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Computed tomographic features of the normal spleen in rabbits (*Oryctolagus cuniculus domesticus*)

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Running head 1: Chernev et al.

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Abbreviations: CT, computed tomography; DICOM, digital imaging and communications in medicine; HU, Hounsfield units; ROI, region of interest; SD, standard deviation.

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ABSTRACT

Computed tomography (CT) is commonly utilised for the investigation of dental and ear disease in the rabbit, and, more recently, for evaluating abdominal pathology. The spleen, however, is an often overlooked organ, with limited information published. The aims of this retrospective study were to document the visibility, size and shape of the normal rabbit spleen, and potential correlations with signalment. Institutional imaging archives were reviewed for diagnostic-image-quality abdominal CT studies of rabbits. Medical records of identified cases were reviewed and all cases with pathology that could influence the spleen were excluded. In 115 cases the inclusion criteria were met. Pre- and post-contrast CT studies were evaluated by two reviewers for visibility of the spleen. For pre-contrast CT images, the inter-rater agreement for identification of the spleen was fair (Fleiss' Kappa = 0.305, 53.7% agreement). For post-contrast CT images, inter-rater agreement was moderate (Fleiss' Kappa = 0.404, 93% agreement). There were significantly more spleens clearly identified on post-contrast studies compared to pre-contrast CT (McNemar test = 27.034, df=1 $p<0.001$). Splenic location, volume, shape, x-ray-attenuation and length were measured, and the splenic-volume-to-body-weight ratio was calculated. The mean splenic volume was 1ml (range 0.2ml to 3.9ml), mean length 40mm (range 20mm to 61mm), mean attenuation (pre-contrast CT 80HU and post-contrast CT 320HU), mean splenic volume/body weight ratio was 0.5ml/kg (range 0.17ml/kg to 1.2ml/kg). There was a significant relationship between splenic volume and body weight (linear regression t=4.00, $p<0.001$), which was weakly positively correlated (Pearson coefficient r=0.55, $P<0.001$). There was no correlation between splenic volume, age, and sex. The most commonly identified splenic shapes were 'banana', 'tongue', and 'elephant trunk'. The rabbit spleen can be identified on CT images, but more reliably on post-contrast CT images, which underlines the usefulness of contrast-enhanced CT in this species. Bodyweight should be taken into consideration when differentiating between normal splenic size and splenomegaly.

Introduction

Domestic rabbits are common pet animals and present for veterinary care with a number of conditions. Being a prey species, rabbits often hide signs of disease and therefore the initial clinical examination can be limited in diagnostic accuracy. Computed tomography (CT) has been developed as an excellent diagnostic tool for rabbits, allowing the diagnosis of many conditions such as dental disease, otitis, pituitary neoplasia, liver lobe torsion, and sacculitis.[1-5] CT examination of rabbits can be performed without sedation in a designated restraining device (VetCatTrap™, University of Illinois, USA), and with the administration of intravenous contrast medium via a catheter in the marginal ear vein.[6] Extravasation of contrast medium from the catheter inserted into the marginal ear vein is a potential adverse effect and does pose a risk for ear necrosis. Therefore, safe bandaging and securing of the catheter is essential and was performed for all patients in this study. In our institution, we have encountered a number of rabbits with splenic abnormalities and diseases, however, there is very limited information available on the morphology of the rabbit spleen.[7-11] The authors could not find any information about the CT anatomy of the rabbit spleen.

The aims of this study were to determine whether the rabbit spleen can be clearly identified on pre- and post-contrast CT sequences and to establish normal CT reference ranges and variations for rabbit splenic morphology.

We hypothesise that the spleen can be clearly identified in pre- and post-contrast CT studies as an elongated parenchymal organ located adjacent to the greater gastric curvature, in the left cranial abdomen.

