Abdominal anatomic features and reference values determined by use of ultrasonography in healthy common rats (Rattus norvegicus)

Tommaso Banzato, DVM, PhD; Luca Bellini, DVM, PhD; Barbara Contiero; Andrea Martin; Sema Balikçi, DVM; Alessandro Zotti, DVM, PhD

Objective—To determine ultrasonographic features and reference values of the anatomy of the abdomen of common rats (Rattus norvegicus).

Animals—20 adult male and 20 adult female rats.

Procedures—A complete abdominal ultrasonographic examination was performed with the rats sedated. The cadavers of 4 rats were used for anatomic comparisons. Two cadavers were dissected and 2 cadavers were frozen and then cross-sectioned by use of an electric bandsaw. Slices were cleaned with water and photographed on each side. Correlations between variables were determined.

Results—The ultrasonographic anatomy of the abdomen was determined, including measurements of the kidneys and adrenal glands and thickness of the walls of the stomach (saccus caecus, fundus, and pylorus), duodenum, and cecum. A significant positive correlation between kidney size and body weight was detected. The dorsoventral measurements of the left and right adrenal gland were significantly different, regardless of sex. Dorsoventral measurements of the right adrenal gland were significantly different between males and females.

Conclusions and Clinical Relevance—The ultrasonographic images and data provided an atlas of the ultrasonographic anatomy of common rats that may be useful to veterinary radiologists, clinicians, and researchers. (Am J Vet Res 2014;75:67–76)

Rattus norvegicus was the first species to be domesticated purely for scientific purposes and presently is the most widely studied species in biomedical research. Moreover, because of some specific characteristics of this species, such as its extreme intelligence and charming nature, rats are commonly kept as pets.

The increasing prevalence of rats as pets has led to an increase in the number of requests for specialty veterinary services for this species. However, a relatively low number of reports are presently available regarding medical treatment and medical imaging of rats, compared with other species. To the authors’ knowledge, the only description of the ultrasonographic features of the abdomen of rats is reported in a textbook of imaging of small exotic mammals; the ultrasonographic diagnosis of pregnancy in rats has also been reported. A description of the liver, spleen, and kidneys obtained by use of high-frequency (40 MHz) ultrasonography has also been published.

The purpose of the study reported here was to provide a comprehensive description of the abdominal ultrasonographic features of healthy common rats, including the most common abdominal reference measurements that are typically used during an ultrasonographic examination in pets such as dogs or cats.

Materials and Methods

Animals—Forty healthy adult common rats (20 males and 20 females; mean ± SD weight, 304.7 ± 59.2 g [males, 312.1 ± 65.2 g; females, 297.4 ± 53.2 g]) kept as pets and referred for examination to the Radiology Unit, Department of Animal Medicine, Production and Health, University of Padua, were included in the study. All rats were from 6 months to 1 year of age. All rats belonged to private owners who voluntarily took part in a survey on the prevalence of subclinical diseases in small exotic mammals. This study was approved by the University of Padua Ethical Committee (protocol No. 41060). The rats were considered healthy on the basis of individual history and clinical examination; moreover, each subject underwent whole-body radiography and a complete abdominal ultrasonographic scan. Food was not withheld prior to examination.

The cadavers of 4 rats (2 males and 2 females; mean ± SD weight, 440 ± 75 g) were also dissected for this study. Each animal was euthanized, upon the owner’s request, because of advanced respiratory tract disease. The rats were anesthetized with a combination
of ketamine and medetomidine administered IM, and a lethal dose of a mixture of embutramide, mebenzoinum iodide, and tetracaine hydrochloride was administered IV.

