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Standardization of Radiographic and Ultrasonographic Features and Measurements in Two Small Mammal Pet-Species: Domestic Rat (*Rattus norvegicus*) and Mixed Breed Dwarf Rabbit (*Oryctolagus cuniculus*)

Coordinator of the School: Ch.mo Prof. Gianfranco Gabai

Supervisor :Ch.mo Prof. Alessandro Zotti

PhD student : Dr. Sema Balıkçı Dorotea

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SUMMARY

Exotic animal practice is one of the fastest growing disciplines in veterinary medicine. The importance of small exotic mammals as veterinary patients has continued to grow and in some practices these animals form a significant percentage of the clientele. At the same time, knowledge regarding the anatomical, physiological, and pathophysiological characteristics of these species has rapidly increased. In addition, the demands by many small exotic mammal owners for quality medical care for their animals has clearly continued to rise. Diagnostic imaging became a fundamental step of the clinical investigation in small mammals as well as in other species such as dogs and cats, and the interpretation of diagnostic images is complex and requires a broad knowledge of anatomy, physiology and pathology of these species. Moreover, an accurate interpretation of diagnostic images depends directly on the differentiation between normal and abnormal anatomy in order to achieve a correct diagnosis. In this PhD thesis, the diagnostic imaging features of some small mammal species such as domestic rat and rabbit are described. The main aims of the work presented in this PhD thesis are to provide practical atlases matching the normal gross and cross-sectional anatomy with the normal ultrasonographic features of domestic rat, to describe the normal radiographic anatomy of the rat and rabbit abdomen and to provide the most common abdominal reference ultrasonographic and radiographic measurements for domestic rat and radiographic abdominal reference values for rabbit species.

This PhD thesis is composed of three chapters. Chapter 1 includes a general introduction to small mammal species and a review of small mammal diagnostic imaging. Chapter 2 comprises the largest part of this thesis and provides some useful anatomical and diagnostic imaging references along with the most valuable radiographic and ultrasonographic abdominal reference measurements in domestic rat while Chapter 3 provides a comprehensive description of the abdominal radiographic features, including the most common abdominal reference measurements in mixed breed dwarf rabbits.

In **Chapter 1**, a general introduction to small mammal species, current state of exotic pet practice, some taxonomic and general characteristics of domestic rat and rabbit species

are introduced. Moreover, the relationship between anatomy and diagnostic imaging is described and finally, a review to small mammal diagnostic imaging is presented.

Chapter 2 includes a review to the diagnostic imaging of domestic rat. This part of the thesis focused on the description of the normal ultasonographic and radiographic features of rat abdomen, including the most common abdominal reference measurements obtained by ultrasonography and radiography. Moreover, a comparison study of kidney measures using ultrasonography and radiography was carried out to see if accurate radiological methods could be used as an alternative in the evaluation of kidney size in rats.

The outline of Chapter 2 was developed as radiographic (relevant skeleton, thorax and abdomen) and ultrasonographic (abdomen) features including some abdominal reference values, kidney measures comparison study (using ultrasonography and radiography) and heart size assessment in domestic rat. The radiograhic part includes the description of the normal radiographic anatomy of the rat abdomen along with providing the measures of kidneys from ventrodorsal views, caudoventral extent of liver, the vertebral heart score (VHS) and caudal vena cava diameter from right lateral views taking into consideration the body weight and sex effect. Whole body ventrodorsal and right lateral radiographs were taken using a high detail screen-film combination.

The ultrasonographic part focuses on the description of the normal ultrasonographic features of the abdomen in the domestic rat. The normal gross and cross sectional anatomy are matched with the normal ultrasonographic features of the rat abdomen. For the anatomic comparisons, four rat cadavers were used in this study. Gross anatomic dissections were performed in two rats (1 male and 1 female) within 24 hours. Other two completely frozen rats (1 male and 1 female) were sectioned into 3mm slices along the transverse plane. All anatomical structures have been identified and labelled with the aid of available literature in the anatomical images and then matched on the corresponding ultrasonographic images.

Moreover, normal reference ultrasonographic measurements of the kidneys, adrenal glands, hypogastric lymph node; wall thickness of urinary bladder, stomach -saccus cecus, fundus, pylorus-, cecum and duodenum are reported.

Based on comparison of kidney measures study, both radiography and ultrasonography appear valuable options for measurement of kidneys in rats.

In **Chapter 3,** a comprehensive description of the normal abdominal radiographic features, including the most common abdominal reference measurements (kidneys, liver,

colon diameter, ventral displacement of kidneys and colon from vertebral column) in clinically healthy dwarf rabbits are provided. The rabbits have a relatively light and fragile skeleton which makes them predisposed to lumbar fractures, vertebral spondylosis, scoliosis and lordosis and these conditions might affect the second lumbar vertebra (L2) which is used as a radiographic anatomical landmark to calculate the ratio for kidney length and width in rabbits. Therefore, the fifth lumbar vertebra (L5) is investigated whether could be used instead of L2 to calculate kidney ratio. As it is suggested in previous studies, L5 could be a reference for assessment of kidney size. Based on the present study results, it might be accurate to use any of those (L2 or L5) vertebrae for ratios in view of anatomical or pathological changes that might affect L2. Furthermore, this Chapter includes a kidney measures comparison study using radiography and ultrasonography in dwarf rabbits. The results of this part of the study provide a valuable outcome in which these measures could be treated interchangeably and one reference range can be used for both. This might particularly be helpful when the ultrasonographic equipment is not readily available in general practice or when the practitioner is unfamiliar with ultrasound machine or ultrasound anatomy of this species. So, an alternative might be to use the radiographic kidney measurements in dwarf rabbits.

To conclude, this PhD thesis provides useful information on the diagnostic imaging in particular radiography and ultrasonography in domestic rats. The existing gaps in the medical imaging in domestic pet rats could be filled in this regard. Moreover, this study provides information on the abdominal radiographic anatomy and some useful measurements of the dwarf rabbit which may be helpful for the veterinary clinician who is dealing with this species.

RIASSUNTO

La clinica per animali esotici è una delle discipline a più rapida crescita in medicina veterinaria. L'importanza dei piccoli mammiferi esotici come pazienti veterinari ha continuato a crescere e in alcuni casi questi animali costituiscono una percentuale significativa della clientela. Allo stesso tempo, le conoscenze anatomiche, fisiologiche e fisiopatologiche di queste specie sono rapidamente aumentate. Inoltre, la richiesta da parte di molti proprietari di piccoli mammiferi esotici di cure mediche di qualità per i loro animali ha chiaramente continuato ad aumentare. La diagnostica per immagini è diventata uno strumento fondamentale nell'indagine clinica nei piccoli mammiferi, proprio come in cani e gatti. L'interpretazione delle immagini diagnostiche è però complesso e richiede un'approfondita conoscenza dell'anatomia, fisiologia e patologia di queste specie. Inoltre, da una corretta interpretazione delle immagini diagnostiche dipende direttamente la differenziazione tra anatomia normale e anormale al fine di ottenere una corretta diagnosi. Nella presente tesi di dottorato, sono descritte le caratteristiche in diagnostica per immagini di alcuni piccoli mammiferi quali il ratto domestico e coniglio. I principali obiettivi del lavoro presentato sono: fornire dei pratici atlanti che affianchino le immagini di anatomia macroscopica normale e le sezioni trasversali con le normali caratteristiche ecografiche nel ratto domestico; descrivere l'anatomia radiografica normale dell'addome di ratto e coniglio; fornire i riferimenti ecografici addominale più utili e le misurazioni radiografiche per il ratto domestico e i valori radiografici addominali di riferimento per il coniglio.

Questa tesi di dottorato si compone di tre capitoli. Il Capitolo 1 consiste in un'introduzione generale sui piccoli mammiferi e una revisione della diagnostica per immagini nei piccoli mammiferi. Il Capitolo 2 costituisce la maggior parte di questa tesi e fornisce alcuni utili valori di riferimento anatomici e diagnostici insieme alle più importanti misurazioni radiografiche ed ecografiche addominali nel ratto domestico. Il Capitolo 3 fornisce una descrizione completa delle caratteristiche radiografiche addominali e le misurazioni addominale di riferimento più utili nel coniglio nano (razza incrocio).

Il **Capitolo 1** inizia con un'introduzione generale sulle specie di piccoli mammiferi esotici, seguono una descrizione dello stato attuale della pratica clinica per animali

esotici, alcune caratteristiche tassonomiche e generali delle specie ratto e coniglio. Inoltre, vengono descritti i rapporti tra anatomia e diagnostica per immagini e viene presentata una breve revisione sulla diagnostica per immagini nei piccoli mammiferi.

Il **Capitolo 2** comprende una revisione sulla diagnostica per immagini nel ratto domestico. Questa parte della tesi è incentrata sulla descrizione delle normali caratteristiche ecografiche e radiografiche dell'addome nel ratto, comprese le misure di riferimento addominali più comuni. Inoltre, è stato effettuato uno studio di confronto tra le misurazioni ecografiche e radiografiche del rene per verificare se metodi radiologici accurati potrebbero essere utilizzati come alternativa nella valutazione delle dimensioni renale nei ratti. Il Capitolo 2 si sviluppa descrivendo le caratteristiche radiografiche (scheletro, torace e addome) ed ecografiche (addome) nel ratto, con alcuni valori di riferimento addominali, seguono uno studio comparativo delle misurazioni del rene (ecografiche e radiografiche) e una valutazione delle dimensioni cardiache nel ratto domestico. La parte radiografica comprende la descrizione dell'anatomia radiografica normale dell'addome in ratto e fornisce le misure renali in proiezione ventrodorsale, l'estensione caudoventrale del fegato, il vertebral heart score (VHS) e il diametro della vena cava caudale in veduta laterale destra prendendo in considerazione l'effetto di peso corporeo e sesso. Per queste analisi sono state scattate delle radiografie dell'intero corpo dei soggetti con una combinazione schermo-pellicola ad elevato dettaglio (proiezioni ventrodorsale e laterale destro). La parte ecografica si concentra sulla descrizione delle caratteristiche ecografiche normali dell'addome nel ratto domestico. L'anatomia macroscopica normale e le sezione trasversale vengono abbinate alle caratteristiche ecografiche dell'addome normale del ratto.

Per questi studi di confronti anatomici, sono stati utilizzati quattro cadaveri di ratto. Le dissezioni anatomiche sono state effettuate su due ratti (1 maschio e 1 femmina) entro 24 ore. Altri due ratti (1 maschio e 1 femmina) sono stati completamente congelati e sezionati trasversalmente in fette di 3 mm di spessore. Tutte le strutture anatomiche sono state identificate con l'ausilio della letteratura disponibile e sono state poi abbinate alle corrispondenti immagini ecografiche. Inoltre, sono stati misurati i normali valori di riferimento ecografici di reni, ghiandole surrenali, linfonodi ipogastrici, spessore della parete della vescica urinaria, stomaco -saccus, fondo, piloro-, cieco e duodeno.

Sulla base dello studio di confronto delle misurazioni renali radiografiche ed ecografiche, è risultato che entrambe le metodiche diagnostiche possono essere utilizzate per una corretta valutazione delle dimensioni renali nel ratto.

Nel **Capitolo 3**, viene fornita una descrizione completa delle caratteristiche radiografiche normali dell'addome in conigli nani clinicamente sani, comprese le misurazioni di riferimento addominali più comuni (reni, fegato, diametro del colon, spostamento ventrale dei reni e del colon dalla colonna vertebrale).

I conigli hanno uno scheletro relativamente leggero e fragile che li rende predisposti a fratture lombari, spondilosi vertebrale, scoliosi e lordosi. Queste condizioni possono coinvolgere la seconda vertebra lombare (L2) che viene comunemente utilizzata come riferimento anatomico radiografico per calcolare il rapporto della lunghezza e larghezza del rene nei conigli. Pertanto, si è voluto valutare se L5 potesse essere usato al posto di L2 come descritto in studi precedenti svolti su altre specie. Sulla base dei risultati del presente studio, utilizzare L5 per calcolare i rapporti dimensionali dei reni potrebbe essere corretto qualora L2 fosse coinvolta da cambiamenti anatomici o patologici.

Inoltre, questo capitolo fornisce i dati relativi al confronto tra le misurazioni renali radiografiche ed ecografiche nel coniglio nano e i relativi valori di riferimento. Dallo studio è risultato che queste due metodiche diagnostiche potrebbero essere utilizzate in modo intercambiabile nella valutazione delle dimensioni renali. Ciò può essere particolarmente utile quando l'apparecchio ecografico non è disponibile nella struttura veterinaria o quando il medico non ha familiarità con la macchina ecografia o la diagnostica per immagini di queste specie.

Per concludere, questa tesi di dottorato fornisce innanzitutto informazioni utili sulla diagnostica per immagini, in particolare radiografia ed ecografia, nel ratto domestico che possono in parte colmare le lacune esistenti in questa disciplina e riguardo a questa specie. Inoltre, questo studio fornisce informazioni sull'anatomia radiografica addominale normale e alcune misurazioni di riferimento nel coniglio nano che possono essere utili per il clinico veterinario che si occupa nella pratica di questa specie.

CHAPTER 1

General Introduction

1.1 Companion animal species

- **Small exotic mammals**

The term ‘exotic animal medicine’ has often been used about those animals that are not dogs, cats, horses or farm animals and is commonly applied only to certain pet species of rabbits and rodents, reptiles and birds. The term ‘exotic’ is defined by several veterinary dictionaries as ‘‘an animal that is not indigenous to a location where it currently lives’’ (Redrobe 2008a).

In the last decade, small exotic mammals namely ferrets, rabbits, and rodents including chinchillas, guinea pigs, mice, rats, hamsters, and gerbils have become more popular and are more frequently kept as pets. These new pets can be categorized in many different ways, including listing them by popularity or by taxonomy (i.e. rodentia, lagomorpha) or alternatively they can be classified by their alimentary habits herbivores, omnivores, carnivores, insectivores.

In addition to those exotic pets mentioned above, there are several other species of small mammals which kept as pets but are much less frequently seen in practice. These less popular pets are the African pygmy hedgehog, the sugar glider, the prairie dog, and the degu. For various reasons (e.g. diet, nocturnal activities, social needs), these pets have not become as popular as other small-mammal pets (Rosenthal 2006).

Historically, the focus on small mammals in veterinary medicine was not individual animal medicine but was colony medicine as seen in laboratory animals (Rosenthal 2006).

Over the last 25 years, the situation has gradually changed: small exotic mammals are being widely kept as pets and not only as research, food, or hunting animals (Rosenthal 2006). Even more, in a recent research, Niemi (2013) suggested to consider laboratory animals as individual veterinary patients and treat them such without compromising the scientific aims of their use (Niemi 2013). In addition, the importance of small exotic mammals as veterinary patients has continued to grow and in some practices these animals form a significant percentage of the clientele. At the same time, knowledge regarding the anatomical, physiological, and pathophysiological characteristics of these species has rapidly increased. In addition, the demands by many small exotic mammal owners for quality medical care for their animals has clearly continued to rise (Reese 2011).

- **Current state of exotic pet practice**

Exotic animal practice is one of the fastest growing disciplines in veterinary medicine (Mayer and Martin 2005). A survey conducted in 2006 reported a dramatic increase in the percentage of households owning exotic pets (Lennox 2006). Veterinarians interested in exotic animal practice have a clear advantage in the increased popularity of these non-conventional pets (Fiskett 2005). Moreover, as a consequence of the increased number of exotic pets, the interest of the veterinarians in exotic animals medicine grew together with the scientific literature concerning these species. Furthermore, also medical standards for exotic pets have steadily improved although they have not attained the level of traditional pet animal practice (Mayer and Martin 2005).

The latter consideration might find an explanation in the constant change in popularity of one exotic species over another. In fact, with this trend-effected popularity it results difficult to give an appropriate emphasis on each species in a traditional comparative medicine course. Nevertheless, the recent formation of The Association of Exotic Mammal Veterinarians, formed in 2000, reflects the importance of having a specialty branch of veterinary medicine dedicated to these new pets (Mayer and Martin 2005).

