitated premature removal of the TT, because fluid was never aspirated from the TT. Pleural effusion of the remaining dog from which the TT was removed on day 7 was characterized by nondegenerate neutrophils and moderate macrophage inflammation, which was consistent with mixed inflammation (nucleated cell count was unavailable as a result of clumping, but it contained 41 g of protein/L).

Bacterial cultures of pleural effusion were available for 6 dogs. Pure cultures of *Staphylococcus pseudintermedius* were grown for samples obtained on day 7 from 2 dogs (one had detectable but not quantifiable colonies, and the other had 260 CFUs/mL) and a sample obtained on day 4 from the dog that chewed its TT (which required TT removal) on day 3 (9.8×10^7 CFUs/mL). Pure cultures of *Streptococcus equi* subsp *zooepidemicus* were cultured from samples obtained on day 7 from the dog that was lethargic on that day (1.3×10^7 CFUs/mL) and on day 6 from a dog that developed pyothorax (4.7×10^8 CFUs/mL). A mixed culture of both of these bacterial species (*S pseudintermedius* was detectable but not quantifiable, and 11.9×10^5 CFUs of

S equi subsp *zooepidemicus*/mL) was grown from a sample obtained on day 4 from a dog that chewed its tube and developed pyothorax. No cultures were available for the dog that chewed its TT (which necessitated premature TT removal) on the evening of day 6. Bacterial culture of a sample from the remaining dog from which the TT was removed on day 7 yielded negative results.

Discussion

Results for the study reported here indicated that minimal amounts of pleural effusion were produced as a result of indwelling TTs. This is contrary to the suggestion that a volume of 2 mL of fluid/kg/d is induced by the presence of indwelling foreign objects.[2,4] Findings for the present study were consistent with the clinical observation that minimal amounts of pleural effusion are evident following many thoracic surgical procedures. Even in the dogs that developed pyothorax, the fluid volume produced remained low, although this may have been attributable to extremely early detection of pleural effusion. Thus, when larger volumes of pleural effusion develop in the presence of a TT in a clinical patient, it is likely secondary to an underlying pathological condition, rather than to the presence of the TT.

Results of cytologic analysis in the present study were most often consistent with an exudate characterized by septic suppurative inflammation. This is in contrast to the commonly accepted belief that indwelling TTs induce a mild inflammatory reaction.

Differences in fluid volume determined by aspiration and calculated from CT images were detected. During the study period, fluid was at times evident on CT images that was not in the vicinity of the TT. When we attempted to aspirate the effusion via the TT, no fluid was obtained, even when the dogs were repositioned. Thus, we concluded that for these situations, the small amount of fluid did not move to a location from which it could be aspirated via the TT.

The hypothesis that the thoracic cavity would remain sterile for the duration of time the TT was in place was rejected because positive results for bacterial culture were obtained for 6 of 8 dogs. For 2 of these 6 dogs, damage to the TT may have resulted in secondary bacterial contamination. However, for the other 4 dogs, there was no known breach of the closed TT system. Despite the fact aseptic technique was used for all handling of the TTs, pure or mixed populations of *S pseudintermedius* or *S equi* subsp *zooepidemicus* were cultured from samples obtained from these 6 dogs. This led us to believe that the indwelling TT was a portal for bacterial entry. Bacteria may have been introduced by chewing of the TT (2 dogs). It is also possible that bacteria were introduced into the thoracic cavity at the time of TT placement. Additionally, because the TT contacts the skin of the thorax, it is possible that bacteria migrated on the TT into the pleural space during the course of the study. Four dogs had pyothorax as determined on the basis of positive results for bacterial culture, intracellular bacteria observed during cytologic analysis, and clinical signs consistent with pyothorax. It is possible the positive culture results obtained for 2 dogs (TT removed on day 7) were indicative of a subclinical infection or secondary to contamination of the sample because no evidence of bacterial organisms was evident during cytologic analysis and only low quantities of bacterial growth were present on cultures. *Staphylococcus pseudintermedius* is a commensal organism of canine skin and mucous membranes[12]; thus, it was not surprising that this organism was isolated. *Streptococcus equi* subsp *zooepidemicus* is not considered to be a commensal organism in dogs.[13]

Despite the fact aseptic technique was used when handling the TTs, pyothorax was a complication confirmed in all dogs for which samples were available for bacterial culture. Common clinical signs associated with pyothorax include fever, lethargy, anorexia, and weight loss.[1] Signs of pyothorax developed at approximately day 4, the day on which pleural effusion became apparent by use of CT in 6 dogs. In the other 2 dogs, pleural effusion was detected on days 5 and 6. In the dogs with samples that yielded positive bacterial culture results, clinical signs of lethargy were evident concurrently or within 1 day after CT detection of pleural effusion. Bacterial culture yielded growth for samples obtained from 6 of 8 dogs on the day of TT removal. This was within 1 to 3 days after pleural effusion was detected. On the basis of results for the study reported here, it appeared that maintaining an indwelling TT for > 4 days would not be recommended.