Methods

Case Selection:

This was a retrospective single-institutional observational study. Ethical approval was obtained from the institution's ethical review committee. Institutional medical and imaging archives were reviewed for rabbit patients with abdominal CT studies during the period between October 2018 and December 2022. Inclusion criteria required a diagnostic quality CT study, including the full abdomen, performed with a standardised protocol, including a pre- and post-contrast series, using a dedicated CT restraining device (VetCatTrap™). All patients were scanned consciously. Complete signalment data (age, breed, sex, neuter status, body weight), recently performed haematology, and biochemistry (at least a week before or after the CT examination), with a white blood cell count within the range from 4.7 x 10^9 cells/L to 12.5 x 10^9 cells/L and red blood cell count from 3.8 x 10^{12} cells/L to 7.43 × 10^{12} cells/L based on established textbook references were part of the inclusion criteria.[12] Patients with abnormal CT findings of the spleen or liver, diagnosis of thymoma or other haemopoietic neoplasia or evidence of systemic inflammation or infection were excluded. Further exclusion criteria included sacculitis or other gastrointestinal diseases, hepatopathies, abdominal abscessation, and biochemical abnormalities such as marked uraemia or hypercalcaemia. In cases where the same patient underwent multiple CT examinations, the included study was the first in which the patient met all the inclusion criteria. All cases were collected by the first author (CC), a small animal intern with advanced imaging training, confirmation of inclusion into the study was made in consensus with a board-certified veterinary radiologist (TS) and a board-certified exotic animal veterinary specialist (KE).

Pilot Study:

A two-part pilot study was performed to establish some basic validations, on which the retrospective study would be based. In the first part of the pilot study, an investigation was performed with two rabbits undergoing a post-mortem examination to verify the CT-identification of the spleen. The rabbits were culled laboratory rabbits that are obtained as cadavers, in batches, for student teaching, promoting the ‘three Rs’ (reduce, reuse, recycle). They were euthanased previously for reasons unrelated to the study or abdominal disease. The fur over the ventral abdomen was clipped, and the cadavers were CT-scanned using the standard pre-contrast protocol used for the retrospective study with the exception of recumbency. The cadavers were scanned in dorsal recumbency to better expose the abdominal wall for skin marking purposes. Using a dedicated DICOM viewing software (Horos, Purview, Annapolis MD, USA, version 3.3.6) CT images were reviewed, and the spleen was identified by two reviewers with full agreement (CC & TS). The z-axis position of the mid-body of the spleen was identified on CT images, and with the help of the CT positioning laser lights, the transverse plane was marked on the skin of the ventral and lateral abdominal wall with a permanent black marker (Sharpie^{TM}, Sanford LP, USA). The cadavers were then frozen in the original CT-scanning position. Six days later, these were sectioned along the marked transverse plane with an oscillating band saw (600 Series, AEW Delford, Norwich, UK) using five-teeth-per-inch blades. The cut surfaces of each cadaver were photographed and visually inspected by the same two reviewers with full agreement (CC & TS) and the spleen was identified. The location of the spleen on the cut surfaces was compared to the position identified on the post-mortem CT images.

In the second part of the pilot study, a post-mortem specimen study of a rabbit spleen was performed to validate size and shape measurements of the spleen, using one rabbit that had been euthanized immediately prior for progressive dental disease and with signed owner consent. A post-mortem mid-abdominal laparotomy was performed, and the spleen was removed; no other abdominal pathology was appreciated. All fat and connecting ligament tissues were removed via blunt dissection. The spleen was measured using a standard ruler on a flat surface. The organ was then submerged into a known volume of water within a calibrated measuring tube, the measurement was repeated, and the mean of both measurements was calculated. A CT examination of the isolated spleen placed on an absorbent pad was performed using the same CT protocol as for the retrospective study part. Two reviewers (CC and TS) blindly and independently reviewed the CT images to determine the volume of the spleen by manually drawing regions of interest (ROI) of the splenic cross-section on transverse-plane post-contrast CT images using the pencil tool of the DICOM viewing software. The splenic cross-section was measured on every reconstructed CT image. The splenic volume was then calculated and recorded using the volume computing tool, which also generated a volume-rendered 3D image. Each reviewer then repeated the volume calculation using every other reconstructed CT image, and then again, using every fourth reconstructed CT image, always including the most peripheral image containing the spleen at each end. The mean splenic volume of both reviewers was calculated for every-image, every-second-image, and every-fourth-image stacks of the CT series.

One reviewer (CC) measured and recorded on sagittally reconstructed images of post-contrast CT series the length of the spleen. One reviewer (CC) recorded minimal, maximal and mean splenic x-ray attenuation in Hounsfield units (HU) in three areas of the spleen using the oval ROI tool.