On the other side, as with the traditional pet species, exotic pet owners span a wide range in their desire to pursue diagnostic and treatment options in caring for their animal requesting and willingly paying for advanced techniques. Therefore, the practice of offering the best possible medical course as an option in every case is the most appropriate approach and this underline, once again, the necessity to have a specialty preparation to deal with exotic pets (Mayer and Martin 2005).

The next paragraphs will summarize the main charactheristics of Rodentia and Lagomorpha orders, and introduce the two species that are the subject of this thesis: the rat and the rabbit.

1.2 Order Rodentia

Rodents comprise the largest mammalian order with over 1800 species (40% of all mammals) and 29 families (Hurst 1999; Sainsbury 2003). Traditionally, the order Rodentia was divided into the suborders Myomorpha, Sciuromorpha and Caviomorpha (Hystricomorpha) based on skull and jaw musculature (Hurst 1999; Nowak 1999a; O'Malley 2005a); however rodents taxonomy is still in evolution. Studies on mandibular anatomy have suggested classifying rodents into just two suborders: Sciurognathi and Hystricognathi (D'Erchia et al. 1996; Keeble 2009; Nowak 1999a; O'Malley 2005a; Vaughan 1986; Wolff and Sherman 2008).

Well-known members of the suborder Sciurognathi include squirrels, chipmunks, prairie dogs, rats, mice, hamsters (Syrian/golden, Siberian/dwarf), and gerbils. Well-known members of the suborder Hystrichognathi include naked mole rats, chinchillas, guinea pigs and degus. Within these species, mouse, rat, gerbil, hamster, guinea pig, chinchilla, and prairie dog are the most common species kept as pets and they are largely popular worldwide (Klaphake 2006; Nowak 1999a).

- **General, anatomic and physiologic characteristics of rodents**

In general rodents have a uniform body structure with various adaptations (Lennox and Bauck 2012). Their small size means rodents have a high ratio of surface area to body weight and consequently a high metabolic rate and energy intake (Hurst 1999).

The word "rodent" is derived from the Latin verb "rodere", which means "to gnaw." Small rodents of the Myomorpha suborder possess a common dental formula: 2(I1/1, C 0/0, M 3/3). The four prominent, incisors are elodont, or continuously growing throughout life, while cheek teeth are anelodont, and do not grow after eruption (O'Malley 2005a; Capello, Gracis, and Lennox 2005). The enamel of most common rodents is white; however, some species may have enamel that is orange to yellow in color. The crowns of the mandibular incisors are longer than the maxillary incisors and may be mistakenly assumed to be overgrown. In general, the crown/length ratio for the upper to lower incisors is approximately 1:3. Many small rodents have bulging eyes and may appear exophthlamic, especially when scruffed. The harderian gland, which lies behind the eyeball, produces lipid- and porphyrin-containing secretions that aid ocular lubrication and play a role in pheromone-mediated behavior. These secretions impart a

red tinge to the tears and fluoresce under ultraviolet light. Normally, the lacrimal secretions are spread over the pelage during daily grooming. However, in stressful situations and in certain disease conditions, there may be an overflow of tears; this can be inaccurately diagnosed as bleeding from the eyes and nose (Lennox and Bauck 2012).

Rodents are monogastric, with many species having a forestomach that is separated from the glandular stomach by a limiting ridge. They have a relatively large cecum and an elongated colon. Most rodents practice some degree of coprophagy. Most common rodent species do not vomit, in part because of the limiting ridge in the stomach; but other factors play a role as well, such as the pressure and strength of the esophageal sphincter and crural sling and the innervation of the diaphragm (Lennox and Bauck 2012; Montedonico et al. 1999; Pickering and Jones 2002). For this reason and because these small mammals have such a high metabolic rate, preoperative fasting is not required or recommended (Lennox and Bauck 2012).

The urinary and reproductive tracts terminate in separate urethral and vaginal orifices in the female. Small rodents are spontaneous ovulators and are polyestrous. Mammary tissue can be extensive in rodents and ranges from over the shoulders to the perianal region. Most female rats and hamsters have 6 pairs of nipples, while gerbils have 4 and mice have 5; however, variations in numbers can be seen (Lennox and Bauck 2012).

The testicles and scrotum are usually large in relation to the overall body size, and the inguinal canal is open, allowing the testicles to pass freely from the abdomen to the scrotum (Keeble 2009; Lennox and Bauck 2012).

Hamsters, rats, and mice possess four front toes and five hind toes, gerbils shows the opposite situation. All rodents have tail: it is longer than the animal’s body in gerbils, rats, and mice; at the contrary, golden hamsters have very short tail, while dwarf hamsters have relatively long tail. Rodents do not pant and have no sweat glands, therefore their ability to withstand high temperatures is limited. Heat dissipation occurs through the ear pinnae and tail. Some smaller rodents may salivate in response to warm temperatures (Lennox and Bauck 2012).

- **The rat: taxonomy and history**

Class: Mammalia
Order: Rodentia
Suborder: Sciurognathi
Family: Muridae
Genus: Rattus
Species: *Rattus norvegicus* and *Rattus rattus* (Keeble 2009).

Rats are grouped into suborder Sciurognathi based on the anatomic and functional differences of the lower jaw (Wolff and Sherman 2008; Keeble 2009). The most common and noted members of genus Rattus are the Norwegian or brown rat (*Rattus norvegicus*) and the black rat (*Rattus rattus*).

The original habitat of the Norwegian or brown rat is Asia (plains of north China and Mongolia) where they lived in burrows. The black rat hails from India and the Malaysian peninsula. In the Middle Ages, the black rat spread to Europe first when trade routes opened from the East. The brown rat followed later, arriving in Europe in the early 18th century when rat baiting became a popular blood sport (Harkness and Wagner 1995).

Following the routes of human migration rats reached the America by the end of the century (Harkness and Wagner 1995). Between the two, brown rat became more successful due to its greater ability to withstand cold and adapt to living in close proximity to humans. Moreover its omnivorous diet meant it could adapt to every urban environment from sewers to buildings (Koolhaas 1999; Sharp and LaRegina 1998).

Beside its spread all around the world that made brown rat one of the most invasive pest, *Rattus norvegicus* was incredibly the first species to be domesticated purely for scientific purposes and it is now the most widely studied experimental animal in biomedical research (O'Malley 2005b). Moreover, *Rattus norvegicus* is currently a common pet and it is considered one of the favourite rodent pets thanks to its larger size and calm nature (Lennox and Bauck 2012).

1.3 Order Lagomorpha

Order Lagomorpha contains two families: the Ochotonidae, which includes the pikas (genus Ochotona), and the Leporidae, which includes rabbits (genus Oryctolagus), hares (genus Lepus) and cottontails (genus Sylvilagus) (Nowak 1999b). Up until the mid-twentieth century the Lagomorphs were classified as a suborder of the order Rodentia but it is now thought that their different dentition and jaw structure makes them more similar to artiodactyls, such as cows and horses (Crossley 2003; Nowak 1999a).

Lagomorphs differ from rodents both in dentition and jaw structure, but they otherwise share many anatomical characteristics and similar behaviours. Unlike rodents they have two rows of upper incisors and the upper maxillary arcades lie further apart than the mandibular arcades, meaning that only one side of the cheek teeth can be occluded at a given time (O'Malley 2005a).

Rabbits differ from hares in having a short gestation period (28–33 days) and giving birth in burrows underground. Hares are generally larger than rabbits, have a gestation period of 40 to 50 days, and give birth over ground to precocious young (Harkness and Wagner 1995; Nowak 1999b; O'Malley 2005a).

- **The rabbit: taxonomy and history**

Class: Mammalia
Order: Lagomorpha
Family: Leporidae
Genus: Oryctolagus
Species: *Oryctolagus cuniculus* (Naff and Craig 2012)

The domestic rabbit (*Oryctolagus cuniculus*) is a descendent of the European wild rabbit (Shively 1979). The genus name is derived from the words orukter (Greek: a tool for digging) and lagos (Greek: a hare) and the species name is derived from cuniculus (Latin: a rabbit or an underground passage) (Vella and Donnely 2011).

Compared with dogs, cats, and ferrets, rabbits are the most recently domesticated small animal species. Perhaps because of their noticed untrainability, lack of natural weapons, and ability to breed in captivity without human assistance or intervention. Following their domestication they were cultivated as a sustainable food source and then, the New

Zealand White ,developed in the early 20th century, (Verhoef-Verhallen 1998) became the model of choice for the acute irritancy test. The New Zealand White breed has since become the breed most commonly used in biomedical research (Naff and Craig 2012).

At various times throughout history, man has regarded the European rabbit as a game species, agricultural commodity, research model, invasive pest, endangered species, and companion animal. Yet despite the European rabbit's familiarity, economic and ecological significance, and scientific importance, its evolutionary history is still debated. The rabbits' legendary reproductive capacity, burrowing behavior, and adaptability to a diverse range of climates and herbivorous diets more than compensate for its small size, appeal to predators, and lack of natural defenses, resulting in their colonization, with varying degrees of success, of every major continent except Antarctica (Naff and Craig 2012).

Rabbits are divided into over 60 fancy breeds and fur breeds. The fur group is divided into normal fur breeds, Rex breeds, and Satin breeds (Vella and Donnely 2011). The normal fur breeds have a coat made up of an undercoat and projecting guard hairs; the Rex breeds have short guard hairs that do not appear above the level of the undercoat; and the Satin breeds have an abnormal hair fiber that produces a sheen (Sandford 1996; Vella and Donnely 2011). Body conformation and ear size vary widely among breeds of rabbits, and rabbit fanciers have coined some unusual terms to describe lagomorph body shape and fall of ear. They refer to the small, chunky body of a dwarf rabbit, like a cobblestone, as cobby; they describe the long, lean body of a Belgian hare as racy; and they often describe giant rabbits as mandolin-shaped because of the high, curved top line over their hindquarters (Williams 1976; Vella and Donnely 2011). Most breeds of rabbits have upright ears, which can be long or short. However, some breeds have soft, pliable ears that hang downward and are incapable of erection which are known as lops (Vella and Donnely 2011).

Rabbits remain popular today in the United States for production of meat and fur, as research animals, and as companion pets (Mitchell 2009). Moreover, there has recently been a marked increase in the popularity of rabbits as companion animals, making these the third most popular mammalian pet in the United Kingdom (Nicholson 2001; Meredith 2006).

- **Similarities to rodents**
  - Acute sense of hearing, smell, and touch;
  - High surface area to body weight – resulting in susceptibility to rapid dehydration and hypoglycemia;
  - Fast metabolism – resulting in high oxygen and food intake;
  - Constantly growing molars in rabbits, guinea pigs, and chinchillas – hence increased incidence of dental problems in these species;
  - Larynx placed high in oropharynx making them obligate nose breathers;
  - Cecotrophy is normal behavior (especially in herbivores) (O'Malley 2005a).

- **General anatomic and physiologic characteristics of rabbit**

In this paragraph the main anatomic and physiologic characteristics of the rabbit are briefly summarized in order to give simple overview on the peculiarities of this species.

Rabbit skin is very delicate compared with that of other exotic pets, dogs or cats. Unless care is taken in clipping fur for surgery or ultrasonography, it is easy to tear or rip the skin. Female rabbits have a large fold of skin over the throat known as a dewlap. Breeding does pull fur from this area to line their nests before kindling. Moist dermatitis often develops in this area. Rabbits do not have footpads, instead coarse fur covers the front and hind toes and metatarsal areas. Rabbits housed on wire floors often develop an ulcerative pododermatitis of this area called sore hocks (Vella and Donnely 2011). Bones of rabbits are relatively fragile compared with their muscle mass. The skeleton represents only 7% to 8% of body weight in rabbits, whereas the skeletal muscles comprise more than 50% of the body weight (Brewer and Cruise 1994; Cruise and Brewer 1994; Vella and Donnely 2011).

All the teeth of rabbits are classified elodont (continuously growing, with no anatomic "roots") and hypsodont (long-crowned). The rabbit dental formula is 2 (I2/1, C0/0, PM3/2, M3/3) = 28 (Vella and Donnely 2011).

The abdominal cavity of rabbits is large. The gastrointestinal (GI) tract has a simple glandular stomach, a long intestinal tract and a large cecum. The cecum is the largest organ in the abdominal cavity and it holds approximately 40% of the GI contents. The stomach serves as a reservoir for ingesta holding approximately 15% of the GI contents and it is rarely empty. The cardia and pylorus are two well-developed structures; the

anatomic arrangement of the cardia and the stomach in rabbit lead to the unability to vomit (Cruise and Brewer 1994; Vella and Donnely 2011).

Gut-associated lymphoid tissue constitutes about 50% of the total lymphoid tissue mass, therefore rabbit spleen is relatively small compared with the spleen in other species (Vella and Donnely 2011).

The thoracic cavity of the rabbit is relatively small in contrast with the abdominal cavity that is large. They have a large thymus occupying the cranial thorax (from ventral to the heart to the thoracic inlet) and it may persist into adult life. The rabbit has a relatively small heart, which accounts for about 0.3% of its body weight. However, the small size of the thorax may paradoxically create the impression of an enlarged heart radiographically (Vella and Donnely 2011).

The serum calcium level of rabbits is unusual because it is not regulated in a narrow range but reflects the level of dietary calcium. Urine is a major route of calcium excretion, which varies directly with the serum calcium level. Whereas the fractional excretion of calcium in most mammals is less than 2%, the range for rabbits is 45% to 60%. Increases in dietary calcium levels directly increase urinary calcium excretion (Vella and Donnely 2011).

1.4 Relationship between anatomy and diagnostic imaging

An image is a likeness or representation of a person or thing. To image is to produce a pictorial representation of a part of the body for diagnostic medical purposes (Schwarz 1993). In the medical professions, diagnostic imaging is the science that produces and records images of structures located within the intact body, to assist in the diagnosis and treatment of disease (Tidwell 1999; Sandhu et al. 2010).

All imaging modalities rely on the interaction of energy with body tissue and the detection and recording of the resulting alterations in signal. The complex mathematical equations involved are converted into a pictorial format and displayed as images for interpretation. This relies on computing power and therefore image quality depends heavily on the equipment available (Tidwell 1999; Sandhu et al. 2010).

Image quality depends on many factors including resolution which is the ability to distinguish between two points. Spatial resolution relates to the distinction between objects according to their physical location while contrast resolution relates to their differences in density (Curry, Dowdey, and Murry 1990).

Anatomy is the science of the physical structure of an animal (Schwarz 1993). In the earlier phases of its development, anatomy signified the cutting apart or disassociation of parts of the body and was purely a descriptive science based on what could be observed with the naked eye. Dissection still remains an important anatomical technique although the introduction of the microscope and other technological advances have allowed development beyond macroscopic or gross anatomy (Ellenport 1975).

Accurate interpretation of diagnostic images depends directly on the differentiation between normal and abnormal anatomy (Nyland, Mattoon, and Wisner 1995). Radiography is currently the most widely available diagnostic imaging modality and requires an understanding of topographic anatomy, the position of various parts of the body relative to each other (Thrall 2013). Computed tomography (CT), Magnetic Resonance Imaging (MRI) and ultrasound are all forms of tomography and therefore depict a section through the body free from superimposition by overlying structures (Tidwell 1999; Thrall 2013). This has altered the way in which anatomical structures are perceived on images (Zook et al. 1981; Assheuer and Sager 1997; Thrall 2013) and interpretation requires a recognition of how the structures should appear in each of these planes (Tidwell 1999). Some illustrations of normal cross-sectional anatomic atlases of the canine, feline, rabbit and other non-pet species have been reported (Assheuer and

Sager 1997; George and Smallwood 1992; Liste et al. 2006; Samii, Biller, and Koblik 1998; Smallwood and George 1993a; Smallwood and George 1993b; Zook et al. 1981; Zook, Hitzelberg, and Bradley 1989; Zotti, Banzato, and Cozzi 2009).

When interpreting diagnostic images, it is often useful to have normal images or specimens available for comparison (Barone et al. 1973; Harcourt-Brown 2003a; Cozzi et al. 2006; Orti, Garcia, and Soriano 2004). Therefore, cadavers have been used for comparison with diagnostic images to determine normal morphological details and sections through a cadaver have been compared with corresponding tomographic images (Zook et al. 1981; George and Smallwood 1992; Smallwood and George 1993a; Tasaki and Westesson 1993; Samii, Biller, and Koblik 1998; Weller et al. 1999; Morrow et al. 2000; Zotti, Banzato, and Cozzi 2009; Chen et al. 2012).