**Ultrasonographic procedures**—Each animal was sedated with a combination of 2 anesthetic drugs injected IM immediately prior to the beginning of the scan. The hair in the ventral portion of the abdomen was carefully clipped from a point approximately 1 cm cranial to the xyphoid cartilage to the caudal-most part of the pubis. A complete ultrasonographic examination was performed following the same ventral approach, with the animal in dorsal recumbency. All images were obtained with a 4- to 8.5-MHz linear array transducer (frequency, 8.5 MHz) connected to a commercial ultrasonic system that was serviced and checked regularly; a standoff pad was not used. Duration of each examination was 30 to 45 minutes. To minimize interoperator variability, all ultrasonographic examinations were performed by the same operator (TB). Different scanning planes were used for evaluation of different abdominal organs; the testicles were evaluated in transverse and longitudinal scanning planes. The urinary bladder, coagulating glands (accessory sex glands), seminal vesicles, uterus, cecum, left and right adrenal glands, stomach (saccus cecus, fundus, and pylorus), duodenum, and liver were evaluated in a transverse scanning plane. The left and right ovaries and the spleen were evaluated in a parasagittal scanning plane, whereas the left and right kidneys were evaluated in transverse, parasagittal, and longitudinal scanning planes. The ultrasonographic measurements of the kidneys, adrenal glands, and hypogastric lymph node and the wall thickness of the urinary bladder, saccus cecus of the stomach, fundus, pylorus, cecum, and duodenum were recorded. To obtain the most precise measurements, image magnification was always used.

**Anatomic dissections**—Gross anatomic dissections were performed in 2 rats (1 male and 1 female). All the dissections were performed within 24 hours of death to minimize postmortem changes. Two other rats (1 male and 1 female) were placed within 20 minutes of death to minimize postmortem changes. The hair in the ventral portion of the abdomen was carefully clipped from a point approximately 1 cm cranial to the xyphoid cartilage to the caudal-most part of the pubis. All images were obtained with a 4- to 8.5-MHz linear array transducer (frequency, 8.5 MHz) connected to a commercial ultrasonic system that was serviced and checked regularly; a standoff pad was not used. Duration of each examination was 30 to 45 minutes. To minimize interoperator variability, all ultrasonographic examinations were performed by the same operator (TB). Different scanning planes were used for evaluation of different abdominal organs; the testicles were evaluated in transverse and longitudinal scanning planes. The urinary bladder, coagulating glands (accessory sex glands), seminal vesicles, uterus, cecum, left and right adrenal glands, stomach (saccus cecus, fundus, and pylorus), duodenum, and liver were evaluated in a transverse scanning plane. The left and right ovaries and the spleen were evaluated in a parasagittal scanning plane, whereas the left and right kidneys were evaluated in transverse, parasagittal, and longitudinal scanning planes. The ultrasonographic measurements of the kidneys, adrenal glands, and hypogastric lymph node and the wall thickness of the urinary bladder, saccus cecus of the stomach, fundus, pylorus, cecum, and duodenum were recorded. To obtain the most precise measurements, image magnification was always used.

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of death on a plastic support in ventral recumbency with the limbs placed close to the body and then stored in a freezer at –20°C. After freezing, these specimens were cross sectioned by means of an electric bandsaw. Contiguous 3-mm sagittal slices were obtained, starting at the nose and ending at the anus. The slices were cleaned with water, numbered, and photographed on the cranial and caudal surfaces to provide the anatomic images used in the study.

Statistical analysis—The ultrasonographic measurements were tested for normal distribution after the outliers (values that were 3 times the SD or greater) had been discarded. Data not normally distributed were log-transformed. The 95% reference intervals were calculated with a parametric approach if the data were normally distributed; otherwise, the robust method was used.

The data were analyzed according to an ANCOVA linear model with the fixed effect of sex (female vs male) and covariate effect of weight. Least squares means and SEM were calculated to compare values in females versus males. To test the difference between right versus left adrenal gland sizes, the adrenal gland data were analyzed with a linear mixed model with weight as covariate effect and animal as random effect, and with the fixed effects of sex (female vs male) and side (right vs left). Statistical analysis was performed with commercially available statistical software. For all comparisons, \( P < 0.05 \) was considered significant.

Results

Reproductive system—Ultrasoundically, the testicles had a mottled parenchyma and the mediastinum was not visible (Figure 1). The head, body, and tail of the epididymis could be imaged in a longitudinal scan. The seminal vesicles and the coagulating glands were identified dorsally or laterally to the urinary bladder as 2 symmetric, ovoid, mildly echoic structures (Figure 2). It was impossible to distinguish the coagulating glands from the seminal vesicles in the ultrasonographic images. The rat prostate is located caudally in the pelvis and thus was not identified ultrasonographically in any study subject.