CT Scanning Procedure:

All rabbits were scanned with a CT 64-slice multi-detector-row CT scanner (Somatom® Definition AS, Siemens, Erlangen, Germany) using a full-body protocol in a patented restraining device (VetCatTrap™, University of Illinois, USA) in sternal recumbency, using all 64 detectors with 0.6mm detector width. For the pre-contrast series, an automated adaptive tube current and tube potential, a collimator pitch of 1.5 and a tube rotation time of 0.33s were used. Images used for this study were reconstructed with an iterative medium frequency kernel (Siemens proprietary name I40f, iteration strength two) with a reconstructed slice width of 1mm and a 0.7mm image interval. For the post-contrast series, an automated adaptive tube current and a fixed tube potential of 120kV, a collimator pitch of 0.55, and a tube rotation time of 1s were used. Images were reconstructed with an iterative medium frequency kernel (Siemens proprietary name J40f, iteration strength three) with a reconstructed slice width of 1mm and a 0.7mm image interval. The image-field-of-view was adapted to the size of the animal and was typically 18 to 20cm in diameter. All images were stored in DICOM format on a local picture archiving and communication system. Iodinated, non-ionic contrast medium (Iopamidol, Niopam 350, Bracco Ltd, UK) was injected intravenously through a catheter in the marginal ear vein with a power injector (Empower CTA Injector system, Bracco Ltd, UK) at a dose of 700mg iodine/kg, with a maximum pressure of 325psi in each rabbit. All rabbits were conscious during the scan and the procedure followed a previously published CT contrast medium injection protocol.[6]

Images Analysis:

All images were reviewed on a computer workstation (iMac 27-inch, Apple, Cupertino, California, USA) with a calibrated LCD flatscreen monitor (retina display), using an open-source DICOM viewer software (Horos, Purview, Annapolis MD, USA, version 3.3.6). The abdominal CT images were viewed in a soft tissue view window (window level 50 HU, window width 350 HU) and were analyzed on pre- and post-contrast sequences. Windowing changes were allowed for reviewers. Reviewers for different tasks included one ACVR-, ECVDI-, and RCVS-boarded veterinary diagnostic imaging specialist (TS), two diagnostic imaging residents (II and TP), and one small animal intern with advanced imaging training (CC). The imaging features of the spleen derived from the first part of the pilot study, in which the spleen identification was verified, were used as a baseline for assessment of spleen identification in the retrospective study part and were made available to the reviewers. The spleen was assessed blindly and independently by three reviewers (TS, II & TP) as *clearly visible* or *not clearly visible*. The assessment was performed in a randomized order on transverse pre-contrast images first, followed by transverse post-contrast images, in all cases. In each case, reviewers could not compare the pre- and post-contrast images when making their assessments. One reviewer (CC) scored transverse post-contrast CT images of the location of the spleen as *caudodorsal*, *caudodorsomedial*, *caudal to the stomach with minimal adjacency to the gastric wall*, *caudodorsolateral, medial, lateral, dorsolateral*, or *dorsomedial to the greater curvature of the stomach*. One reviewer (CC) used multi-planar reconstructions of post-contrast CT series to measure and record the length of the spleen. As the rabbit spleen anatomically in situ is not uniformly straight, but curves along the gastric wall, up to three lines were measured and angled along the center of the organ. The three lines were then added together, and a total length was determined. The plane selected for measurement was the one that allowed the easiest and most accurate assessment of the length. The method of measurement differs from that used in our pilot study, where a single line was used to measure the isolated spleen. One reviewer (CC) determined the volume of the spleen by manually drawing regions of interest (ROI) of the splenic cross-section on transverse-plane post-contrast CT images using the pencil tool. Based on the results of the pilot study, the splenic cross-section was measured on every other image, unless the cranial or caudal tip had a strong deviation, in which case, every image was measured at the tip. The splenic volume was then calculated and recorded using the volume computing tool of the DICOM viewer software, which also generated a volume-rendered 3D image. The spleen-volume-to-body-weight ratio was calculated in ml/kg, recorded for each case, and the mean of all ratios was calculated and recorded.