The conclusive step of this comparative examination is that the information must be recorded pictorially using photography before the specimen is discarded (Zook et al. 1981; George and Smallwood 1992) or alternatively a suitable technique must be found to preserve the material allowing re-examination at a later date (Miklošová and Sivrev 1999). This can be achieved in a variety of ways: aquatic formalin solutions have good display, preserving, disinfecting and deodorant qualities and are used in various forms in most anatomical and preparatory laboratories throughout the world (Gillbe 1973; Zook et al. 1981; Miklošová and Sivrev 1999; Morrow et al. 2000); alternatively, material can be frozen at temperatures varying from -20° to -70° (Ström et al. 1988; Rodríguez et al. 2002; Zotti, Banzato, and Cozzi 2009).

1.5 Diagnostic Imaging of Small Mammals

At present time some references describing the anatomy, physiology(O'Malley 2005c; Quesenberry and Carpenter 2011) and pathology (Harcourt-Brown 2003a; Quesenberry and Carpenter 2011; Harkness et al. 2010) of small mammals are available, thus enabling a great increase in the demand for veterinary specialty health services for these species.

In the last years, diagnostic imaging became a common means in the diagnosis and management of diseases also in small mammals (Krautwald-Junghanns et al. 2011). Recent technical advances made possible to routinely use radiography (RX) and ultrasonography (US) in small exotic mammals and to date RX and US are the most common imaging modalities used in small mammals (Redrobe 2001) while more advanced imaging tools, such as computed tomography (CT) and MRI are quickly gained popularity within the scientific research community, veterinary teaching hospitals, and veterinary referral hospitals. However, these imaging options are rarely used by the general practitioners due to the high cost associated with the examination and requirement of generalized anesthesia for absolute immobilization (Fehr 2011).

The most frequent fields of application for imaging techniques in small mammals are in the diagnosis of dental (Crossley et al. 1998; Verstraete 2003; Capello and Gracis 2005; Capello 2008; Capello and Cauduro 2008; Gracis 2008; Lennox 2008) and skeletal diseases (Helmer and Lightfoot 2002; Ritzman and Knapp 2002; Simova-Curd et al. 2008; Johnson 2012; McCullough et al. 2012; Zehnder and Kapatkin 2012; Sasai et al. 2015), cardio-respiratory diseases (Capello and Lennox 2011; Casamian-Sorrosal et al. 2014), gastrointestinal (Hauptman, Tichy, and Knotek 2001; Harcourt-Brown 2003b; Harcourt-Brown 2007a; Huynh and Laloi 2013; Huynh and Pignon 2013; Decubellis and Graham 2013; Graham et al. 2014), uro-genital (Johnson-Delaney 1998; Beregi, Zorn, and Felkai 1999; Beregi et al. 2001; Knotek, Wildnerová, and Jekl 2004; Fisher 2006; Wang et al. 2007; Harcourt-Brown 2007b; Aksoy et al. 2009; Dimitrov 2010; Bean 2013) and adrenal gland disorders (Neuwirth et al. 1993; Gaschen et al. 1998; Kuijten, Schoemaker, and Voorhout 2007). Moreover, diagnostic imaging is also used in detection of pregnancy in small mammal species (Ypsilantis et al. 2009).

Interpretation of the various imaging features is mandatory in such a scenario. However, this does require a thorough knowledge of the normal and pathological imaging aspects of each species studied in the clinical investigation.

At the present time, apart from short introductory chapters in standard companion animal veterinary textbooks, there is a paucity of relevant material available on normal imaging features and on the clinical and diagnostic use of imaging modalities in small exotic mammals. However, the imaging modalities are more focused on RX and US rather than on CT and MRI (Girling 2002; Silverman and Tell 2005; Redrobe 2008b; Krautwald-Junghanns et al. 2011; Fischetti 2012; Capello and Lennox 2013). Conversely, there are a significant number of individual publications regarding the use of imaging modalities on small exotic mammals in the specialized research literature (Kiessling and Pichler 2010; Sandhu et al. 2010). However, the specialized research information mentioned above has only a limited use in the clinical veterinary setting.

1.6 References of CHAPTER 1

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CHAPTER 2

Diagnostic Imaging In Domestic Rat (Rattus norvegicus)

This chapter was adapted from:

Tommaso Banzato; Luca Bellini; Barbara Contiero; Andrea Martin; **Sema Balikçi**; Alessandro Zotti. "Abdominal anatomic features and reference values determined by use of ultrasonography in healthy common rats (*Rattus norvegicus*)" American Journal of Veterinary Research, 2014;75: 67–76.

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2.1 INTRODUCTION

2.1.1 Comparison between diagnostic images and cadaver material

In the past decade, there has been a steady increase in the use of cross-sectional imaging techniques such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI). These imaging modalities have reformed the ability to noninvasively explore the contents of body cavities by depicting structures located within the intact body (Samii, Biller, and Koblik 1998; Sandhu et al. 2010). Therefore body regions must be exposed to allow visual identification for comparison (King 2008).

Accurate interpretation of imaging studies of the thorax and/or abdomen requires a thorough knowledge of the regional cross-sectional or gross anatomy (Samii, Biller, and Koblik 1998; King 2008) and this can be achieved by comparing the images with the corresponding section through a cadaver (Zook et al. 1981; Zook, Hitzelberg, and Bradley 1989; George and Smallwood 1992; Smallwood and George 1993a; Smallwood and George 1993b; Samii, Biller, and Koblik 1998; Weller et al. 1999; Morrow et al. 2000; Zotti, Banzato, and Cozzi 2009).

Cross-sectional anatomical sections of the rabbit neck, thorax and abdomen, canine neck and head and feline thorax and abdomen have been produced by freezing the cadaver material and then slicing it using a bandsaw (Zotti, Banzato, and Cozzi 2009; Zook et al. 1981; Samii, Biller, and Koblik 1998) and compared with transverse CT and MRI images (Zook et al. 1981; Zotti, Banzato, and Cozzi 2009; George and Smallwood 1992; Samii, Biller, and Koblik 1998; Assheuer and Sager 1997). Oblique sections have also been produced for comparison with ultrasound images obtained in the corresponding plane (Weller et al. 1999).

To make the ultrasonogaphy and radiography an effective diagnostic modality in pet rats, normal species-specific data must be characterized. For this reason, we used the cadaver material to provide an atlas of normal gross and cross-sectional anatomy of the rat abdomen along with the comparison with ultrasonographic and radiographic images. In this way, our study could represent a comprehensive and efficient source for practice and research.

2.1.2 Diagnostic imaging in rats

The importance of rats as veterinary patients has continued to grow and in some practices these animals form a significant percentage of the clientele (Reese 2011a). At the same

time, knowledge regarding the anatomical, physiological,(O'Malley 2005a; Lennox and Bauck 2012; Harkness et al. 2010; Laber-Laird, Swindle, and Flecknell 1996) and pathological (Brown and Donnelly 2012; Wesche 2009) characteristics of this species has rapidly increased, thus enabling a great increase in the demand for veterinary specialty health services for rats. In addition, the demands by many rat owners for quality medical care for their animals have even progressed to specific requests for advanced radiographic and ultrasonographic imaging tests (Reese 2011a).

There is a great deal of knowledge about these animals and their use as laboratory research species, but less is known about diagnosing and treating them as individual pets (Evans and Souza 2010). However, apart from short introductory chapters in standard companion animal veterinary textbooks, there is a paucity of relevant material available on the clinical and diagnostic use of imaging modalities in rats compared with other species (Girling 2002; Orr 2002; Capello, Gracis, and Lennox 2005; Silverman and Tell 2005a; Tully 2009; Reese 2011b; Fischetti 2012; Capello and Lennox 2013). Radiography and ultrasonography are the most common imaging modalities used in rats. They are complementary imaging techniques: rarely one technique is used instead of another but more commonly both modalities are used to maximize diagnostic information (Redrobe 2001). Radiography is useful in determining size, shape, and position of organs within the body and is routinely performed on small mammals including rats without complications. The radiographic images provide valuable information about the diverse areas of dental and skeletal disease in rats; moreover it also aid in the diagnosis of respiratory, gastrointestinal and urinary diseases, thereby significantly impacting treatment decisions and prognosis determination in this species (Reese 2011b).

The radiologic description of normal rat anatomy is reported in several textbook and book chapters (Silverman and Tell 2005a; Nautrup 2011; Capello and Lennox 2013). Grossi et al. (2009) have described the technique of upper gastrointestinal series radiological examination using contrast enhanced radiology. In this study the authors have defined the anatomic findings along with position of the esophagus, stomach and duodenum of rats.

Ultrasound imaging is a versatile, well-established, and widely used diagnostic tool in human and veterinary medicine. Along with the advances in ultrasound imaging technology during the last decade, accurate and reliable images of rat hearts (Pawlush et al. 1993; Litwin et al. 1994; Burrell et al. 1996; Forman et al. 1997; Watson et al. 2004; Stein et al. 2007) and other target organs such as kidneys (Winters, McDonald, and Krauter 1997; Wang et al. 2007; Chen et al. 2012), spleen and liver (Chen et al. 2012)

have been published. Ypsilantis et al. (2009) have described the technique and the findings of ultrasonographic examination of the rat uterus for diagnosis of early and midterm pregnancy. However, the publications mentioned above are restricted to the experimental research considering the rat as laboratory animal and not as a pet. For the author's knowledge, there is only one reference in which the ultrasonographic features of the abdomen of domestic rat has been described briefly. However, no reference values of adrenal glands, wall thicknesses of stomach parts, duodenum, cecum or urinary bladder were provided. Moreover, no reference values of hypogastric lymph node was reported (Reese 2011c).

Conversely, there is a significant number of individual publications regarding the use of imaging modalities on laboratory rats in the specialized research literature (Kiessling and Pichler 2010; Sandhu et al. 2010; Chen et al. 2012; Ypsilantis et al. 2009; Grossi et al. 2009; Johnson 2008; Watson et al. 2004; Burrell et al. 1996; Litwin et al. 1994; Pawlush et al. 1993; Wang et al. 2007). Consequently, the information mentioned in the specialized research, on laboratory rats, has only limited use in the clinical veterinary setting (Reese 2011a).

Whereas, more advanced imaging techniques such as computed tomography and magnetic resonance imaging are less commonly utilized in pet rats and they are limited to experimental research due to high cost and necessity of anesthesia (Redrobe 2001).

However, as it is mentioned before there is no comprehensive description of ultrasonographic and radiographic 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. Moreover, there are no reports on plain radiographic kidney size or vertebral heart size in healthy rats.

This PhD project was developed with the main objective to increase and deepen the general knowledge regarding imaging techniques in domestic rat, in particular radiographic and ultrasonographic imaging modalities that are more diffuse in clinical practice.

Since the specialized research information mentioned above has only limited use in the clinical veterinary setting, references provided in this PhD project would provide considerable aid to veterinary radiologists, clinicians and researchers.

2.1.3 Kidney measurements and comparison between radiographic and ultrasonographic kidney dimensions

Renal diseases can occur in domestic pet rats and they are often underdiagnosed (Johnson-Delaney 1998). Chronic progressive nephrosis (Johnson-Delaney 1998), infiltrative neoplasms such as lymphoma or leukemia often cause kidney enlargement (Car, Eng, and Everds 2005; Boorman and Everitt 2005) whereas chronic interstitial nephritis is a common, naturally occurring disease in rats and is usually associated with reduced kidney size (Reese and Hein 2011a).

Radiology and ultrasonography are useful tools in diagnosing these pathological conditions (Johnson-Delaney 1998).

Diagnostic techniques are well described in other pet species: for example, radiographs are useful to identify changes in size, shape and opacity of the kidneys and associated retroperitoneal diseases in dogs and cats (Feeney and Johnston 2007). Ultrasonography has developed as the imaging modality of choice for diagnosing diseases of the urogenital tract in many species, and has been suggested as a useful tool for rats as well (Reese 2011a; Suzuki et al. 1987). Excretory urography using intravenous administration of contrast material has also been reported in rats (Knotek, Wildnerová, and Jekl 2004). However, this latter study did not determine radiographic renal size.

The normal kidney size on survey radiographs has not been defined in rats as it has for other species (Finco et al. 1971; Barrett and Kneller 1972; Shiroma et al. 1999; Eshar, Briscoe, and Mai 2013; Hackendahl and Citino 2005).

The ratio of the kidney length to the length of the second lumbar vertebra (L2) on a ventrodorsal (VD) radiograph is commonly used to evaluate kidney size in dogs, cats, ferrets and other species. The use of ratios was suggested because proportional increase in kidney dimensions offsets longer lengths of L2 which correlate with patient size. Therefore the ratios were considered to be independent of patient's size (Feeney and Johnston 2007; Eshar, Briscoe, and Mai 2013; Hackendahl and Citino 2005; Ackerman 1983).

Kang *et al.* conducted a comparative study of methods to assess human kidney size comparing the actual kidney size with the predicted kidney size obtained by survey radiography, intravenous pyelograms, abdominal ultrasound and spiral computed tomography (Kang et al. 2007). The authors stated that spiral computed tomography was more accurate in determining kidney size than other radiological methods. Moreover, ultrasonography was found to underestimate the kidney size whereas plain radiography

was found to either overestimate or underestimate the kidney size due to magnification effect (Kang et al. 2007). A study comparing ultrasonographic and radiographic measures of the urinary bladder in dogs found that radiographic bladder dimensions were greater than ultrasonographic measures (Atalan, Barr, and Holt 1999a). Similarly, a study comparing prostate dimensions measured using radiographic and ultrasonographic means found that when the effect of magnification was eliminated, there were no significant differences between two imaging modalities (Atalan, Barr, and Holt 1999b). These findings suggested that significant differences between ultrasonographic and radiographic measures of renal size could occur in rats and that further studies were warranted.

Since radiography remains the most widely available and utilized imaging modality in veterinary practice and has the additional advantage of providing images of the entire body of small mammals at once, radiographic reference ranges for rats are desirable (Redrobe 2001).

2.1.4 Radiographic measurement of cardiac size in domestic rats

All acquired and congenital heart diseases diagnosed in humans, dogs and cats can be also found in domestic rats (Nautrup et al. 2011).

Standard radiographic images are an indispensable diagnostic aid in the cardiological evaluation in rats (Nautrup 2011). The results of the radiographic examination of these patients may be the first indication that their health issues are due to either respiratory or cardiac disease. With the aid of radiographic images, a suitable treatment protocol can be determined and treatment success can be monitored by using control radiographic images (Nautrup et al. 2011).

A more objective assessment of the heart size can be done using the so-called vertebral heart score (VHS), which is normally carried out using a lateral radiographic image (Nautrup et al. 2011). In dogs, the VHS has proven to be particularly useful for assessing the heart size and is used clinically as a standard evaluation method (Buchanan and Bücheler 1995). However, while some reports have described the evaluation of cardiac size using VHS in dogs (Buchanan and Bücheler 1995; Bavegems et al. 2005), cats (Litster and Buchanan 2000a; Litster and Buchanan 2000b; Ghadiri et al. 2008), ferrets (Stepien, Benson, and Forrest 1999; Onuma et al. 2009) and rabbits (Onuma et al. 2010; Moarabi et al. 2015), none have provided similar information for rats.