Figure 3—Photographs and ultrasonographic images of the uterus and ovaries in a rat. In the photographs, notice the gross appearance after removal of the overlying organs (A) and in cross section (B). The ultrasonographic image of the uterine horns (C) was obtained in a transverse scanning plane, and the ovary was sectioned transversely for a photograph of the gross anatomic features (D). The ultrasonographic image of the ovaries and follicles (E) was obtained in a parasagittal scanning plane. Cr = Cranial. Me = Medial. See Figures 1 and 2 for the remainder of the key.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>Males</th>
<th>Females</th>
<th>BWrc</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left kidney length</td>
<td>38</td>
<td>19.47 ± 2.3</td>
<td>19.87 (0.44)</td>
<td>19.08 (0.44)</td>
<td>0.02</td>
<td>14.97–23.97</td>
</tr>
<tr>
<td>Left kidney width</td>
<td>40</td>
<td>10.74 ± 1.1</td>
<td>10.91 (0.22)</td>
<td>10.57 (0.22)</td>
<td>0.01</td>
<td>8.56–12.93</td>
</tr>
<tr>
<td>Left kidney height</td>
<td>31</td>
<td>10.96 ± 1.2</td>
<td>11.02 (0.29)</td>
<td>10.91 (0.25)</td>
<td>0.01</td>
<td>8.68–13.23</td>
</tr>
<tr>
<td>Pelvis left kidney</td>
<td>33</td>
<td>0.87 ± 0.24</td>
<td>0.852 (0.802–0.905)</td>
<td>0.838 (0.784–0.896)</td>
<td>NS</td>
<td>0.48–1.35</td>
</tr>
<tr>
<td>Right kidney length</td>
<td>37</td>
<td>19.45 ± 2.18</td>
<td>19.91 (0.33)</td>
<td>19.06 (0.40)</td>
<td>0.02</td>
<td>14.40–23.51</td>
</tr>
<tr>
<td>Right kidney width</td>
<td>37</td>
<td>10.75 ± 0.88</td>
<td>10.78 (0.18)</td>
<td>10.71 (0.17)</td>
<td>0.01</td>
<td>8.85–12.63</td>
</tr>
<tr>
<td>Right kidney height</td>
<td>28</td>
<td>11.04 ± 1.00</td>
<td>11.05 (0.25)</td>
<td>11.02 (0.22)</td>
<td>0.01</td>
<td>8.93–13.12</td>
</tr>
<tr>
<td>Pelvis right kidney*</td>
<td>29a</td>
<td>0.84 ± 0.21</td>
<td>0.653 (0.815–0.892)</td>
<td>0.746 (0.711–0.783)</td>
<td>NS</td>
<td>0.55–1.16</td>
</tr>
<tr>
<td>Urinary bladder wall</td>
<td>32</td>
<td>0.73 ± 0.13</td>
<td>0.76 (0.03)</td>
<td>0.71 (0.03)</td>
<td>NS</td>
<td>0.48–0.99</td>
</tr>
</tbody>
</table>

Values for males and females are estimated least squares means (SE or 95% confidence intervals for normally or not normally distributed data, respectively). *Within a row, values differ significantly (\( P < 0.05 \)) between males and females. BWrc = Body weight regression coefficient. No. = Number of data. NGR = Non-Gaussian distribution and RI estimated with robust method. NS = Not significant (\( P > 0.05 \)). RI = Reference interval. aBack transformed after logarithmic transformation. bOne outlier was removed from analysis.
ficult to identify because of the overlying gastrointestinal tract structures, whereas in pregnant females, the uterus was clearly visible. The ovaries were visible as triangular structures located caudal to the caudal pole of the kidneys; the follicles were sometimes visible as tiny round anechoic structures in the ovarian parenchyma (Figure 3).