One reviewer (CC) reviewed the volume-rendered CT image of the spleen and scored the shape of the spleen as reminiscent of a banana, tongue, elephant trunk, wing of a seagull, wedge, amorphous, cat sternum, candy bar or cartoon bone. Lastly, one reviewer (CC) recorded minimal, maximal, and mean splenic x-ray attenuation in HU in three areas of the spleen on pre- and post-contrast CT using the oval ROI tool, calculated and recorded the mean of all pre-contrast and mean of all post-contrast mean splenic x-ray attenuations.

Statistical analysis was performed by one co-author (TP) who had statistical training as part of their PhD using R v4.2.1 (RSTUDIO TEAM 2022. RStudio: Integrated Development Environment for R. Boston, MA: RStudio, PBC) and RStudio v2022.12.0.353 (R CORE TEAM 2022. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing). A p-value of less than 0.01 was considered significant. To test for normality, a Shapiro-Wilk Test was used. A linear regression model for analysis of splenic volume with signalment and splenic length was performed. A McNemar test was used for statistical analysis of the pre- and post-contrast visibility data and both Fleiss' Kappa and percentage agreement were used to calculate inter-rater agreement. A Pearson correlation coefficient was used to investigate the relationship between splenic volume, and body weight, and splenic volume, and length.

Results

In the first part of the pilot study, in both rabbits, the spleen could be identified on the cut surfaces as a soft tissue organ along the dorsocaudal aspect of the greater curvature of the stomach. The location of the spleen identified on the post-mortem CT image at the level of the cut matched the visual appearance on the cut surfaces. In the second part of the pilot study, the CT-volume-rendered-based volumetric calculation revealed a mean splenic volume of 2.0183ml for both reviewers (range 1.9290ml to 2.0987ml). The mean splenic volume from the water submersion test was 2.05ml (range 2ml to 2.1ml). The length of the gross organ was 42mm. The length measured using the line measuring tool on the dorsal-plane CT images was 40mm. The shape of the spleen was reminiscent of a wedge, both on visual inspection and on the volume-rendered CT image. The mean attenuation value of the isolated spleen was 125HU (range -8HU to +217HU).

A total of 173 pre- and post-contrast CT studies of rabbits with haematology and biochemistry screening data meeting the inclusion criteria were identified and reviewed during the study period from October 2018 to November 2022. Of these, 115 studies met all inclusion criteria. Studies were excluded for having no post-contrast sequence (n=3), having a non-diagnostic CT scan (n=13), having a disease specified in the exclusion criteria (n=38), or not having the body weight or age recorded within the specified period relating to the CT scan date (n=4).

Regarding the assessment of splenic visibility on pre-contrast CT series, the inter-rater agreement was fair (Fleiss' Kappa = 0.305), with a percentage agreement of 53.7% **(Figure 1)**. Regarding post-contrast CT splenic visibility assessment, there was moderate inter-rater agreement (Fleiss' Kappa = 0.404), with a percentage agreement of 93% **(Figure 2)**. There were significantly more spleens identified on post-contrast series compared to pre-contrast series (McNemar test = 27.034, df=1 $p<0.001$).

The spleen was found in a variety of locations in relation to the stomach **(Figure 3)**. The most frequent location of the spleen in post-contrast studies was the caudodorsal aspect of the greater curvature of the stomach in 47 rabbits (40.87%), followed by the caudodorsomedial aspect of the greater curvature of the stomach in 19 rabbits (16.52%), then caudal to the stomach in 19 rabbits (16.52%), the caudodorsolateral aspect of the greater curvature of the stomach in 13 rabbits (11.30%), the medial aspect of the greater curvature of the stomach in eight rabbits (6.96%), the lateral aspect of the greater curvature of the stomach in 4 rabbits (3.48%), the dorsolateral aspect of the greater curvature of the stomach in three rabbits (2.61%), and the dorsomedial aspect of the greater curvature of the stomach in two rabbits (1.74%). If the spleen was detached from the gastric wall, the segmentally branching splenic artery and vein, which are contained within the gastrosplenic ligament, were often visible **(Figure 2B)**.