2.1.5 Aims

The aims of the work presented in this chapter were to:

I. Prepare and preserve gross and cross sectional rat anatomical specimens for comparison with the diagnostic images (radiography and ultrasound);

II. Describe the normal radiographic anatomy of the rat abdomen along with providing the measures of kidneys, caudoventral extent of liver, the vertebral heart score (VHS) and caudal vena cava diameter from right lateral views;

III. Provide a comprehensive description of the abdominal ultrasonographic features, including the most common abdominal reference measurements (kidneys, adrenal glands, hypogastric lymph node; wall thickness of urinary bladder, stomach - saccus cecus, fundus, pylorus-, cecum and duodenum) that are typically used during an ultrasonographic examination in pets such as dogs or cats;

IV. Compare the linear dimensions of the kidney measured by ultrasonographic and radiographic methods.

2.2 MATERIALS AND METHODS

2.2.1 Animals

Forty healthy adult domestic pet rats, including 20 males and 20 females, with a mean age of 10 month (range 6-12 months) and a mean body weight of 304.7 ± 59.2 grams (males, 312.1 ± 65.2 g; females, 297.4 ± 53.2 g) were used in this project. The rats were client-owned who voluntarily took part in a survey on the prevalence of subclinical diseases in small exotic mammals and were referred for examination to the Radiology Unit, Department of Animal Medicine, Production and Health, University of Padua.

Although other diagnostic tests (e.g. hematology, urinalysis, ecocardiographic examination) were not performed, the rats were considered healthy based on the following: age at presentation (6 to 12 months), adequate diet history, and no reported or observed clinical disease signs on clinical examination; moreover, each subject underwent whole-body radiography and a complete abdominal ultrasonographic scan. Four of the rats were pregnant, and were discovered during the ultrasonographic examination.

This study was approved by the University of Padua Ethical Committee (protocol No. 41060; 12 July 2010).

2.2.2 Cadaver material

The cadavers of 4 rats (2 males and 2 females; mean ± SD weight, 440 ± 75 g) were 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 (Ketamine, 60 mg/kg, Ketavet 100, Intervet Italia, Segrate (MI), Italy.) and medetomidine (Medetomidine, 100 μg/kg, Domitor, Orion Corp, Espoo, Finland.) administered intramuscularly, and a lethal dose of a mixture of embutramide, mebenzonium iodide, and tetracaine hydrochloride (Tanax, 0.3 mL/kg, Intervet Italia, Segrate (MI), Italy.) was administered intravenously.

2.2.3 Anatomic dissections

The anatomic dissections were carried out at the necropsy room of the Faculty of Veterinary Medicine of the University of Padua.

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 on a plastic support in ventral recumbency with the limbs placed close to the body and then stored in a freezer at –20°C. Once completely frozen, the cadavers were removed from the freezer, separated from their supports and then sectioned along the transverse plane by means of an electric bandsaw. Contiguous 3-mm sagittal slices were obtained, starting at the nose and ending at the anus. Appropriate health and safety measures were observed while using the bandsaw.

Both sides of each slice were cleaned with water, numbered, and photographed on the cranial and caudal surfaces to provide the anatomic images used in the study (a damp cloth was used to remove frosting from the surface of each slice prior to photography). All the sections were then preserved for future reference.

Anatomic references, such as Popesko (1977); Barone (1989); Popesko, Rajtová, and Horak (1992) were used to aid in the identification of the structures. Each anatomic structure was labeled according to the ‘‘Nomina Anatomica Veterinaria, 1992”.

2.2.4 Imaging procedures

Both radiographic and ultrasonographic imaging procedures were held for all animals in Department of Animal Medicine, Production and Health, University of Padua.

The animals were not fasted prior to the examinations. Animals were sedated for both procedures with a combination of medetomidine (100 μg/kg, Domitor, Orion Corp, Espoo, Finland) and butorphanol (2 mg/kg, Dolorex, Intervet Italia, Segrate (MI), Italy) injected intramuscularly in order to:

- cause less stress for the patient;
- provide exactly positioned radiographic images that can be reliably evaluated;
- repeat fewer radiographs are necessary;
- avoid manual fixation (radiation safety).

Subsequent to radiographic examination, the rats were moved to the ultrasound room and placed in dorsal recumbency on an electric heating pad. Hair was shaved prior to ultrasonography.

2.2.4.1 Radiographic examinations

a) Equipment

- ✓ *X-ray facilities:*

  A computed radiography system was used (Kodak point-of-care CR- 360 system Carestream Health, Inc-Rochester, USA).

  0.03 seconds exposure time was used in order to reduce the motion artifact, since the respiratory rate of rats is much higher than in the dog and cat. Relatively low voltage of 45-kVp was used because of the undersized tissue mass of the rat patient results in a low degree of contrast in the radiographic image. To compensate for the low contrast observed in these animals' radiographs, values between 40 kV and 60 kV have proven to be suitable to obtain diagnostic images (Reese and Hein 2011b). A focal film distance (FFD) of 107 cm was used in order to avoid the geometric blur and the proportion of scatter radiation (Reese and Hein 2011b).

- ✓ *Film cassettes and grids:*

  For the examination of rats the film cassette dimension used was 27×34 cm and the same film cassette was utilized for two projections (ventrodorsal, right lateral) dividing it into 2 parts. No scattered radiation grid was used due to the low body weight of the rats. The animals were put directly on the cassette.

- ✓ *Exposure factors and development:*

  Exposure factor values were selected based on the values recommended by other studies (Reese and Hein 2011b; Silverman and Tell 2005b). Therefore a low kV (45 kVp) along with high mAs (6 mAs; 200-mA; 0.03 s) were used in this study. Digital images were assessed on a DICOM workstation. To have a better contrast resolution a magnification, windows/level adjustments were mostly used.

- ✓ *Radiation safety:*

  The general aspects of radiation safety was carried out by wearing of lead-lined protective clothing (e.g. aprons, thyroid shield, gloves). In order to be protected from the radiation exposure, when taking the radiographic images of the rats, an attention was paid to go behind a protective wall.

b) Positioning

The methods of positioning and the projections used for the examination of rats in this study basically followed the same principles as those used in small animal radiology (e.g. dog, cat). For all 40 rats, the whole body survey radiographs were done in right-

sided lateral recumbency (RL) and with the second plane being the ventrodorsal (VD) projection.

For the RL recumbent position, each rat was placed on the cassette in lateral recumbency. The dependent limbs were extended, minimizing superimposition on the thorax and abdomen. The contralateral limbs were similarly extended. The head was fixed in a straight lateral position by gently extending the neck. The tail was extended caudally and taped if necessary.

For the VD projection, each rat was placed on the cassette in dorsal recumbency. The pelvic limbs were symmetrically extended caudally. The thoracic limbs were symmetrically extended cranially. The head was placed in a straight ventrodorsal position.

c) Anatomic reference

Radiographic images of rats in this study were anatomically labeled to coincide with illustrations from A Colour Atlas of the Anatomy of Small Laboratory Animals: Vol. II (Rat, Mouse, and Golden Hamster), authored by (Popesko, Rajtova, and Horak 2002). Individual organs may not always be completely and clearly visualized on radiographs; therefore gross anatomic dissections, provided in this study, have been used as references for the rat. These gross anatomic dissections can be used as general guidelines.

d) Evaluation

All RL and VD radiographs were reviewed and subjectively evaluated. Vertebral and sternal numbers with normal morphology were recorded for each animal.

Concerning the thorax, the vertebral heart size (VHS) was obtained on lateral radiographs. Since ventrodorsal position (VD) was difficult to measure in many animals. Because in the rats the thymus remains present throught the life and it has soft tissue shadow on the cranial side of the heart on radiography (Reese and Hein 2011c). Therefore, only right lateral position (RL) dimensions of the heart were evaluated in this study. Only radiographs in which there was no evidence of rotation of the body were used. Thus, 35 RL radiographs were included to measure the VHS in rats. Long axis (length) (L), short axis (width) (W), sum of L and W (L+W), T5-8 length, ratio of L+W/T5-8 and VHS were measured and compared according to body weight and gender (female vs. male). Measurements obtained in RL view are described below and illustrated in Figure 1.

- The long axis of the heart (L) was measured in millimeters (mm) from the ventral border of bifurcation of the mainstem bronchi to the most distant ventral contour of the cardiac apex;
- The maximal short axis of the heart (W) was measured in millimeters perpendicular to the long axis, between 3rd and 5th ribs;
- L+W: The sum of long axis and short axis of the heart in millimeters;
- T5-8: Total length of 4 vertebrae bodies in millimeters, measured from cranial edge of T5 (fifth thoracic vertebra) to caudal edge of T8 (eighth thoracic vertebra) in RL view;
- Ratio (L+W/ T5-8): sum of length and width measurements compared to T5-8 measurement in RL view. The ratio calculation is adapted from (Stepien, Benson, and Forrest 1999) to evaluate the cardiac size in rats.

Measurements (L and W) were repeated using a previously published method by (Buchanan and Bücheler 1995). Briefly, the number of vertebrae corresponding to the length of the L (mm) and W (mm) from the cranial margin of the 4th thoracic vertebra was provided. The L and W vertebral unit measurements were then added to obtain a total score, vertebral heart score (VHS). The overall size of the heart was thus espressed as total units of vertebral length to the nearest 0.1 vertebrae (Figure 1).

The maximal diameter of the caudal vena cava (cvc) was measured in RL radiographic views.

Concerning the abdomen, to make a more efficient evaluation it was divided in 3 approximate sections: 1) Cranial abdomen, 2) The middle section and 3) Caudal abdomen as suggested by Reese and Hein (2011d). Then, certain organs related to these sections were described and their location was determined in both views (Figure 2 and 3).

Kidney dimensions (length and width) were provided in mm and also as ratios, compared to the body length of second lumbar vertebra (L2) (Figure 4). More information regarding the kidney measurements are provided in the following sections. The caudoventral extent of the liver beyond the costal arch was recorded in mm on RL view (Figure 5) and then, was compared to the L2 length to provide a ratio.

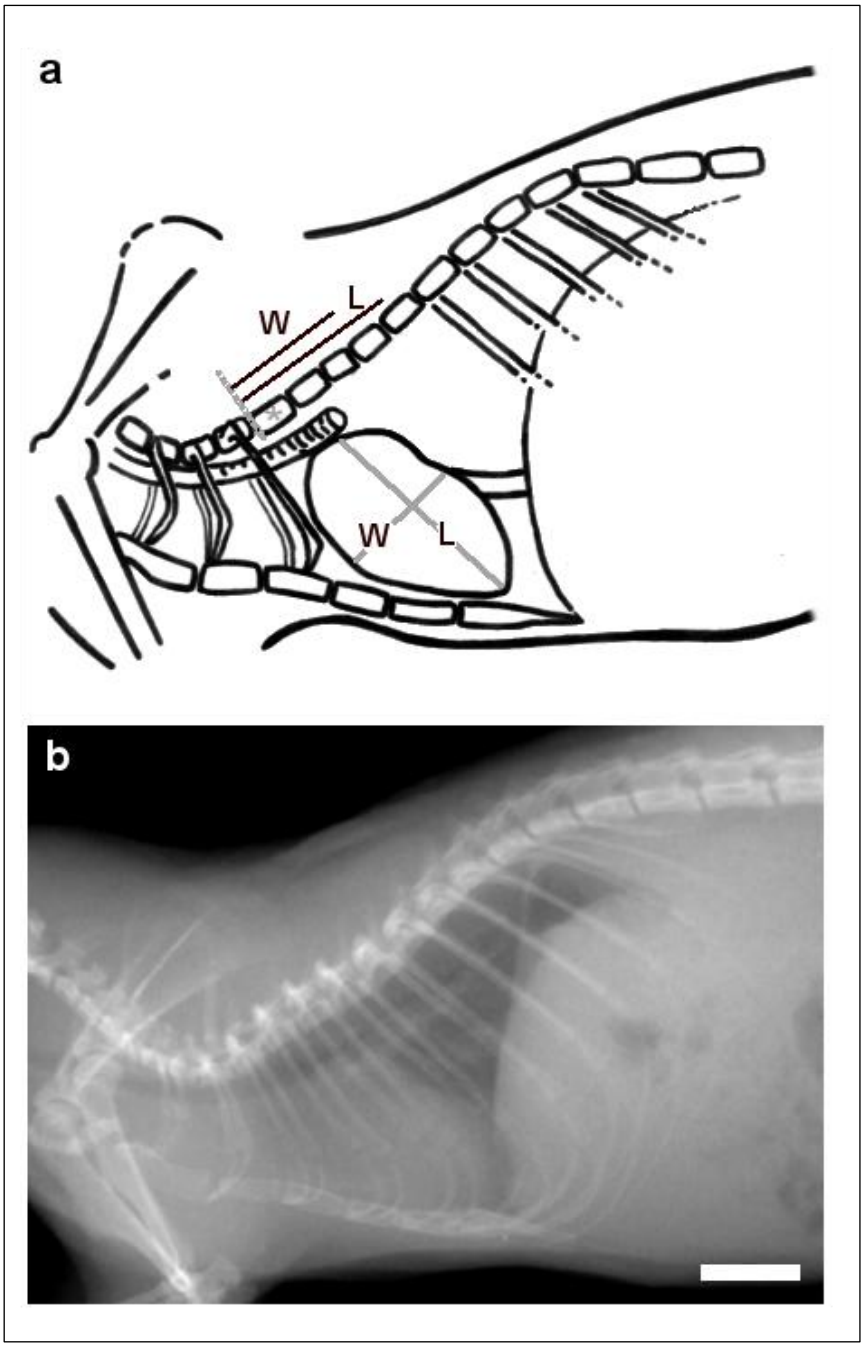

Figure 1(a) Diagram of right lateral view of the thorax of a rat illustrating the vertebral heart size measurement method. The length (L) and width (W) heart measures are transposed onto the vertebral column and recorded as the number of vertebrae beginning with the cranial edge of T4 (*). These values are then added to obtain the vertebral heart size. (b) Lateral radiographic view of a normal rat thorax. Bar, 10 mm.

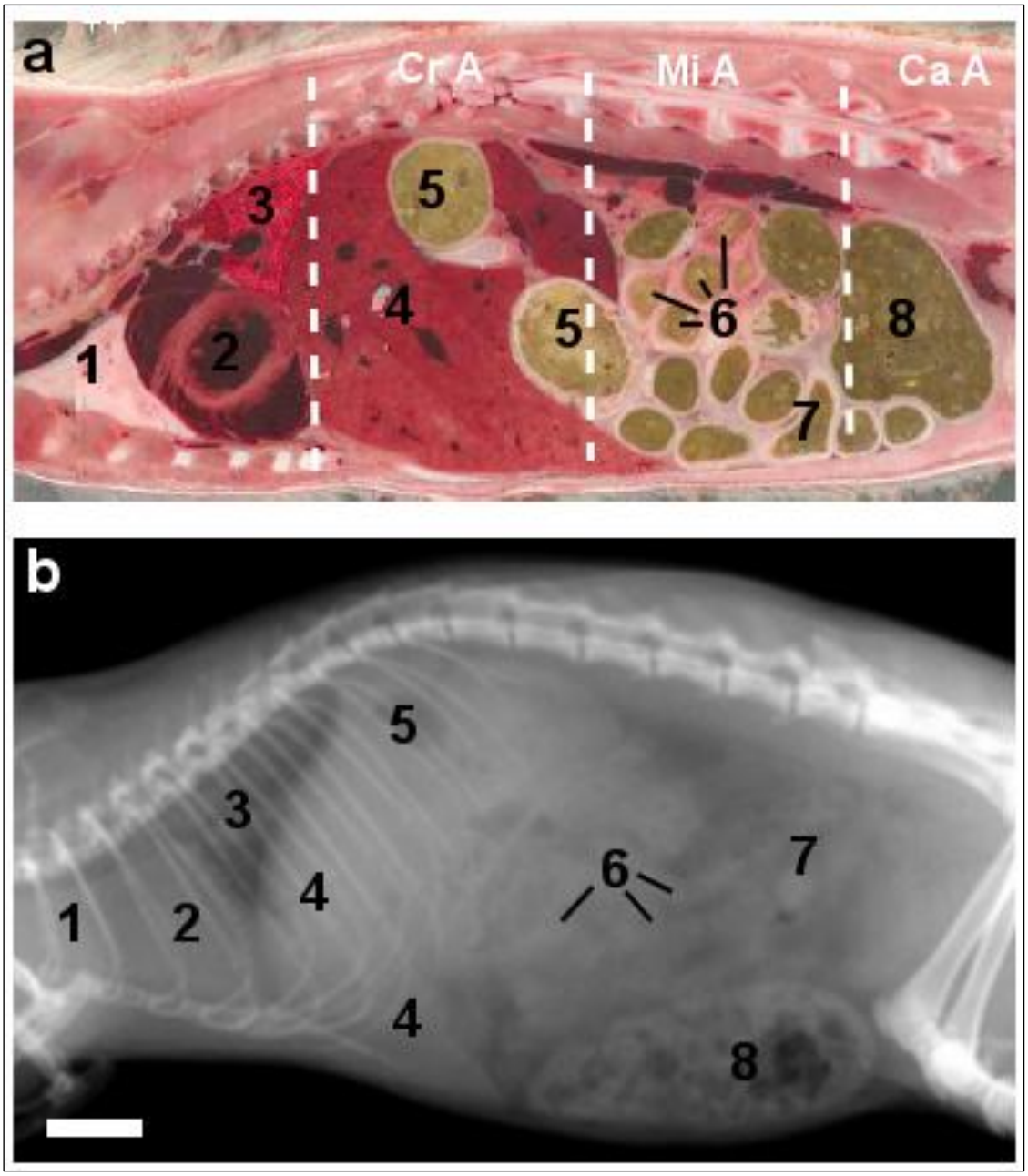

Figure 2 Longitudinal lateral cross section anatomy (a) and right lateral radiograph in a rat (b). Lateral cross section image is 0.5 cm lateral to midline and modified from http://www.invivopharm.com. Note subdivision of the abdomen into cranial, middle and caudal sactions. Notice the caudoventral extension of the liver beyond the costal arch (b). Cr A: cranial abdomen, Mi A: middle abdomen, Ca A: caudal abdomen, 1: thymus, 2: heart, 3: lung, 4: liver, 5: stomach, 6: small intestines, 7: colon, 8: cecum. Bar, 10 mm.

