Applicability of $^{99m}$Tc-Labeled Human Serum Albumin Scintigraphy in Dogs With Protein-Losing Enteropathy


**Background:** Diagnosis of protein loss into the gastrointestinal tract using noninvasive techniques is challenging. In people, scintigraphy not only is a sensitive tool to confirm protein-losing enteropathy (PLE), but it also allows for localization of protein loss.

**Hypothesis/Objectives:** To investigate the feasibility of $^{99m}$Tc-labeled human serum albumin (HSA) scintigraphy in dogs with PLE in comparison with control dogs.

**Animals:** A total of 8 clinically healthy control research dogs and 7 client-owned dogs with gastrointestinal clinical signs and hypoalbuminemia (serum albumin concentration <2.0 g/dL).

**Methods:** Prospective case-control study. After IV injection of 400 MBq freshly prepared $^{99m}$Tc HSA (30 mg/dog), images of the abdomen were obtained 10, 60, 120, and 240 minutes postinjection. Additional images of the salivary and thyroid glands were obtained to rule out free $^{99m}$Tc. A scan was considered positive for PLE when radiopharmaceutical exudation was detectable in the intestinal tract.

**Results:** Only 1 control dog showed exudation of the radiopharmaceutical into the intestinal tract. No free $^{99m}$Tc was detected in any dog. In dogs with PLE, focal small intestinal and diffuse small intestinal radiopharmaceutical exudation into the bowel was detected in 2 and 3 dogs, respectively, whereas in 2 dogs, there was disagreement about whether radiopharmaceutical exudation was focal or diffuse.

**Conclusion and Clinical Importance:** $^{99m}$Tc-labeled HSA scintigraphy was feasible to diagnose PLE in dogs.

**Key words:** Enteral protein loss; Chronic enteric disease; Canine; Diagnostic imaging; Nuclear medicine.

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**Abbreviations:**
- $\alpha$-1 PI: $\alpha$-1-proteinase inhibitor
- CCECAI: canine chronic enteropathy clinical activity index
- HSA: human serum albumin
- MBq: megabecquerel
- PLE: protein-losing enteropathy
- Tc: technetium
- WSAVA: World Small Animal Veterinary Association

**Diagnosis of PLE** is cumbersome because fecal samples must be shipped frozen to the laboratory for measurement of fecal $\alpha$-1-proteinase inhibitor ($\alpha$-1-PI) or technically demanding tests such as $^{51}$Cr-albumin excretion must be conducted. $^{1,12}$ Fecal $\alpha$-1-PI is synthesized in the liver and has a molecular mass similar to that of albumin (approximately 50,000 Daltons). Thus, when intestinal loss of albumin occurs, $\alpha$-1-PI is lost as well, but not hydrolyzed by digestive or bacterial proteinases in the intestinal lumen. Therefore, fecal $\alpha$-1-PI clearance or concentration can be used as an estimate of intestinal protein loss. There is no significant correlation between fecal $\alpha$-1-PI and serum albumin concentration. $^{13}$ Evaluation of fecal $\alpha$-1-antitrypsin clearance and $^{99m}$Tc-HSA scintigraphy in PLE in people determined that scintigraphy had higher sensitivity (100 versus 46%) and negative predictive value (100 versus 63%) compared to fecal $\alpha$-1-antitrypsin clearance. $^{14}$ The latter test is time intensive due to the prolonged physical half-life and therefore extended hospitalization. Finally, a positive laboratory test result cannot distinguish between focal and generalized protein loss.

Few reports have described the imaging findings in dogs with PLE. The intestinal hyperechoic mucosal striations seen ultrasonographically in a series of 23 dogs were associated histologically with lacteal dilatation in...
96% and clinically with PLE in 78% of the dogs. A pilot study using Doppler examinations for pattern recognition and feature extraction of celiac and cranial mesenteric arterial waveforms was performed in 11 dogs with chronic enteropathy. It detected a lack of increase in mean diastolic flow, abnormal arterial waveform shapes, and suboptimal increases in diastolic blood flow during digestion.