Urinary tract—The urinary bladder (Figure 2) was visible only when filled with urine and was not identified in 8 of the 40 animals; the urinary bladder wall thickness reference values were determined (Table 1). The urinary bladder wall thickness was measured in the dorsal portion of the urinary bladder; no layers were visible. All the measurements of the bladder wall thickness were made at the end of each scanning procedure, so the urinary bladder was moderately to severely distended with urine.

Both kidneys were evaluated in all study subjects (Figure 4). The cortex and the medulla were clearly distinguished in the transverse scan, with the cortex being more echogenic than the medulla, whereas no distinction was possible in longitudinal and parasagittal scans. The renal sinus, pelvis, and crest were evident in both scanning planes. The renal pelvis height was measured
in the transverse plane in the central portion of the kidney as the width of the anechoic crescent-shaped band between the renal sinus and the renal crest. Visualization of the kidneys was often hindered by the overlying...
intestinal structures when a ventral approach was used, whereas a lateral approach enabled better visualization. The ultrasonographic reference values for kidney size (length, width, and height) and renal pelvis height were determined (Table 1). Significant positive correlations were detected between body weight and ultrasonographic kidney size (length, height, and width). Pelvis height of the right kidney was significantly different in males and females.

Gastrointestinal tract—In the stomach, the saccus cecus, fundus, and pylorus could be easily recognized in transverse scans (Figure 5). The duodenum was visible when imaging the right portion of the liver in a transverse scan. The ultrasonographic layering of both the stomach and the duodenal wall was similar to that described in dogs and cats; the serosa, submucosa, and interface with the lumen were hyperechoic, whereas the muscularis mucosa and the mucosa were hypoechoic. The mucosa was always the thickest ultrasonographic layer in the stomach and duodenum. The ileum and jejunum were located in the umbilical region and were difficult to distinguish from the large intestine (Figure 6).

The cecum was visible as a large, gas-filled structure, sometimes located in the right hemiabdomen and sometimes occupying the caudal portion of the abdomen (Figure 7). Only small portions of the cecum wall were clearly visible (likely because the wall is thin). When visible, the ultrasonographic layering of the cecum was similar to that of the stomach and duodenum, with the difference that all the ultrasonographic layers appeared of similar thickness. Ultrasonographic reference values for wall thickness of the saccus cecus, fundus, pylorus, duodenum, and cecum were determined (Table 2).

Adrenal glands and hypogastric lymph node—The adrenal glands were located immediately cranial to the cranial pole of the corresponding kidney (Figure 8). The left adrenal gland was visible only in 32 of the 40 animals, because of the overlying stomach. Both adrenal glands were ovoid and ranged from mildly echoic to almost anechoic. The hypogastric lymph node was visible immediately caudal to the aortic bifurcation, between the 2 internal iliac arteries and veins, and was visible as a poorly echoic, ovoid-shaped structure (Figure 9).

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**Table 2—Ultrasonographic measurements (mm) of the gastrointestinal tract of rats.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>Males</th>
<th>Females</th>
<th>BWrc</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saccus cecus of the stomach</td>
<td>35</td>
<td>1.09 ± 0.20</td>
<td>1.04 (0.05)</td>
<td>1.15 (0.05)</td>
<td>NS</td>
<td>0.70–1.49</td>
</tr>
<tr>
<td>Fundus wall</td>
<td>34</td>
<td>1.07 ± 0.25</td>
<td>1.01 (0.97–1.06)</td>
<td>1.14 (1.09–1.19)</td>
<td>NS</td>
<td>0.76–1.59&lt;sup&gt;sem&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pylorus wall</td>
<td>36</td>
<td>2.20 ± 0.41</td>
<td>2.15 (0.10)</td>
<td>2.25 (0.10)</td>
<td>NS</td>
<td>1.40–3.00</td>
</tr>
<tr>
<td>Duodenum wall</td>
<td>38</td>
<td>1.54 ± 0.21</td>
<td>1.47 (1.43–1.51)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.59 (1.54–1.64)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NS</td>
<td>1.13–1.93&lt;sup&gt;sem&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cecum wall</td>
<td>38</td>
<td>0.89 ± 0.13</td>
<td>0.89 (0.03)</td>
<td>0.89 (0.03)</td>
<td>NS</td>
<td>0.62–1.15</td>
</tr>
</tbody>
</table>

<sup>a</sup>One outlier was removed from analysis. <sup>b</sup>Back transformed after logarithmic transformation. See Table 1 for remainder of key.
The ultrasonography reference values for the dorsoventral dimensions of the right and left adrenal glands measured in a transverse scanning plane and the length and width of the hypogastric lymph node were determined (Table 3). Right adrenal gland height was significantly different from left adrenal gland height, regardless of sex, whereas right adrenal gland height differed significantly between males and females.