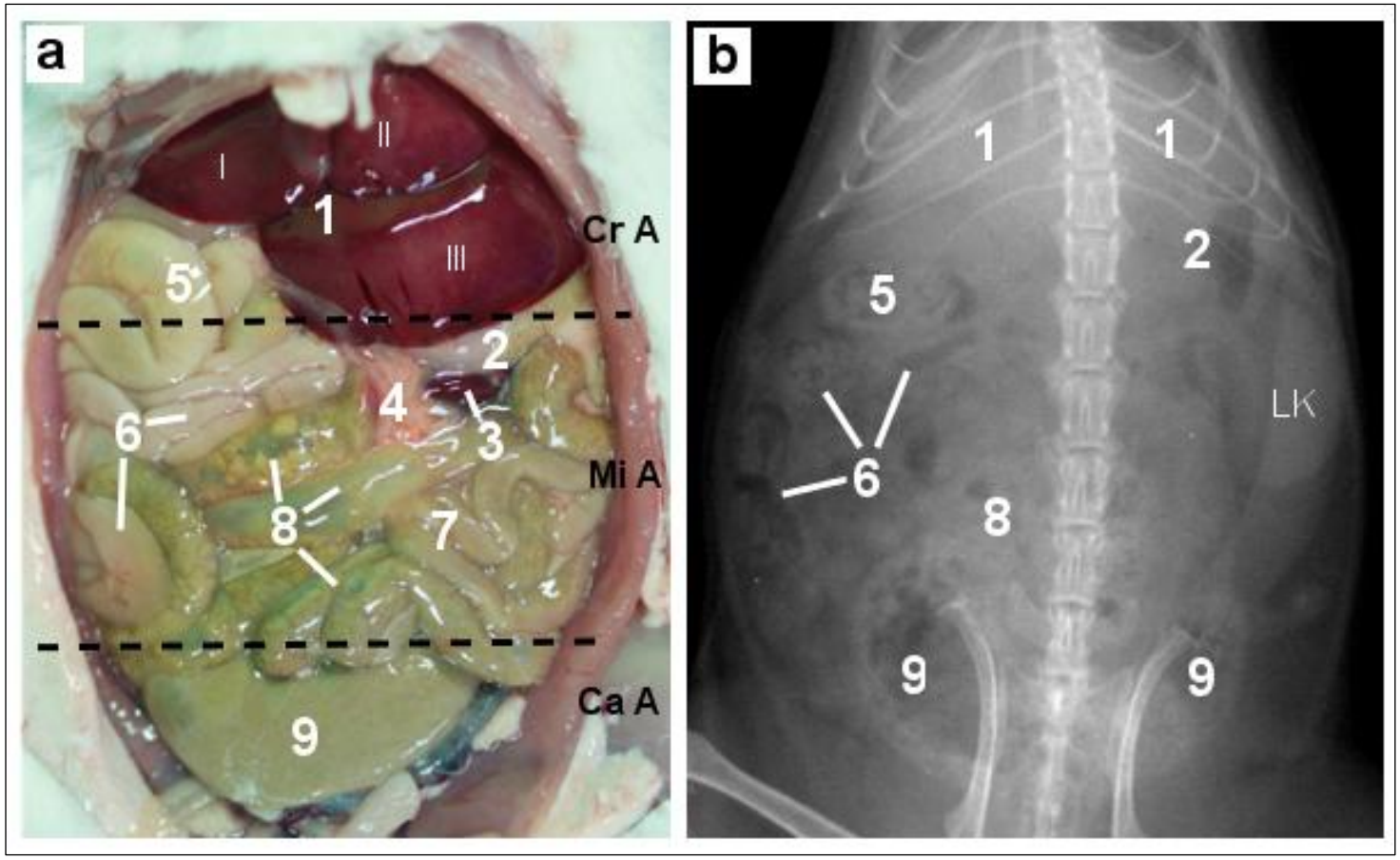

Figure 3 Gross appearance of the abdominal visceral anatomy (a) and ventrodorsal abdominal radiograph in a rat (b). Note subdivision of the abdomen into cranial, middle and caudal sections (a). Cr A: cranial abdomen, Mi A: middle abdomen, Ca A: caudal abdomen, 1: liver (I: right medial lobe, II: left medial lobe, III: left lateral lobe), 2: stomach, 3: spleen, 4: pancreas, 5: duodenum, 6: jejenum, 7: ileum, 8: colon, 9: cecum, LK: left kidney. Bar, 10 mm.

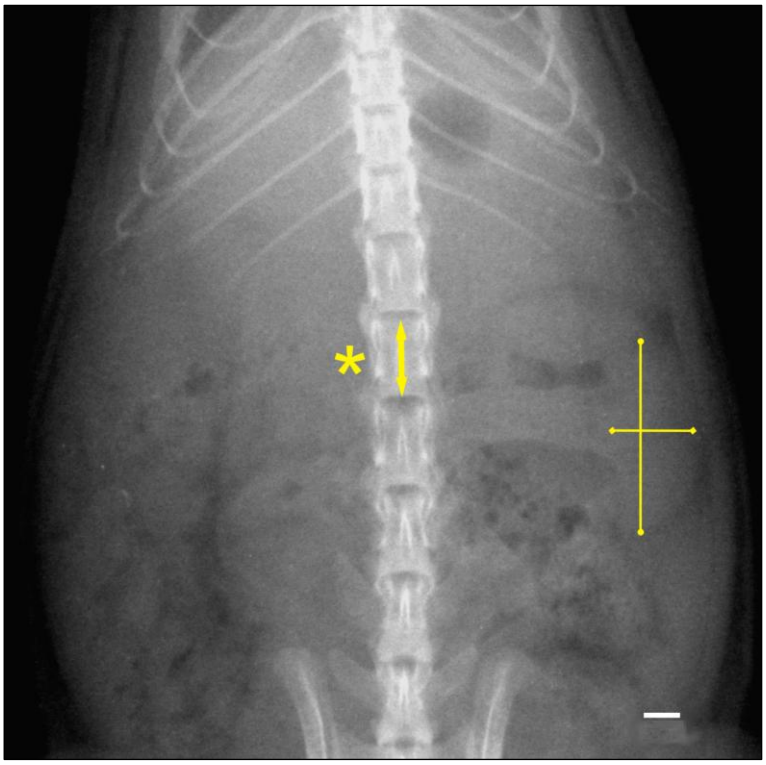

Figure 4 Ventrodorsal (VD) abdominal radiograph in a rat. The image illustrates the measurements performed in the present study: L2 (star), length of L2 (arrow tip), kidney length (round tip), kidney width (diamond tip). Note that the right kidney was not sufficiently visible in this patient due to the presence of adjacent viscera. Bar, 10 mm.

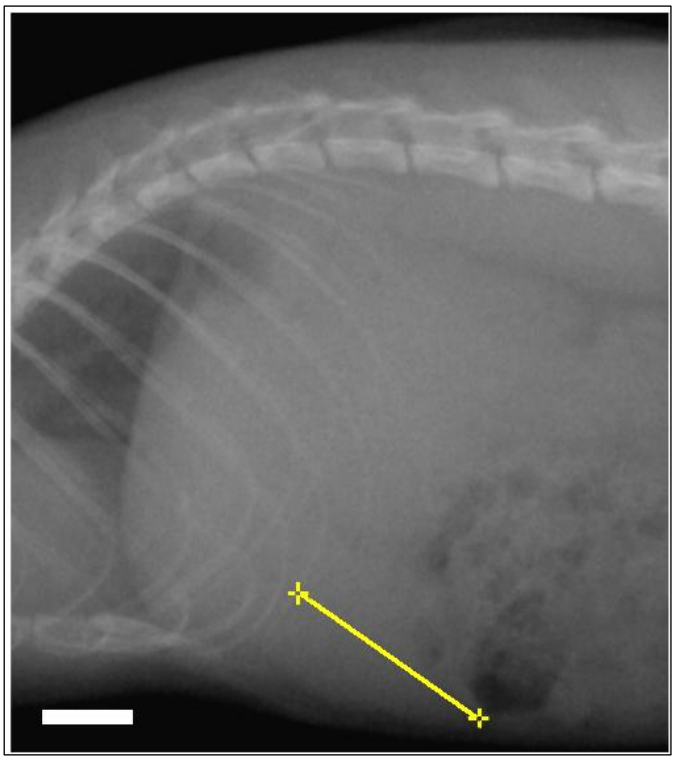

Figure 5 Right lateral radiograph in a rat. The image illustrates the measurement (yellow line) of the caudoventral extension of the liver beyond the costal arch. Bar, 10 mm.

2.2.4.2 Ultrasonographic examinations

Each rat was placed on an electric heating pad (Pet-Mat, Dale Ecotech) in dorsal recumbency, its abdomen was carefully clipped from a point approximately 1 cm cranial to the xyphoid cartilage to the caudal-most part of the pubis, and a generous amount of warmed ultrasound coupling gel (Ecoultragel Pirrone & Co, Italy) was applied to the skin for a better contact of transducer.

The rats were monitored appropriately (rectal temperature, respiratory rate, arterial hemoglobin O2 saturation, pulse rate, palpebral reflex) during the ultrasonographic examination.

An abdominal ultrasonographic examination was performed with a linear-array multi-frequency transducer (4- to 8.5-MHz), operated at 8.5 MHz, connected to a commercial sonographic scanner (Zonare, Zonare Medical Systems Inc, Mountain View, Calif.) that was serviced and checked regularly.

A standoff pad was not used. Duration of each examination was 30 to 45 minutes. The ultrasonographic examination took place in a standardized way for all animals. A systematic abdominal examination beginning with the liver, followed by the left kidney, left adrenal gland, spleen, iliac and hypogastric lymph nodes, urinary bladder, female or male genital tract, right kidney, right adrenal gland, and the gastrointestinaltract (GIT) (duodenum, stomach, colon, cecum) was performed and then pancreas imaging was attempted. To minimize interoperator variability, all ultrasonographic examinations were performed by the same operator.

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;
- The left and right kidneys were evaluated in transverse, parasagittal, and longitudinal scanning planes.

Measurements

The ultrasonographic measurements of kidneys, adrenal glands, hypogastric lymph node, wall thickness of the urinary bladder, saccus cecus of the stomach, fundus, pylorus,

cecum and duodenum were recorded. 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. The urinary bladder wall thickness was measured in the dorsal portion of the urinary bladder. The measurements were calculated using electronic callipers of the ultrasound machine. To obtain the most precise measurements, image magnification was always used.

Figures 6-8 illustrate how the ultrasonographic measurements of kidney length, width and height, renal pelvis height, adrenal and hypogastric lymph node length and the wall thickness of fundus, pylorus, duodenum, colon and urinary bladder were taken.

2.2.5 Kidney measures and comparison between radiographic and ultrasonographic kidney dimensions

- **Ultrasonographic kidney measurements**

The ultrasonographic examination was performed with the animal in dorsal recumbency. Kidney length and width were measured in longitudinal, and the height in parasagittal scan (Figure 6a and b). 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 (Figure 6c). Kidney length was defined as the maximum longitudinal length, the distance between the cranial and caudal poles, and was measured in longitudinal scan plane, whereas kidney width was measured perpendicular to the length, the distance between lateral and medial margins (Figure 6a).

- **Radiographic kidney measurements**

Rats in which one or both kidneys were visible on the radiographs were included. Measurements were performed using a DICOM workstation with electronic calipers (Visbion Ltd, Surrey, UK).

All measurements were performed on the VD view for both kidneys. The kidney length, width and the length of the body of second lumbar vertebra (L2) were recorded. The radiographic kidney length was considered as the maximum distance between cranial and caudal poles of the kidney and the kidney width as a perpendicular axis to the length of the kidney from the most medial margin to the most lateral margin. The length of the body of the L2 was measured from cranial end-plate to the caudal end-plate (Figure 4). A

ratio was calculated by dividing the length or width of the kidney by the length of the body of L2.

- **Comparison between radiographic and ultrasonographic kidney measurements**

The comparison between radiographic and ultrasongraphic kidney measurements was carried out using statistical tests which are explained in detail below.

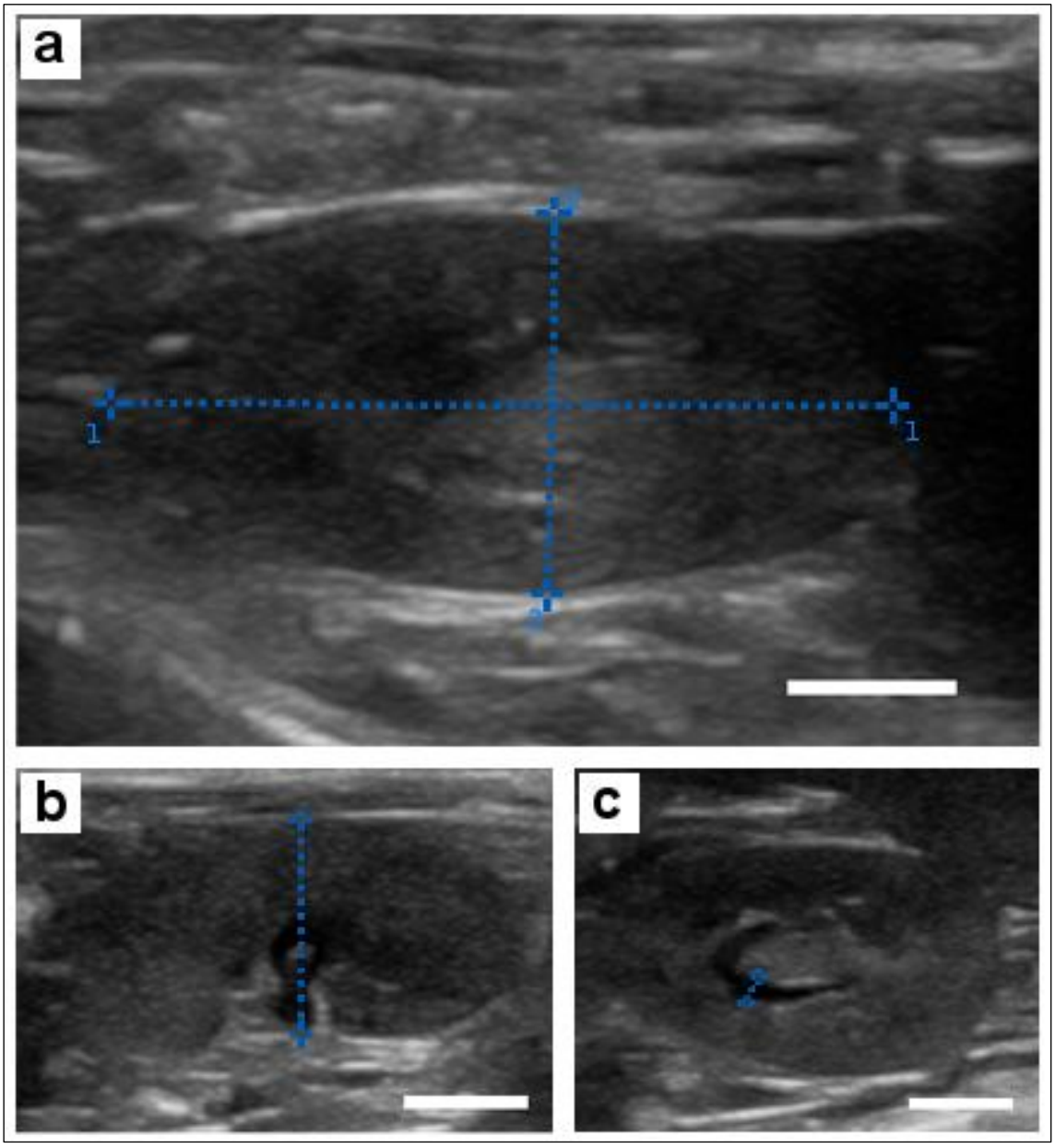

Figure 6 Sonograms of the kidney in a rat. (a) Sagittal plane sonogram of the kidney. Electronic cursors depict the length (1) and the width (2) of the kidney in the rat. (b) Parasagittal plane sonogram of the kidney. Electronic cursor shows the height of the kidney in the rat. (c) Transverse plane sonogram of the kidney. Electronic cursor ilustrates the measurement of renal pelvis height in the rat. Bars, 5 mm.