The present study had some limitations. Power analysis ($\alpha = 0.05$ and $\beta = 0.2$) determined that 8 subjects were needed to provide significant results; however, 2 dogs were removed prematurely from the study. Additionally, only polyvinylchloride TTs were used in the study. Other materials, such as red rubber or silicone, may cause a different response.

In the present study, indwelling TTs induced minimal production of pleural fluid in healthy dogs, and the amount produced was much less than the generally accepted value of 2 mL/kg/d. Presence of a TT resulted in the development of pyothorax in most dogs; thus, an indwelling TT may act as a portal for bacterial entry. Pleural effusion and clinical signs of pyothorax developed 4 and 6 days, respectively, after TT placement. On the basis of these results, it is recommended that TTs be removed in clinical patients by day 4 after placement to decrease the risk for development of pyothorax.

Acknowledgments

Supported by the Western College of Veterinary Medicine Companion Animal Health Fund.

The authors declare that there were no conflicts of interest.

Footnotes

a. Dexdomitor, Zoetis Canada Inc, Kirkland, QC, Canada.
b. Hydromorphone 10 mg/mL, Sandoz Canada Inc, Boucherville, QC, Canada.
c. Marcine 0.5%, Hospira Healthcare Corp, Saint-Laurent, QC, Canada.
d. Argyle thoracic catheter, Covidien LLC, Mansfield, Mass.
e. 2-0 Ethilon, Ethicon, Johnson & Johnson, Markham, ON, Canada.
f. Plastic tubing clamp, Stone Manufacturing & Supply Co Inc, Kansas City, Mo.
g. Tubing adapter, Smiths Medical ASD Inc, Saint Paul, Minn.
h. 3-Way high-flow stopcock with rotating Luer, Icumedical, San Clemente, Calif.
i. Tegaderm film, 3M Health Care, Saint Paul, Minn.
j. Stockinette 6-inch, QMD Medical, Montreal, QC, Canada.
k. 3M VetRap bandaging tape 4-inch, 3M Animal Care Products, Saint Paul, Minn.
l. Antisedan, Zoetis Canada Inc, Kirkland, QC, Canada.
m. Ultram 50 mg, Janssen Inc, Toronto, ON, Canada.
n. Aquilion 16, Toshiba Corp, Otawara-Shi, Tochigi, Japan.
o. Osirix MD, Pixmeo, Geneva, Switzerland.
p. Stata13, Stata Corp LP, College Station, Texas.
q. Clavaseptin 250/500 mg, Vetoquinol N.-A. Inc, Lavaltrie, QC, Canada.
r. Baytril 150 mg, Bayer Inc, Toronto, ON, Canada.

References

1. Hawkins EC. Respiratory system disorders. In: Nelson RW, Couto CG, eds. *Small animal internal medicine*. 4th ed. St Louis: Elsevier Mosby, 2014;337–355.
2. Fossum TW. Pleural cavity and diaphragm. In: Fossum TW, ed. *Small animal surgery*. 4th ed. St Louis: Elsevier Mosby, 2013;991–1032.
3. Radlinsky MG. Thoracic cavity. In: Tobias KM, Johnston SA, eds. *Veterinary surgery small animal*. St Louis: Elsevier Saunders, 2012;1787–1812.
4. Stillion JR, Letendre JA. A clinical review of the pathophysiology, diagnosis, and treatment of pyothorax in dogs and cats. *J Vet Emerg Crit Care (San Antonio)* 2015;25:113–129.
5. Valenciano AC, Arndt TP, Rizzi TE. Effusions: abdominal, thoracic, and pericardial. In: Valenciano AC, Cowell RL, eds. *Cowell and Tyler's diagnostic cytology and hematology of the dog and cat*. 4th ed. St Louis: Elsevier Mosby, 2014;244–265.
6. Stockham SL, Scott MA. Cavitary effusions. In: Stockham SL, Scott MA, eds. *Fundamentals of veterinary clinical pathology*. 2nd ed. Ames, Iowa: Blackwell Publishing, 2008;831–865.
7. Brooks AC, Hardie RJ. Use of PleuralPort device for management of pleural effusion in six dogs and four cats. *Vet Surg* 2011;40:935–941.
8. Utter GH. The rate of pleural fluid drainage as a criterion for the timing of chest tube removal: theoretical and practical considerations. *Ann Thorac Surg* 2013;96:2262–2267.
9. Younes RN, Gross JL, Aguiar S, et al. When to remove a chest tube? A randomized study with subsequent prospective consecutive validation. *J Am Coll Surg* 2002;195:658–662.
10. Baumann MH. What size chest tube? What drainage system is ideal? And other chest tube management questions. *Curr Opin Pulm Med* 2003;9:276–281.
11. Goh SH, Potter S, Wood JO, et al. HSP60 gene sequences as universal targets for microbial species identification: studies with coagulase-negative staphylococci. *J Clin Microbiol* 1996;34:818–823.
12. Bannoehr J, Guardabassi L. *Staphylococcus pseudintermedius* in the dog: taxonomy, diagnostics, ecology, epidemiology and pathogenicity. *Vet Dermatol* 2012;23:253–266.
13. Priestnall S, Erles K. *Streptococcus zooepidemicus*: an emerging canine pathogen. *Vet J* 2011;188:142–148.