The first successful scintigraphic examination of human patients with PLE was performed in 1986. Radiopharmaceuticals bound to a protein accumulate in the gastrointestinal tract because protein loss occurs into the digestive tract resulting in increased radioactivity in the intestinal lumen on scintigraphic imaging. In 1997, a scintigraphic evaluation of 4 dogs with PLE was performed using 111Indium-labeled transferrin. Although it appeared to be a feasible method for evaluating dogs with suspected PLE, costs and prolonged isolation of the dogs due to the long physical half-life of 111In were considerable limitations.

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Materials and Methods

The study was approved by the state ethics and welfare committee of Hessia (number 63/2011). Owner consent allowing for sedation and anesthesia during scintigraphy, followed by endoscopy or surgery, was obtained before inclusion into the study.

Clinically healthy, clinic-owned beagle dogs were used as controls, based on normal physical examination and CBC, serum biochemistry profile, urinalysis and urine protein/creatinine ratio, pre- and postprandial bile acid concentrations, and ACTH stimulation test as well as unremarkable abdominal ultrasonographic examination and deworming treatment 1 week before scintigraphy.

From October 2011 to December 2013, client-owned dogs with PLE were prospectively enrolled. To meet the inclusion criteria, dogs had to be diagnosed based on the presence of clinical signs (eg, weight loss, chronic diarrhea >3 weeks, edema, ascites) and hypoalbuminemia (serum albumin concentration <2.0 g/dL). All dogs had the following tests performed to assess the cause of the hypoalbuminemia: canine chronic enteropathy clinical activity index (CCECAI) scores, serum biochemistry profile, urinalysis and urine protein/creatinine ratio, pre- and postprandial bile acid concentrations, ACTH stimulation test, thromboelastography, serum cobalamin concentration, canine-specific pancreatic lipase concentration, fecal examination (floation, sedimentation), and abdominal ultrasonography. Anti-inflammatory and immunosuppressive medication had to be discontinued for at least 1 month before inclusion.

Scintigraphy

To perform abdominal scintigraphy, 400 megabecquerel (MBq) 99mTc-HSAa was prepared according to the manufacturer’s instructions and incubated for 30 minutes at room temperature and 1 vial (30 mg HSA) was injected IV via the cephalic vein to avoid interference with the abdominal scan. Venral and right lateral images were obtained 10, 60, 120, and 240 minutes after injection of the radiopharmaceutical. The dogs were either lightly sedated using diazepam or heavily sedated using diazepam and propofol to effect. Static images were acquired for a total of 5 minutes at each time point. Images were obtained with a large-field-of-view gamma camera fitted with a low-energy all-purpose collimator, integrating a work station equipped with dedicated software. A 256 × 256 × 16 matrix was used. The possibility of in vivo breakdown of the radiopharmaceutical and false-positive localization of free pertechnetate was monitored by visualizing the stomach during all studies and by visualization of salivary and thyroid glands 240 minutes after injection. All scans were evaluated independently by 2 diplomates in veterinary diagnostic imaging (NO, KvP) experienced in reading scintigraphic images with regard to the presence of intestinal radiopharmaceutical exudation, 1 of them blinded regarding the status of the dogs. Location of exudation was defined as small intestinal if seen ventrally and right cranially or large intestinal if seen longitudinal dorsally and along the left lateral abdominal wall. The intensity of the radiopharmaceutical exudation was graded visually and compared to liver activity, as follows: 1 = mild exudation (less than liver); 2 = moderate exudation (equal to liver); 3 = marked exudation (more than liver). After scintigraphy, intestinal tissue samples were obtained either via gastroduodenoscopy and ileocolonoscopy (all control dogs and 4 dogs with PLE) or surgically (3 dogs with PLE) by full thickness biopsies. All histopathologic examinations were performed by 1 board-certified veterinary pathologist according to the World Small Animal Veterinary Association (WSAVA) recommendations.

Results

Eight healthy beagle dogs were included; they ranged in age from 1.8 to 3.5 years (median, 2.4 years), 6 were female and 2 were male, and all were castrated or spayed.