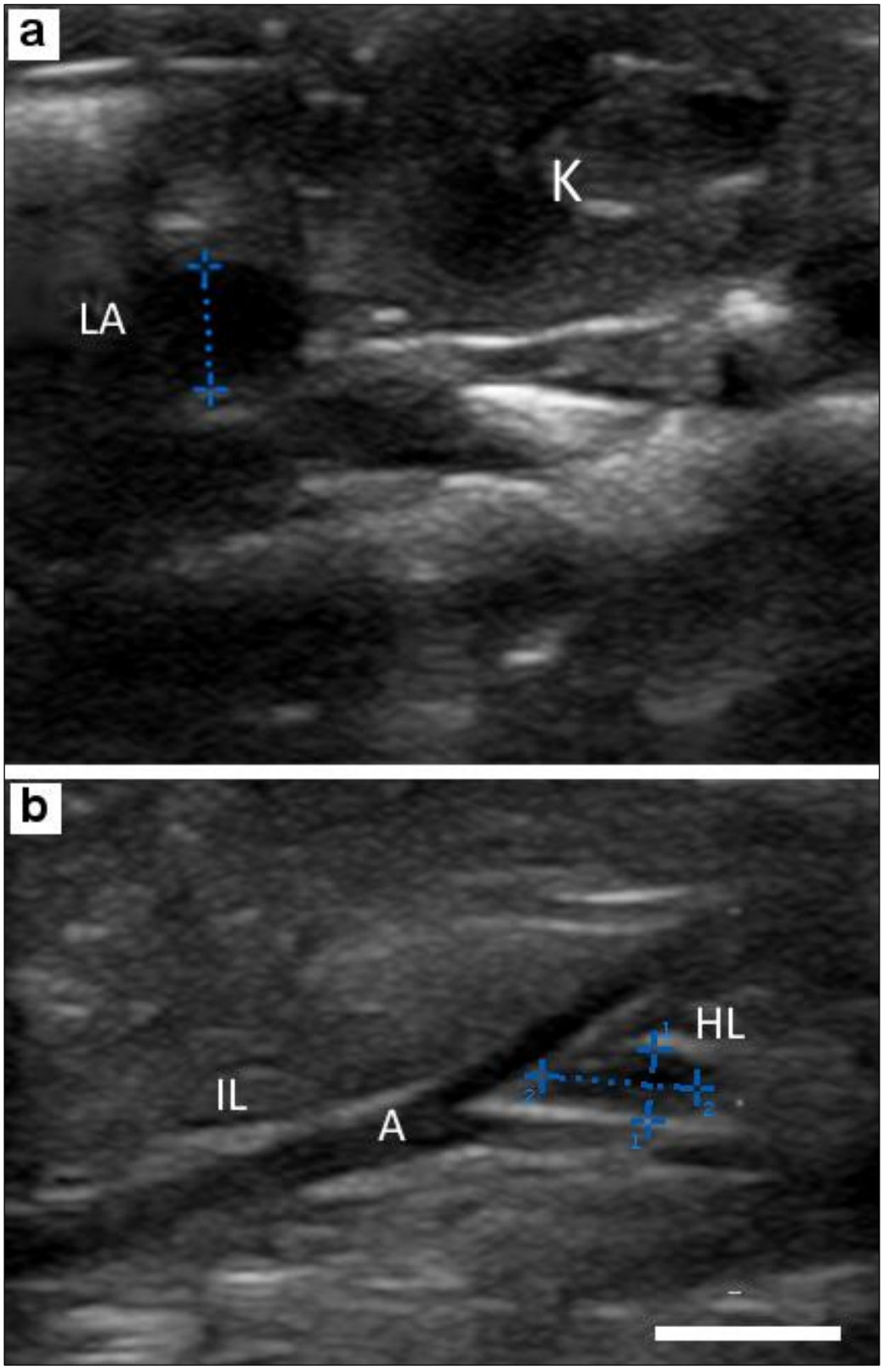

Figure 7(a) Transverse plane sonogram of the adrenal gland in a rat. Electronic cursor shows the dorsoventral diameter of the adrenal gland. LA: left adrenal gland, K: left kidney. (b) Sagittal plane sonogram of the hypogastric lymph node in a rat. Electronic cursors show the width (1) and the length (2) of the hypogastric lymph node in the rat. IL: iliac lymph node, A: aortic bifurcatio, HL: hypogastric lymph node. Bar, 5 mm.

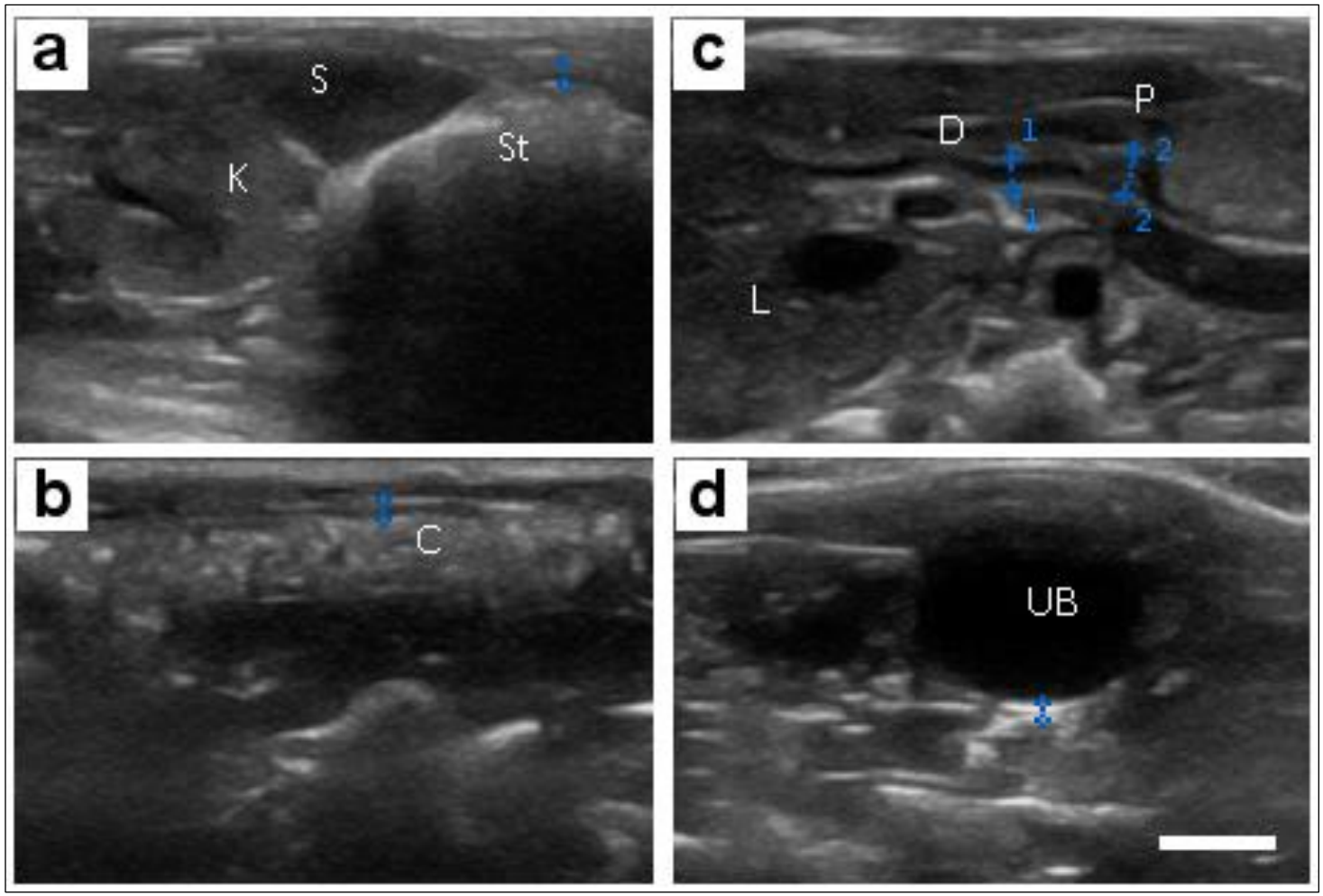

Figure 8 Transverse plane sonograms in a rat. Electronic cursors show the measurements of wall thicknesses of the (a) Stomach fundus (b) cecum (c) duodenum (electronic cursor 1), pylorus (electronic cursor 2) and (d) urinary bladder in a rat. K: left kidney, S: spleen, St: stomach fundus, C: cecum, L: liver, D: duodenum, P: pylorus, UB: urinary bladder. Bar, 5 mm.

2.2.6 Statistical analysis

Statistical analysis of this study was performed to standardize as much as possible some ultrasound and radiographic parameters of reference in order to give them practical use in the veterinary clinic.

- **Statistical analysis for ultrasonographic data**

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 gender (female versus male) and covariate effect of weight. Least squares means (LSMEAN) and standard error of the mean (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 gender (female versus male) and side (right versus left). Statistical analysis was performed with commercially available statistical softwares (MedCalc for Windows, version 12.5, MedCalc Software, Ostend, Belgium and SAS 9.1, SAS Institute Inc, Cary, NC.).

For all comparisons, $P < 0.05$ was considered significant.

- **Statistical analysis for radiographic data**

All data obtained from radiographs were tested for normal distribution using Shapiro-Wilk test analysis and graphic methods such as histogram and Q-Q plot. For the normally distributed data the descriptive statistics used were mean, standard deviation (SD) and 95% confidence limits (CLs) of the mean. In case of non-normal distribution, data were described as median and interquartile range. However, for both normally and non-normally distributed data, the minimum, maximum and reference intervals were calculated. Reference intervals were calculated with parametric method in normally distributed data, whereas in non- normally distributed data, reference intervals were estimated with robust method. The radiographic kidney data, cardiac (L, W, L+W, T5-8, ratio (sum/T5-8) data, except VHS (because it was not normally distributed) and caudal extent of liver and ratio (caudal extent of liver/L2 body length) were analyzed according

to ANCOVA linear model with the fixed effect of gender (females vs males) and covariate effect of weight. LSMEAN and SEM were calculated to compare female versus male values, and F-values were calculated to evaluate the effect of gender and body weight on:

- kidney dimensions (kidney length and width) and ratios obtained by radiography;
- Long and short axis of heart;
- Sum of long and short axis of heart;
- Ratio (L+W/T5-8);
- Caudal vena cava diameter;
- Caudal extent of liver and ratio (caudal extent of liver/L2 body length).

However, for non-normally distributed data (VHS) the non parametric test Mann Whitney was used to compare male versus female values.

Moreover, Spearman correlation coefficient test was run to see the correlation between body weight and kidney size, L2 body length, long axis, short axis of the heart, sum of long and short axis, the ratio (sum/T5-8), VHS, caudal extent of liver, ratio (caudal extent of liver/L2) and caudal vena cava. Furthermore, the Spearman correlation coefficient test was also used to determine whether measured heart size (L, W and sum of L and W) was dependent on skeletal size (T5-8).

Statistical analysis was performed with commercially available statistical softwares: SAS 9.1 (SAS Institute Inc, Cary, NC) and MedCalc for Windows (version 12.5, MedCalc Software, Ostend, Belgium). For all tests, a p-value <0.05 was considered significant.

- **Statistical analysis for comparison of kidney measures**

The paired Student's t-test was used to determine whether there were significant differences between kidney dimensions measured by radiographs or ultrasound and differences between radiographic left versus right kidney sizes. Spearman's rank correlation was used to assess the relationships between kidney length/width measured by ultrasonographic and radiographic methods and correlation between left and right kidney. Also these tests were calculated using SAS 9.1 and MedCalc sofwares and a p-value <0.05 was considered significant.

2.3 RESULTS

2.3.1 Radiographic anatomy of the rat

- **Relevant skeletal system**

All rats had 7 cervical vertebrae, 13 thoracic vertebrae, and 6 lumbar vertebrae. The sacrum consisted mainly of 4 fused segments. Since the tail of the rats was not included in the focus beam, the caudal vertebrae were not determined. The last 2 rib was always floating, except 8 animals (4 males and 4 females) had 3 floating ribs. The sternum consisted of a manubrium sterni, 4 sternebrae and a xiphoid process.

- **Thorax**

The thorax of the rat is very short regarding the abdomen. The precardial section is only the width of 1 to 3 intercostal spaces.

In RL view, the aortic arch and caudal vena cava (CVC) were observed without diffuculty in most animals as radiopaque structures. The CVC had a mean diameter $\pm$ SD of 2.98 ± 0.47 mm, ranging from 2.05 to 3.92 mm (n=28). Statistical results showed that the CVC was highly correlated with body weight (r=0.56, P=0.0016) and the gender did not have significant effect on CVC diameter (P=0.86). However, there was a significant effect of body weight on the CVC diameter (P=0.007). There was weak but statistically significant correlation between CVC diameter and long axis, short axis, long plus short axis of the heart (r=0.39, P=0.03; r=0.38, P=0.04; r=0.44, P=0.01, respectively). In VD view, neither the aorta nor CVC were identified.

In RL view, the precardial lung region had low radiodensity. The pulmonary vasculature was better defined in RL view rather than VD view. In VD view, the apex of the heart was positioned on the left side of the animal. In RL view, the angle between longitudinal axis of the heart and sternum was small and the heart apex was for the most part in close contact with sternum. The heart of all the rats used in this study appeared to project between the 3rd and 5th intercostal spaces.

Heart size assessment

The reference values for clinically normal radiographic cardiac size were recorded for 35 of the 40 rats used in our studies. Table 1 summarizes all the data collected for this objective: long axis (length) (L) and short axis (width) (W) of the heart, sum of long and

short axis (L+W), vertebral heart size (VHS), T5-8 vertebrae bodies length and L+W/T5-8 ratio. The reference intervals were 15.94-22.88 mm for L, 10.19-16.1 mm for W, 26.6-38.68 mm for L+W, 6.3-8.5 vertebral units for VHS, 14.52-18.16 mm for T5-8 vertebrae bodies length and 1.72-2.26 for L+W/T5-8 ratio (Table 1).

ANCOVA linear model test was run to evaluate the effect of gender and body weight on the cardiac data and also skeletal size (T5-8 vertebrae bodies length). ANCOVA test results showed that there was no significant effect of the gender on body weight, L, W, L+W, T5-8 vertebrae bodies length and L+W/T5-8 vertebrae bodies length ratio values (for all $P>0.05$). Moreover, the Mann-Whitney test showed no significant effect of the gender on VHS values ($P=0.8$) (Table 2).