The mean length of all spleens was 40mm (range 20mm to 61mm). The splenic length ranged from 20mm to 29mm in 20 rabbits (17.39%), from 30 to 39mm in 53 rabbits (46.09%), from 40mm to 49mm in 31 rabbits (26.96%) and from 50mm to 59mm in two rabbits (1.68%). In one rabbit (0.84%), the splenic length was 61mm. Mean x-ray attenuation of all spleens was 80HU (range 31HU to 108HU) for pre-contrast series and 320HU (range 116HU to 507HU) for post-contrast series CT.

The mean rabbit splenic volume based on CT-volumetry was one milliliter. The frequencies of splenic volume were as follows: 35 rabbits (30.43%) had a splenic volume from 0.2ml to 0.6ml, 24 (20.87%) from 0.61ml to 0.8ml, 35 (30.43%) from 0.81ml to 1.2ml, 15 (13.04%) from 1.21ml to two ml, and six rabbits (5.22%) had a volume greater than two ml. The mean splenic-volume-to-body-weight ratio was 0.5ml/kg (range 0.17ml/kg to 1.2ml/kg). The splenic-volume-to-body-weight ratio was below 0.2ml/kg in 15 rabbits (13.04%), from 0.2 to 0.4ml/kg in 61 rabbits (53.04%), from 0.41 to 0.6ml/kg in 28 rabbits (24.35%), from 0.61 to 0.8ml/kg in 5 rabbits (4.35%), from 0.81 to one ml/kg in five rabbits (4.35%), and above one ml/kg in one rabbit (0.87%). The volume of the rabbit spleen was significantly related to body weight (linear regression $t=4.00$, $p<0.001$), with a weak positive correlation coefficient (Pearson coefficient: $r=0.55$, $P<0.001$). Additionally, there was a significant relationship between rabbit splenic volume and splenic length (linear regression: $t=5.173$, $p<0.001$), with a weak positive correlation coefficient (Pearson coefficient: $r=0.55$, $P<0.001$). The shape of the spleen on volume-rendering CT images was reminiscent of a banana in 32 cases (27.83%), a tongue in 27 rabbits (23.48%), an elephant trunk in 15 rabbits (13.04%), the wing of a seagull in 13 rabbits (11.30%), a wedge in 10 rabbits (8.7%), an amorphous structure in nine rabbits (7.83%), a cat sternum in four rabbits (3.48%), a candy bar in three rabbits (2.61%), and a cartoon bone in two rabbits (1.74%) (**Figure 4**).

Discussion

The rabbit spleen can be clearly identified on post-contrast abdominal CT images, confirming one of our hypotheses. Although detailed histological studies of the rabbit spleen were not performed, it is described in standard anatomic references as a dark red-brown organ.[7,13-17] The spleen of all mammals is composed of two histologically distinguished compartments. The red pulp acts as a red blood cell depot and is functionally linked to erythrocyte and iron metabolism. The white pulp acts as a storage for mature white blood cells and facilitates processes of leucocyte and lymphocyte activation and metabolism.[18] A highly vascularised, predominantly red-pulp-type splenic tissue corresponds to the strong contrast enhancement following administration of intravenous iodinated contrast medium found in our study, with a 400% increase in x-ray attenuation, compared to pre-contrast values, making it more clearly visible than other abdominal organs.

Our hypothesis, that the spleen would be clearly identified on pre-contrast CT images, was not confirmed. Although the rabbit spleen does have a relatively high mean x-ray attenuation value of 80HU, likely related to the high haemoglobin iron content in the red pulp, this is probably not sufficient to clearly distinguish it from neighbouring organs. Other organs, such as the kidneys, more easily stand out due to the surrounding fat which has a much lower x-ray attenuation. The splenic parenchyma was relatively homogenous in x-ray attenuation in our study. This is consistent with ultrasound studies which describe a homogeneous echogenicity of the splenic parenchyma.[8,11]