Seven dogs with PLE were included. Mild-to-moderate ascites was present in 4 dogs with PLE. Urine protein/creatinine ratio and pre- and postprandial serum bile acid results were within the reference range in all dogs. The ACTH stimulation test result was diagnostic for atypical hypoadrenocorticism in 1 dog. Canine-specific pancreatic lipase concentration was high in 3 dogs (range, 382–434 μg/L; reference range, <200 μg/L). Fecal examination was positive for Giardia sp. (comprontigen ELISA) in 2 dogs and for Trichuris vulpis (fecal flotation) in 1 dog. All dogs with PLE were in a hypercoagulable state as detected by thromboelastography. Hypocoalbuminemia (range, 96–281 mg/dL; reference range, 300–800 mg/dL) was found in 4 dogs. Abdominal ultrasonographic examination detected ascites in 4 dogs and hyperechoic mucosal striations in 3 dogs. In 1 dog, focal loss of wall layering was detected in the jejenum.

In the healthy control dogs, all scans detected normal radiopharmaceutical accumulation in the liver, spleen, kidneys, urinary bladder, and large vessels. No
radiopharmaceutical accumulation was observed in the stomach, salivary glands, thyroid glands, and the intestinal tract in all but 1 control dog (Fig 1). Moderate radiopharmaceutical exudation in the small intestine was recognized by the diplomate blinded to the status of the dogs, but not by the other diplomate. Moderate lymphoplasmacytic mucosal infiltration of the duodenum and ileum was present in 2 control dogs.

Both diplomates detected radiopharmaceutical exudation in the intestinal tract in all dogs with PLE in addition to normal radiopharmaceutical accumulation in the liver, spleen, kidneys, urinary bladder, and large vessels. No free pertechnetate accumulation was present in stomach, salivary glands, and thyroid gland in dogs with PLE. Radiopharmaceutical exudation was diffuse small intestinal (Fig 2), focal small intestinal or large intestinal (Fig 3) and could be seen as soon as 10 minutes postinjection. The best time points to visualize radiopharmaceutical exudation varied among individual dogs and were at 10 minute (n = 1), 60 minute (n = 1), 120 minute (n = 1), 240 minute (n = 3), and at all time points equally (n = 1). There was agreement regarding diffuse versus localized and small versus large intestinal radiopharmaceutical exudation between both diplomates in all but 2 dogs with PLE. Mild, moderate, and marked exudation was seen in the intestinal tract in 2, 2, and 3 dogs, respectively. Full thickness biopsy specimens with subsequent histologic examination were surgically obtained in 3 dogs with PLE, including 2 dogs with focal tracer exudation in which a partial enterectomy also was performed. Partial enterectomy was performed in 1 dog 24 hours after scintigraphy with intraoperative tracer measurement at the site of highest accumulation and in the other dog based on palpation of the intestinal tract.

Discussion

The results of our study suggest that $^{99m}$Tc-HSA scintigraphy may be a useful method to diagnose protein loss into the gastrointestinal tract in dogs with hypoalbuminemia. Each dog with hypoalbuminemia showed radiopharmaceutical exudation into the intestinal tract, whereas only 1 control dog was positive; no free pertechnetate was detected. We could not only confirm enteric protein loss in all dogs with hypoalbuminemia, but also localize the site of protein loss within the gastrointestinal tract. Determination of the site of protein loss influenced our further procedures and treatment substantially: in the 2 dogs with localized radiopharmaceutical exudation, subsequent partial enterectomy was proposed, a combination of procedures already established in human medicine. Both dogs experienced a rapid increase in serum albumin concentration after surgery with the sole treatment comprising postoperative antibiotics and pain medications, and continuous use of a hypoallergenic diet. In the remaining 5 dogs, the site of protein loss could be limited to either small or large intestine. The dog with diffuse radiopharmaceutical exudation into the large intestine was diagnosed with marked T. vulpis infection.
Treatment with fenbendazole (50 mg/kg once daily) on 3 consecutive days resolved clinical signs and hypoalbuminemia. The possibility of false-positive results caused by intestinal bleeding was considered unlikely based on the absence of anemia, melena, and hematochezia; testing for occult blood was not performed.