However, the body weight had significant effect on L, W, L+W and T5-8 vertebrae bodies length values ($P=0.0009$ for L, $P<0.0001$ for the other values), whereas the body weight did not have significant effect on L+W/T5-8 vertebrae bodies length ratio ($P=0.26$).

Spearman correlation coefficient test was run to investigate the relationship between body weight and L, W, L+W, T5-8 vertebrae bodies length, VHS and the ratio (L+W/T5-8 vertebrae bodies length). Moreover, the relationship between the skeletal size and L and W was determined using Spearman correlation coefficient test. Based on the results, significant positive correlations were detected between body weight and L ($r=0.6$, $P=0.0001$), W ($r=0.7$, $P<0.0001$), L+W ($r=0.6$, $P<0.0001$) and T5-8 ($r=0.9$, $P<0.0001$). However, there was no significant correlation between body weight and L+W/T5-8 ratio ($r=0.2$, $P=0.1$) or body weight and VHS ($r=0.2$, $P=0.22$) whereas L and W were highly correlated with T5-8 ($r=0.6$, $P<0.0001$; $r=0.7$, $P<0.0001$ respectively).

Table 1. Measurements from right lateral radiographs in rats.

Variable	No	Mean±*SD*[a] Median (q1-q3)[c]	95% CL[b]	Range (min-max)	Reference interval
Length (mm)	35	19.41±1.76	18.8-20.02	15.2-22.81	15.94-22.88
Width (mm)	35	13.14±1.5	12.62-13.66	10.97-17.83	10.19-16.1
Length+Width (mm)	35	32.64±3.08	31.58-33.7	26.6-41.26	26.6-38.68
T5-8 length (mm)	35	16.33±0.92	16.01-16.65	14.32-18.39	14.52-18.16
Ratio	35	1.99±0.13	1.94-2.04	1.72-2.42	1.72-2.26
VHS * (vertebral units)	35	7.4 (7.2-8)	7.36-7.72	6.4-9.2	6.3-8.5

SD[a]= Standard deviation
CL[b]= Confidence limit
(q1-q3)[c]= q1; inferior interquartile, q3; superior interquartile
VHS*= Not normally distributed.

Table 2. Comparison of weight and measurements obtained from right lateral radiographs of normal male (No=19) and female (No=16) rats.

Variable	Males	Females	p-value
Weight (mg)	311.26±66.83[†]	286.87±42.97	0.21
Length (mm)	19.72±1.82	19.03±1.68	0.59
Width (mm)	13.16±1.69	13.12±1.3	0.28
Length+Width (mm)	33.04±3.47	32.15±2.56	0.99
T5-8 length (mm)	16.52±1.04	16.11±0.74	0.66
Ratio	1.99±0.14	1.99±0.13	0.83
VHS * (vertebral units)	7.4 (7.2-8)	7.35 (7.2-7.8)	0.8

[†]Values are represented as mean ± standard deviation for normally distributed data and median (inferior-superior quartile) for non normally distributed data. P values < 0.05 were considered significant (ANCOVA linear model).
VHS * = Not normally distributed. The effect of gender was analyzed by the Mann Whitney non parametric test.

- **Abdomen**

The abdomen was divided in three sections as described in "materials and methods-section". The organs located in each section (cranial, middle and caudal) were identified and topographically described.

Cranial abdomen

The liver and the stomach situated in the cranial abdomen. In VD view, in 26 of 40 radiographs the stomach was intrathoracic, between 11^{th} and 12^{th} intercostal space. However, in 14 of 40 radiographs it was distended beyond the costal arch up to the 1^{st} lumbar vertebral body.The contour of the stomach was pear-shaped in VD view and oval in RL view.

The liver had homogeneous soft-tissue shadow and was positioned mainly on the right side of the body (Figure 2) where it extended up to 20.13 mm caudal to the last rib with its sharp caudoventral tip in right lateral view (for females the mean$\pm$SD 14.78$\pm$3.22 and for males the mean$\pm$SD 17.39$\pm$2.20). A ratio was calculated dividing the length of caudal extension of the liver to second lumbar vertebra. The mean$\pm$SD of the ratio was 2.39$\pm$0.48, with a range of 1.5-3.23 (for females the mean$\pm$SD 2.19$\pm$0.56 and for males the mean$\pm$SD 2.55$\pm$0.33). However, its exact caudal margin on ventrodorsal view could not clearly be determined. Based on the ANCOVA test results, there was a significant effect of gender on caudal extent of liver linear measure and the calculated ratio(for both P=0.01) (Figure 9), however there was no significant effect of body weight on caudal extent of liver linear measure or ratio (P=0.3 and P=0.17, respectively).

Middle abdomen

The pancreas, kidneys, adrenal glands, spleen, ovaries, small and large intestines and cecum are located in the middle section of the abdomen. The pancreas, adrenal glands and the ovaries were not identified in any of the radiographic projections performed. The spleen was discernible in only 5 VD radiographs whereas it was not identified in any RL radiographs.

The right kidney was located between T13 and L1 vertebrae in most subjects and was conspicuous in 19 of 40 animals in VD view. The left kidney was in most animals situated between L2 and L3 vertebrae in both RL and VD view. In 24 of 40 radiographs, the left kidney was discernibile in VD view. Kidney dimensions (length and width) were provided in millimeters and also as ratio (kidney length and width /L2 body length). More information regarding the kidney measurements are provided in the further sections

(2.3.3. Kidney measurements). The small intestines were located mainly on the right side of the abdomen. In RL view, they were mostly caudal to the liver and in contact with the ventral abdominal wall. They were distinguished from large intestines by their small diameter and curved appearance; however, it was not possible to discern the components of small intestine. Nevertheless, in VD view they were situated in the middle of abdomen and in some instances they reached the caudal section of the abdomen. The large intestine was inconspicuous in most radiographs, with the exception of descending colon which was discernible in 10 of 40 radiographs when it was filled with hard faeces. The cecum was often well visualized in both views. In RL view, it was located beyond the small intestines or the caudoventral edge of the liver. In VD view, the cecum had a "C" shape and lied on the mid-caudal left abdomen.

Caudal abdomen

The caudal section of the abdomen includes the urinary bladder, the uterus in the female or the prostate and seminal vesicles in male subjects and the rectum.The uterus or the prostate and seminal vesicles were not identified in any radiographical projection. The urinary bladder was conspicuous in 12 of 40 right lateral radiographs and it displayed as a radiopaque pear-shaped structure; however, it was not identified in any VD view. The rectum was discernible in 10 of 40 lateral radiographs when it was filled with hard faeces.

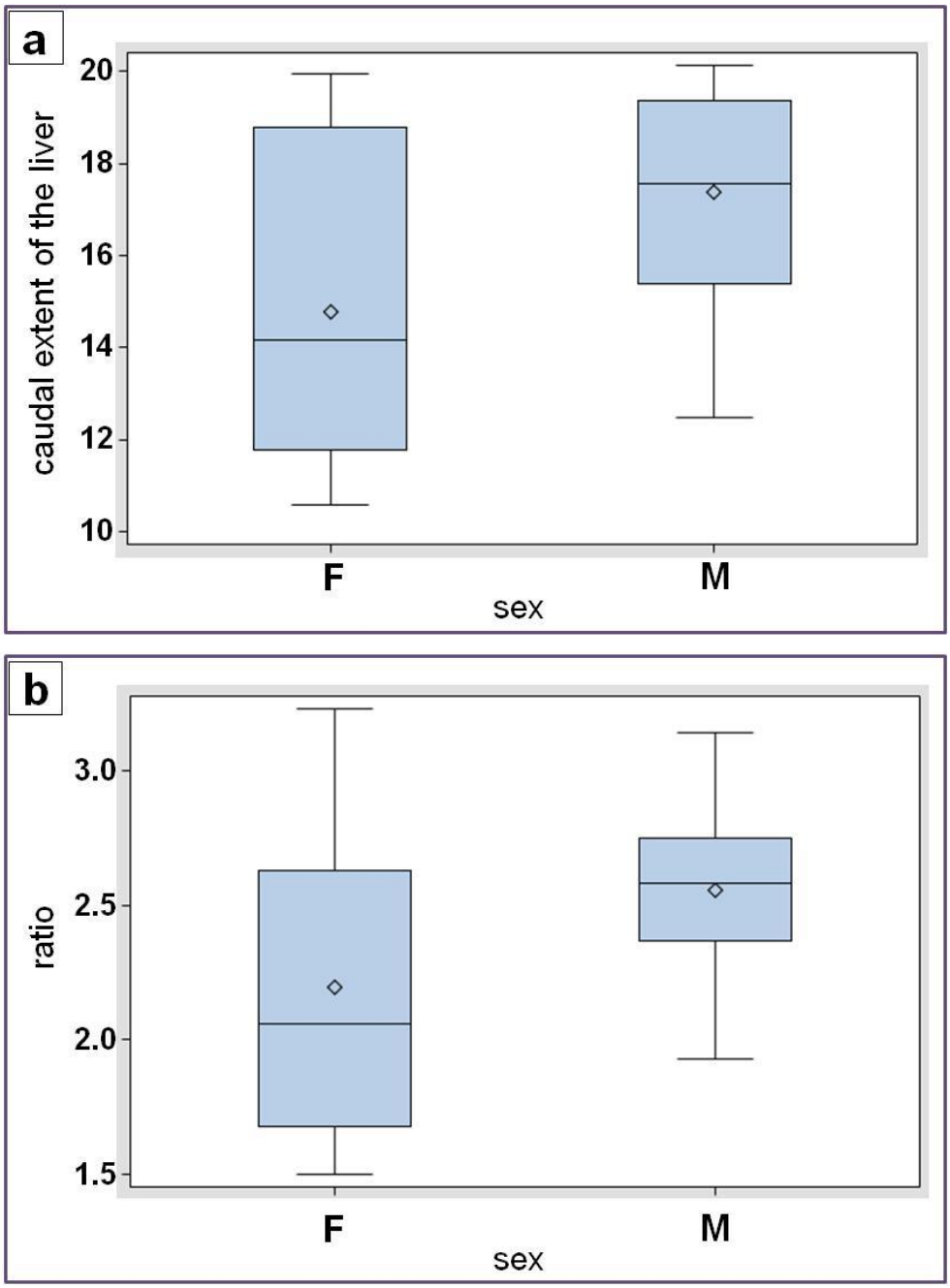

Figure 9 Comparison of radiographic caudal extent of liver linear (mm) and ratio measurements based on sex in 34 rats. Caudal extent of the liver linear measures (a) and ratios (caudal extent liver length/L2) (b) differed significantly between males (n=19) and females (n=15) (for both P=0.01). Whiskers represent range, boxes represent interquartile range, diamonds within circles represent mean and crossline represents median value.

2.3.2 Ultrasonographic anatomy of the abdomen

- **Reproductive system**

Female genital tract

The ultrasonographic examination of the female genital tract was initiated from the caudal aspect of the body of the animal. The analysis included the vagina, the cervix, the uterine horns and the ovaries.The vagina lied primarily within the pelvic cavity and so was not possible to image sonographically. The cervix and the caudal portion of the uterine horns were visible in all females. In nonpregnant females, the cranial portion of the uterine horns was difficult to identify because of the overlying gastrointestinal tract structures, whereas in pregnant females the uterus was clearly visible. The uterine horns (left and right uterine horns) were hypoechoic tube-like structures which can be identified in the transverse plane lying between the urinary bladder and rectum (Figure 10c). The ovaries were identified as triangular hypoechoic structures located caudally to the caudal pole of the kidneys, on the dorsolateral abdominal wall. The follicles were in some cases visible as tiny round anechoic structures in the ovarian parenchyma (Figure 10e).

Male genital tract

Ultrasonographic imaging on the male genital tract enables to evaluate the testicles, the epididymis and the seminal vesicles.

Ultrasonographically, the testicles had a homogeneous, mottled parenchyma of medium echogenicity. The mediastinum testis was inconspicuous. The head, body, and tail of the epididymis could be imaged in a longitudinal scan. It projects as hypoechoic structure laying dorsally from a pole of the testicle to the other (Figure 11).

The seminal vesicles were identified dorsally or laterally to the urinary bladder as two symmetric, ovoid, mildly echoic structures (Figure 12). The coagulating glands are located medially to the seminal vesicles, however it was not possible to distinguish the seminal vesicles from the coagulating glands in the ultrasonographic images with any certainty. The rat prostate is located caudally in the pelvis and thus was not identified ultrasonographically in any studied subject.

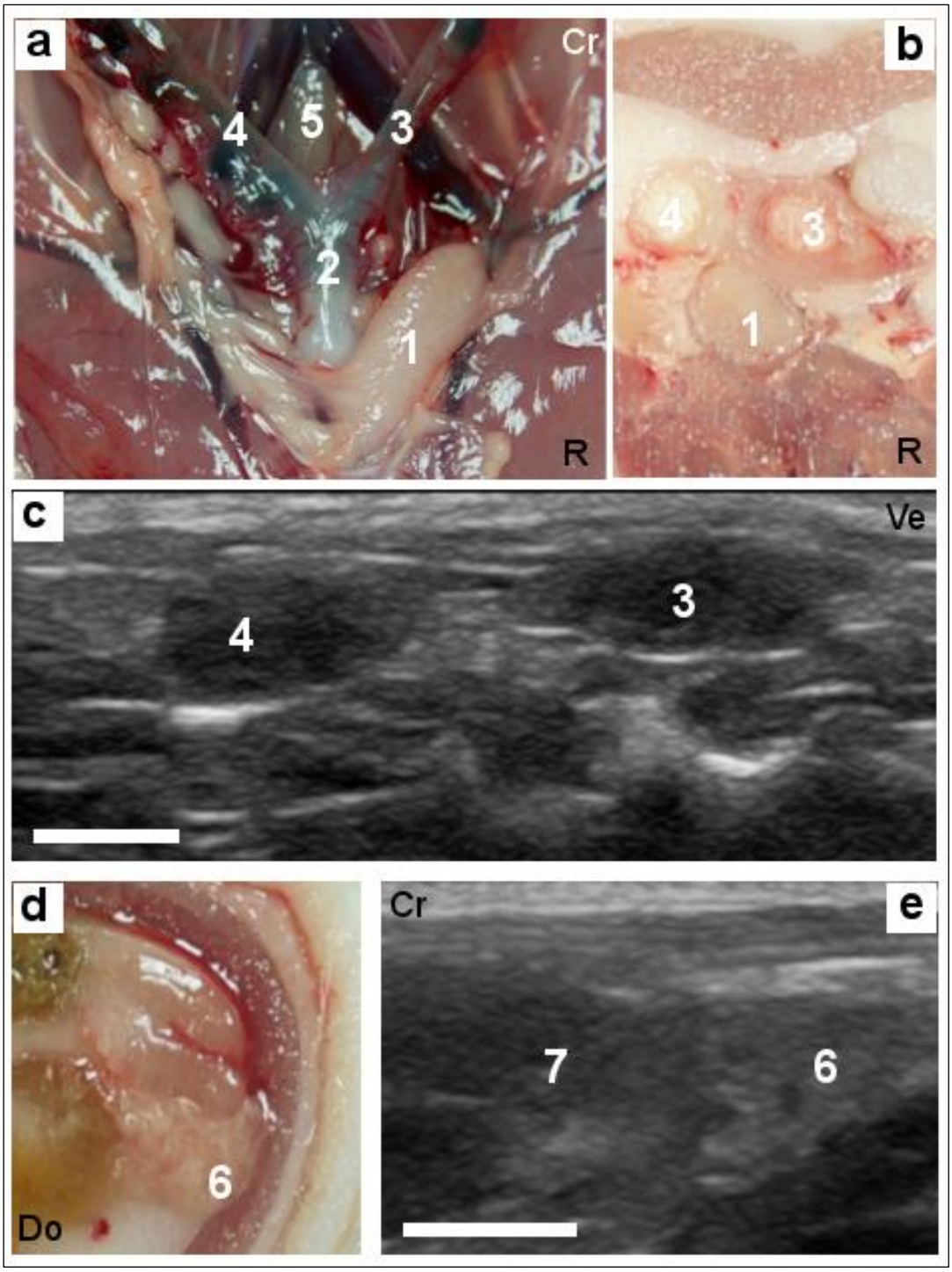

Figure 10 Photographs and ultrasonographic images of the uterus and ovaries in a rat. In the photographs, notice the gross appearance of the uterine horns after removal of the overlying organs (a) and in cross section (b). The ultrasonographic image of the uterine horns (c) was obtained in a transverse scanning plane. The ovary was sectioned transversely for the gross anatomic features (d). The ultrasonographic image of the ovary (with follicles) (e) was obtained in a parasagittal scanning plane. 1: urinary bladder, 2: cervix, 3: right uterine horn, 4. Left uterine horn, 5: hypogastric lymph node, 6: left ovary, 7: caudal pole, Cr: Cranial, Ve: ventral, Do: dorsal, R: right. Bars, 5 mm.