Liver, spleen, and pancreas—The liver had a coarse echoic texture, and portal vessels with thin hyperechoic walls were visible. Individual liver lobes were impossible to visualize (Figures 5 and 8). The aorta, caudal vena cava, and portal vein were clearly identified in the liver parenchyma.

The spleen had a fine, homogeneous texture and could be imaged from its dorsal to the ventral edges on the left side of the abdomen. A direct comparison between the echogenicity of the spleen and the liver was impossible because the stomach was interposed between them (Figure 8). For this reason, comparison was performed by coupling the images of the liver and spleen in the same screenshot, which revealed that the liver had lower echogenicity than did the spleen. The cranial pole of the right kidney was in direct contact with the liver, enabling direct comparison between the echogenicity of the right kidney cortex and that of the liver (Figure 5). The renal cortex of the right kidney was more echoic than the liver parenchyma. Likewise, the echogenicity of the cortex of the left kidney was higher than that of the spleen (Figure 10).

The body and left and right lobes of the pancreas could be imaged in all study subjects (Figure 10). The body of the pancreas was visible, both in longitudinal and transverse scans, adjacent and immediately caudal to the duodenum. The left lobe was visible as an echoic, roughly triangular structure ventral to the left kidney. The right lobe was visible as an echoic and irregular structure embedded between the medial surface of the right kidney and the right lobe of the liver. The echogenicity of the pancreas was greater, compared with that of the liver and the renal cortex.

Discussion

Most of the clinically relevant organs and structures of the abdomen were identified in all study subjects. Although some authors suggest the use of a 10- to 15-MHz linear transducer for the ultrasonographic examination of the abdomen in rats, results of the present study indicated that the lower-frequency transducers commonly used in veterinary practices may also be used for diagnostic imaging in this species. It is likely, however, that the use of higher frequency transducers might provide better image detail, thus enabling an improved corticomedullary distinction in those organs like the kidneys, the adrenal glands, and the lymph nodes along with a more detailed evaluation of the wall of the gastrointestinal tract.

The ultrasonographic reference values were consistent with the gross anatomic measurements described for laboratory rats, suggesting that neither the different strain analyzed nor the sedation procedures affected organ size.

Table 3—Ultrasonographic measurements (mm) of the hypogastric lymph node and adrenal glands of rats.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>Mean ± SD</th>
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<th>Females</th>
<th>BWrc</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node length</td>
<td>31</td>
<td>4.19 ± 1.26</td>
<td>4.01 (3.71–4.34)</td>
<td>4.03 (3.73–4.35)</td>
<td>NS</td>
<td>2.23–7.55</td>
</tr>
<tr>
<td>Lymph node width</td>
<td>31</td>
<td>1.53 ± 0.75</td>
<td>1.32 (1.19–1.47)</td>
<td>1.47 (1.33–1.63)</td>
<td>NS</td>
<td>0.48–2.98</td>
</tr>
<tr>
<td>Right adrenal gland</td>
<td>37</td>
<td>3.41 ± 0.49</td>
<td>3.32 (0.11)</td>
<td>3.6 (0.11)</td>
<td>NS</td>
<td>2.45–4.26</td>
</tr>
<tr>
<td>Left adrenal gland</td>
<td>32</td>
<td>3.07 ± 0.36</td>
<td>3.0 (0.08)</td>
<td>3.1 (0.09)</td>
<td>NS</td>
<td>2.37–3.77</td>
</tr>
</tbody>
</table>

DV = Dorsoventral.