Subsequent monitoring of radiopharmaceutical exudation over time is essential to define the site of protein loss because bowel transit of excreted radiopharmaceutical may be confounding. This applies not only to identification of the site of protein loss in localized radiopharmaceutical exudation but also to the differentiation between localized and diffuse radiopharmaceutical loss. The 2 discrepant results were caused by variation in assessment of a diffuse or localized problem. Diffuse protein loss may go unnoticed or be misinterpreted as focal disease without progressive monitoring as shown in Figure 2. Moreover, the variable position and size of the spleen may be confounding when not monitored subsequently.

Accumulation of $^{99m}$Tc-HSA in inflamed intestines so far has not been reported. To decrease the possibility of false-positive results caused by transit of activity rather than a second site of protein loss and to increase the detection rate for PLE, serial images were performed over a 4-hour time period. The finding of marked activity in the kidneys and urinary bladder (which are visible in every image) is well known when using $^{99m}$Tc-HSA but does not occur when other radiopharmaceuticals such as $^{111}$In-transferrin are used. Proteinuria or free radiopharmaceutical activity as a cause has been ruled out as described.

Possible pitfalls of $^{99m}$Tc-HSA scintigraphy for diagnosing PLE are (1) false-positive findings due to gastrointestinal bleeding, (2) in vivo breakdown of the radiopharmaceutical producing a false-positive result mimicking radiopharmaceutical exudation, and (3) bowel transit of the radiopharmaceutical which may result in either incorrect localization or false-negative results. These pitfalls were excluded as much as possible by (1) having no dog with melena or anemia, (2) obtaining scintigraphic images of thyroid glands, and (3) obtaining multiple images over a 4-hour period.

A potential limitation of this study is the use of HSA. Infusion of supraphysiological doses of HSA (250 g/L) was fatal or produced life-threatening adverse reactions in healthy dogs. Furthermore, all healthy dogs given supraphysiological doses of HSA produced anti-HSA antibodies which might preclude future use of HSA. However, infusion of similar...
supraphysiological doses of HSA to 185 critically ill or hypoalbuminemic dogs resulted in no adverse reactions. In our study, only 30 mg HSA per dog was used and no reaction was seen, neither in hypo- nor in normo-albuminemic dogs. A low dose of HSA or binding to $^{99m}$Tc rendering the sites for unwanted reaction unavailable might have been the reason. No measurement of anti-HSA antibodies was carried out, but their production later is possible and thus repeated scintigraphy for re-examination with $^{99m}$Tc-HSA was not performed. Other radiopharmaceuticals might circumvent this problem, such as $^{99m}$Tc-dextran or $^{111}$In-transferrin which have been used in human medicine. Species-specific canine albumin is newly available for therapeutic use and could be used as radiopharmaceutical in the future. Finally, in human medicine, $^{99m}$Tc-labeled to autologous serum albumin has been used, thus avoiding the production of antibodies and therefore opening the possibility for scintigraphic re-examinations. Other limitations of the study are the use of control dogs not age, sex, or breed matched to the dogs with PLE. This might have given some few false-positive results considering that specificities of 51–72% are reported in humans when patients with other forms of hypoproteinemia are used as controls. In 1 control dog, discrepant findings between both diplomates were seen, and having 1 examiner not blinded to the status of the dog might have added some bias. Finally, low case numbers precluded calculating inter- and intra-observer variability.

In conclusion, noninvasive $^{99m}$Tc-HSA scintigraphy can be used to detect gastrointestinal protein loss in dogs with hypoalbuminemia and potentially localize the site of protein loss within the gastrointestinal tract. Future studies are needed to see whether early PLE or other intestinal diseases without marked hypoproteinemia can be detected using this technique.

**Footnotes**

- Albumon HSA Tc-99 labelling kit, Medi-Radiopharma Ltd., Erd, Hungary
- Gamma Diagnost Tomo, Siemens LEM, Siemens AG, Erlangen, Germany
- Siemens ICON, software version 7.5, Erlangen, Germany

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**Conflict of Interest Declaration:** Authors declare no conflict of interest.

**Off-label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.
References


Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

Table S1. Signalment, clinical signs, histology and scintigraphic findings in 7 dogs with PLE undergoing 99m-Tc-HSA scintigraphy.