Our hypothesis, that the spleen is visible as an elongated organ located along the caudodorsal stomach wall, was confirmed. The rabbit spleen has been described as an elongated organ with a three to five cm length and a width of 0.6 to 1.2cm, with the dorsal extremity adjacent to the last two ribs and cranial to the left kidney and the remainder of the spleen being located at the level of the costal arch under the abdominal wall, between the greater curvature of the stomach, the jejunum and the caecum.[7,11,13,16,17] The spleen is attached to the greater curvature of the stomach via the gastrosplenic ligament. The phrenicosplenic ligament is only rudimentarily developed.[11,14-17] The splenic ligaments themselves are not visible on CT, but the segmentally branching splenic artery and vein contained within the gastrosplenic ligament are visible if the spleen is not directly adjacent to the stomach. On CT images, the distal third of the spleen may or may not be adjacent to the stomach. This is probably due to the variation of the filling of the stomach and the way it displaces the spleen in vivo. With the spleen being connected to the stomach via the gastrosplenic ligament, it is likely that the positional variation described here is due to mild rotation of the stomach. It is possible that the gastrosplenic ligament is sufficiently long to allow a degree of splenic displacement, or that it does not run the complete length of the spleen. The shape of the normal rabbit spleen is depicted as similar to the shape of a tongue in anatomic references.[7,13,14] In our study, a banana shape was most frequently identified, with the tongue being a close second. These variations are likely related to the degree of splenic congestion and compression by adjacent organs, in particular by the stomach with different degrees of filling.

The splenic volume can be easily and quickly calculated from CT images and provides additional useful information, to distinguish normal splenic size from splenomegaly and to follow up disease processes of the spleen. Our pilot data suggest that the volume calculation is accurate, even if splenic surface contouring is not performed on every single slice image, and in our retrospective study, the calculation could be performed very quickly. This is also supported by another study on canine liver CT volumetry, using the same, widely available software.[19]

Limitations of this study include the lack of histopathological confirmation of splenic normalcy and the inability to record splenic weight, due to the retrospective nature of the study and the study population of live patients. Some of these limitations were partially mitigated by performing the pilot studies. On the other hand, using clinical cases allowed contrast enhancement assessment, provided splenic morphology data in living animals, which might be different in a post-mortem study, and provided data that are more likely useable in clinical cases, such as the splenic-volume-to-body-weight ratio.

In conclusion, the spleen of the rabbit can be clearly and reliably identified on post-contrast CT studies as an elongated, most commonly banana- or tongue-shaped parenchymal organ located along the greater gastric curvature, caudodorsal or deviated medially or laterally to it. It is normally approximately 40mm long, has a volume of approximately one millilitre, and a splenic-volume-to-body-weight ratio of approximately 0.5ml/kg.

List of author contributions:

Category 1

(a) Conception and Design: Chernev, Schwarz, Eatwell, Richardson

(b) Acquisition of Data: Chernev, Procter, Isaac

(c) Analysis and Interpretation of Data: Chernev, Schwarz, Isaac, Procter, Keeble, Eatwell, Richardson, Koterwas

Category 2

(a) Drafting the Article: Chernev, Schwarz

(b) Revising the Article for Intellectual Content: Chernev, Schwarz, Isaac, Procter, Keeble, Eatwell, Richardson, Koterwas

Category 3

(a) Final Approval of the Completed Article: Chernev, Schwarz, Isaac, Procter, Keeble, Eatwell, Richardson, Koterwas

Category 4

(a) Agreement to be accountable for all aspects of the work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Chernev, Schwarz, Isaac, Procter, Keeble, Eatwell, Richardson, Koterwas

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Figures

Figure 1. A) Pre-contrast CT image of a rabbit spleen (arrows) scored as *not clearly visible.* Limited visibility is likely related to splenic position in proximity of the caudolateral wall of

the stomach (St), left kidney (K) and left abdominal wall. B) The corresponding post-contrast CT image delineates the spleen slightly better.

Figure 2. A) Post-contrast CT image of a rabbit spleen (arrows) that was scored as *not clearly visible*, likely due to the small size and poor contrast enhancement. B) Post-contrast CT image of a spleen (arrows) that was scored *clearly visible* together with the vessels within the gastrosplenic ligament (arrowheads). K: left kidney.

Figure 3. Post-contrast CT images of different rabbit spleens (arrows) with A) a medial, B) a dorsal, C) a dorsolateral and D) a caudal location in relation to the stomach (St). K: right kidney.

Figure 4. Three-dimensional volume-rendered CT images of different rabbit spleens, varying in shape from being reminiscent of A) a banana, B) a tongue, C) an elephant trunk, D) a wedge, E) a wing of a seagull, F) a cat sternum, G) a candy bar and H) a cartoon bone.