See Table 1 for remainder of key.
or shape. However, all the study subjects were sedated with a combination of medetomidine and butorphanol. Medetomidine may cause a 3-fold increase of urine output in rats, therefore, some of the reported measurements such as the renal pelvis height and the urinary bladder wall thickness may have been influenced by the chemical restraint procedure. Furthermore, we are aware of the influence of sedation on the size and echogenicity of the spleen of dogs. To the best of our knowledge, similar data regarding the rat spleen are not available; however, because this was a clinical study, clinicians should be aware that the data and images of the spleen could have been affected by sedation.

It is the authors’ belief that the ultrasonography reference measurements and the thorough description of the abdominal organ ultrasonography patterns will enable a deeper insight into the imaging and medical knowledge of this species. The ultrasonographic appearance of the parenchyma of the testicles was remarkably different from the normal ultrasonographic
appearance of the parenchyma of the testicles of dogs and cats.\textsuperscript{10} Clinicians should be aware of this difference and interpret the mottled appearance of the parenchyma as a normal feature and not as a pathological finding. It has been reported that in small rodents the mediastinum testis is inconspicuous and can only be imaged with an optimal scanning angle.\textsuperscript{4} We can hypothesize that the lack of mediastinum visualization could be a specific characteristic of the rat testicle, caused by the low-frequency transducer, or both. Reproductive system tumors are among the most common neoplasias in both male and female rats.\textsuperscript{13,14} The matched anatomic and ultrasonographic images should provide the clinician with reference material for clinically normal rats that is useful in evaluating the genitourinary system.

Significant relationships between kidney height, width, and length and body weight were observed (Table 1). Chronic interstitial nephritis is a common, naturally occurring disease in rats and is usually associated with reduced kidney size.\textsuperscript{12} Infiltrative neoplasms such as lymphoma or leukemia often cause kidney enlargement.\textsuperscript{13,14} The matched anatomic and ultrasonographic images and the reference intervals and the body weight regression coefficients may be useful to clinicians in detecting and interpreting different pathological features in the kidneys.

The ultrasonographic layering of both the stomach and the duodenum was similar to that described in dogs and cats.\textsuperscript{13} Reference values for gastrointestinal tract wall thicknesses have been reported for dogs and cats and were also related to body weight in dogs.\textsuperscript{16} Furthermore, ultrasonography is presently considered a reliable tool with which to diagnose and differentiate enteritis from infiltrative intestinal diseases in pets.\textsuperscript{17} Results of the present study suggested no relationship between body weight and the thicknesses of the layers of the gastrointestinal tract wall in rats. Because some gastrointestinal tract neoplasms\textsuperscript{14,18} and several infectious diseases\textsuperscript{1} occur in rats, the data reported here may help clinicians in the ultrasonographic assessment of the gastrointestinal tract of rats.

The normal ultrasonographic pattern of the liver parenchyma and its similarity in echogenicity with that of the right kidney cortex were apparent (Figure 5). The inversion of the relative echogenicity (with the liver parenchyma becoming more echogenic than the kidney cortex) has high specificity (90%) and sensitivity (100%) in the detection of experimentally induced fatty liver disease in rats.\textsuperscript{19} Moreover, several proliferative and nonproliferative diseases of the hepatobiliary system of rats have been described.\textsuperscript{20}

Pituitary tumors that cause hyperadrenocorticism, adrenal gland hyperplasia,\textsuperscript{21} pheochromcytomas,\textsuperscript{21} and adrenal gland adenomas\textsuperscript{21} have been described as spontaneous diseases in rats; the reference values reported here may provide useful information for veterinarians in this regard. The right adrenal gland is larger than the left in both males and females, with the right adrenal gland significantly larger in females. No difference in shape was detected in relation to side, and no relationship between gland size and body weight was evident, independent of sex.

The figures and the data reported here may provide veterinary clinicians and researchers with useful reference material for ultrasonographic evaluation of common pet rats. However, further studies comparing low versus higher frequency transducer images and reference data may provide further information regarding the normal ultrasonographic appearance of this species.

\textbf{References}


17. Penninck D, Smeyers B, Webster CR, et al. Diagnostic value of