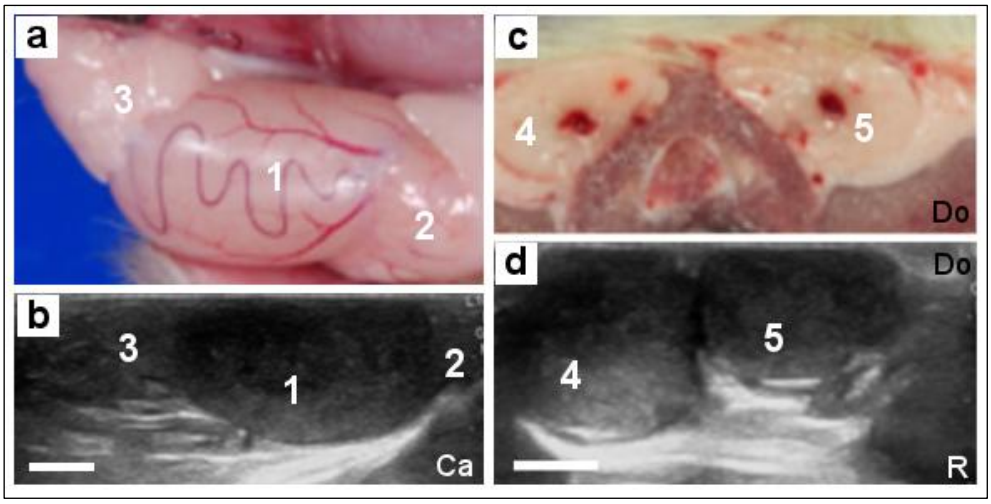

Figure 11 Photographs and ultrasonographic images of the testicles in a rat. In the photographs, notice the gross appearance of one intact testicle (a) and the right and left testicles in cross section (c). The ultrasonographic images were obtained in a longitudinal scanning plane (b) and a transverse scanning plane (d). 1: testicle, 2: head of the epididymis, 3: tail of the epididymis, 4: left testicle, 5: right testicle, Ca: Caudal, Do: Dorsal, R: Right. Bars, 5 mm.

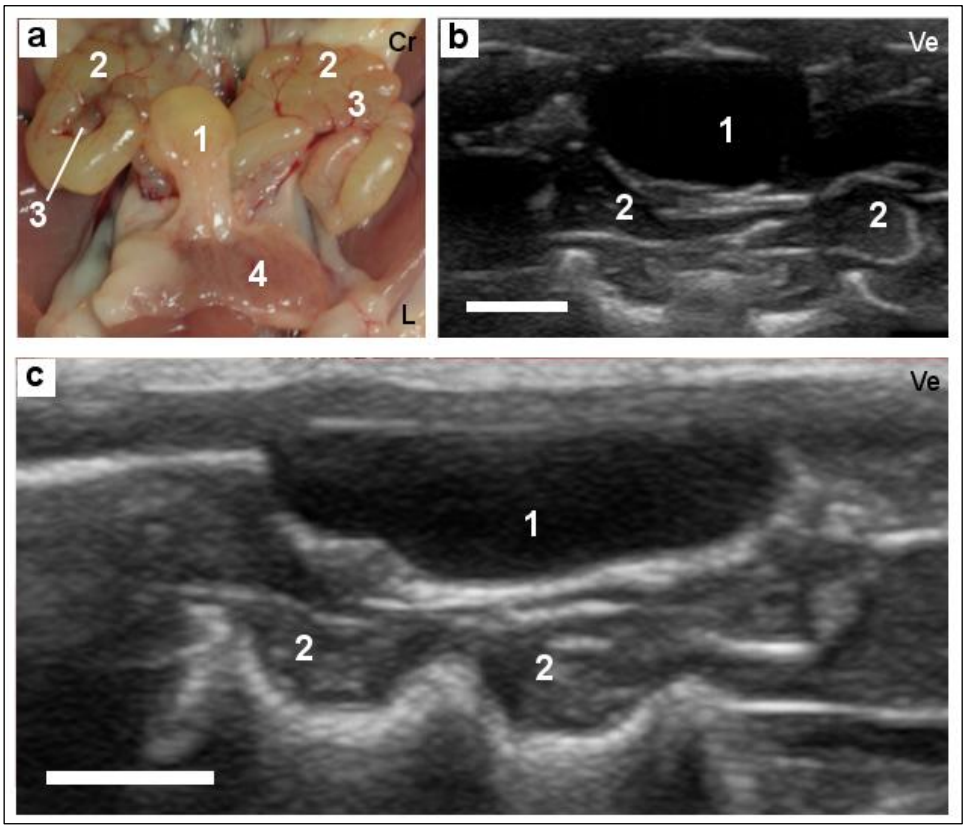

Figure 12 Photograph and ultrasonographic images of the seminal vesicles and coagulating glands in a rat. (a) The photograph shows the gross appearance of seminal vesicles and coagulating glands after removal of the overlying organs. The ultrasonographic images were obtained in a transverse scanning plane (b) and a sagittal plane (c). 1: urinary bladder, 2: seminal vesicles, 3: coagulating glands, 4: prostate, Cr: cranial, Ve: ventral, L: left. Bars, 5 mm.

- **Urinary tract**

Ultrasonographical examination of urinary tract in rats included the kidneys, the ureter, the urinary bladder and the urethra.

The kidneys in rats are smooth with a single hilus and have a similar structure to that found in dogs and cats (Figure 13).

Both kidneys were ultrasonographically evaluated in all studied subjects. Kidneys were imaged in the sagittal (longitudinal), parasagittal and transverse planes (Figure 13). The cortex and the medulla were clearly distinguished in the transverse scan, with the cortex being of medium echogenicity and the medulla being hypoechoic, whereas no distinction was possible in longitudinal and parasagittal scans. The echogenic renal sinus was evident in all scanning planes whereas the hypoechoic renal pelvis was evident in parasagittal and transverse scans , and renal crest (renal papilla) was discernible in transverse scan. The renal papilla was hypoechoic and was surrounded by the horseshoe-shaped renal cortex (Figure 13e). Visualization of the kidneys was often hindered by the overlying voluminous intestinal structures when a ventral approach was used, whereas a lateral approach enabled better visualization. The length, width, height of the kidneys and renal pelvis height in the rats are provided in Table 3. Ultrasonographic imaging of the ureters was not possible in most rats. The urinary bladder was visible only when filled with urine (anechoic) and it was not identified in 8 of the 40 animals examined. The wall of a full urinary bladder appeared as a hypoechoic band defined by a very thin echoic lines on the inner and outer surfaces (Figure 14). The urinary bladder wall thickness reference values were determined in 32 rats during the ultrasonographic examination and they are provided in Table 3. The urethra was not visualized in any subjects.

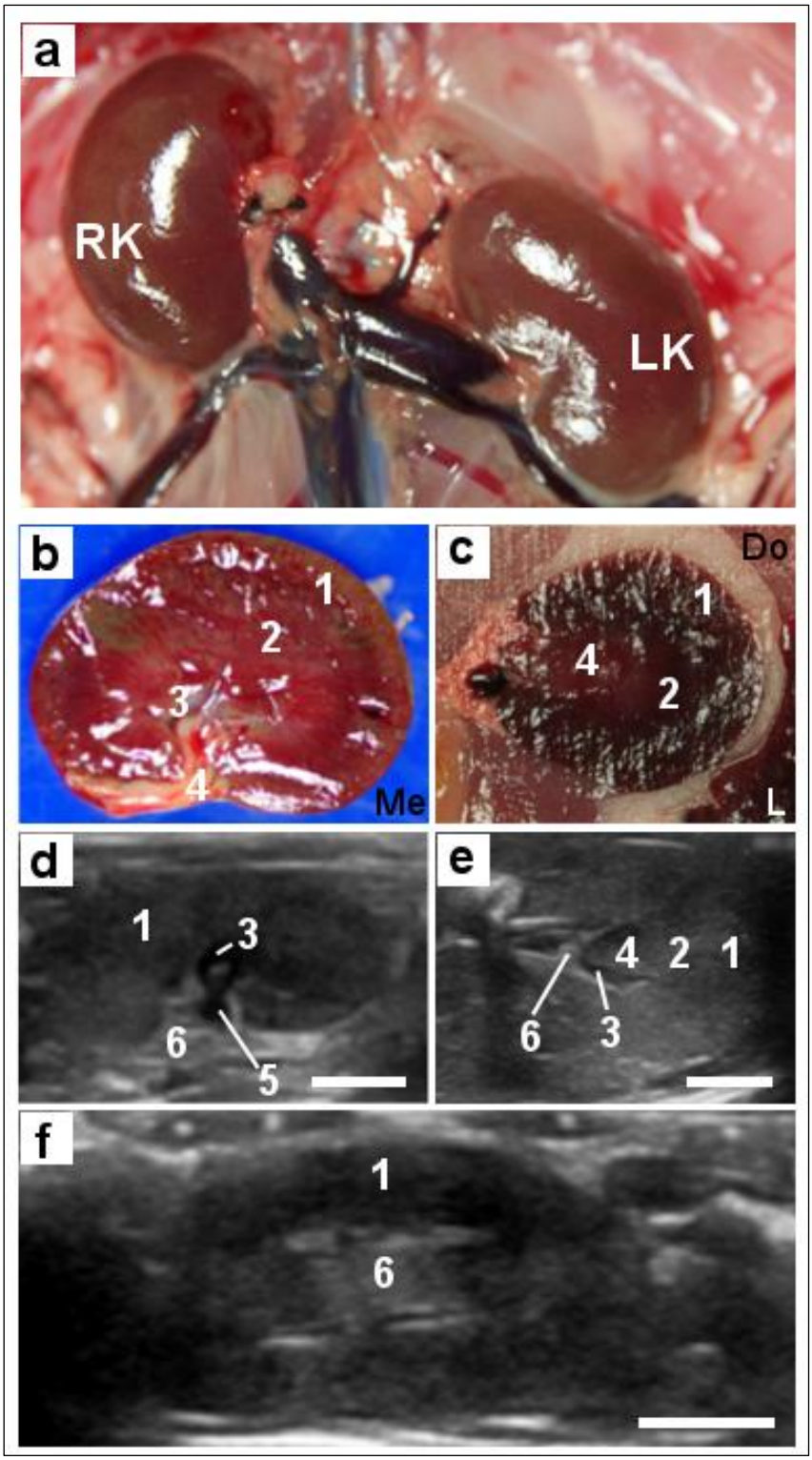

Figure 13 Photographs and ultrasonographic images of the kidneys in a rat. (a) The gross anatomy of both kidneys, notice the right kidney is located more cranial than the left. (b) A parasagittal section through the long axis of the right kidney. (c) Cross section of the right kidney at the level of the hilus. The ultrasonographic images were obtained at the level of the hilus in a parasagittal scanning plane (d), at the level of the hilus in a transverse scanning plane (e) and in a sagittal scanning plane (f). RK: right kidney, LK: left kidney, 1: renal cortex, 2: renal medulla, 3: renal pelvis, 4: renal papilla (crest), 5: ureter, 6: renal sinus, Me: medial, Do: dorsal, L: left. Bar, 5 mm.

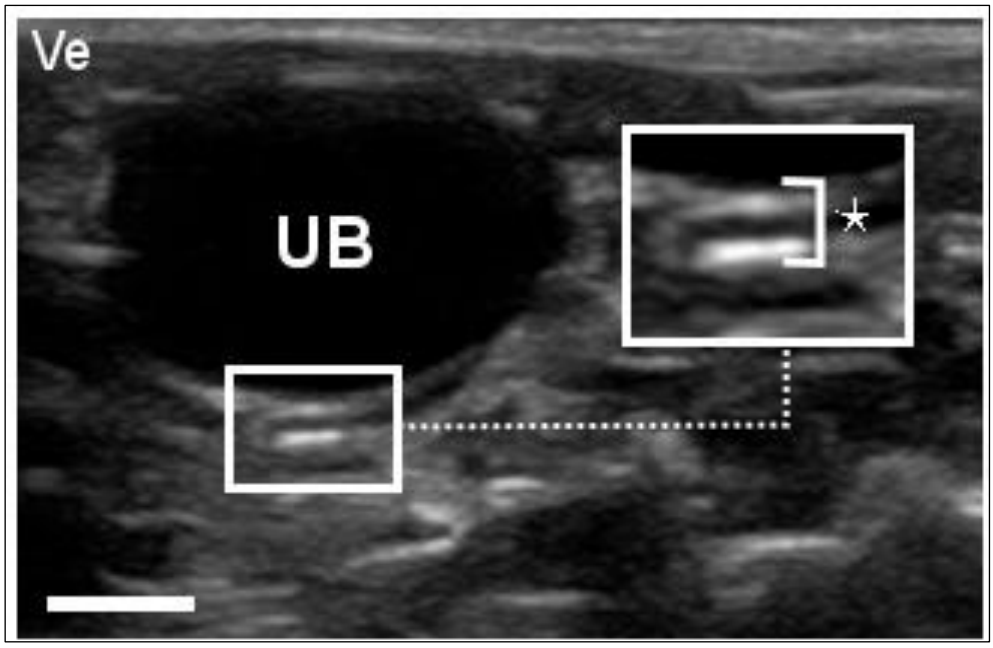

Figure 14 Transverse plane ultrasonographic image of the urinary bladder in a rat. Distended urinary bladder containing anechoic urine within the lumen. *Inset: notice in magnified detail the layer of the urinary bladder wall; a hypoechoic band defined by thin echoic lines on the inner and outer surfaces. UB: urinary bladder lumen, Ve: ventral. Bar, 5 mm.

Table 3. Ultrasonographic measurements (mm) of the urinary tract of rats.

Variable	No	Mean±SD	Males	Females	BWrc	RI
Left kidney length	38	19.47 ± 2.3	19.87 (0.44)	19.08 (0.44)	0.02	14.97–23.97
Left kidney width	40	10.74 ± 1.1	10.91 (0.22)	10.57 (0.22)	0.01	8.56–12.93
Left kidney height	31	10.96 ± 1.2	11.02 (0.29)	10.91 (0.25)	0.01	8.68–13.23
Pelvis left kidney	33	0.87 ± 0.24	0.852 (0.802–0.905)[a]	0.838 (0.784 – 0.896)[a]	NS	0.48–1.35[NGR]
Right kidney length	37	19.45 ± 2.18	19.81 (0.39)	19.06 (0.40)	0.02	14.40–23.51
Right kidney width	37	10.75 ± 0.88	10.78 (0.16)	10.71 (0.17)	0.01	8.85–12.63
Right kidney height	28	11.04 ± 1.00	11.05 (0.25)	11.02 (0.22)	0.01	8.93–13.12
Pelvis right kidney*	29[b]	0.84 ± 0.21	0.853 (0.815–0.892)[a]	0.746 (0.711–0.783)[a]	NS	0.55–1.16[NGR]
Urinary bladder wall	32	0.73 ± 0.13	0.76 (0.03)	0.71 (0.03)	NS	0.48–0.99

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.

[a]Back transformed after logarithmic transformation. [b]One outlier was removed from analysis.

- **Gastrointestinal tract**

Stomach

The stomach was relatively easy to locate during the ultrasonographic examination because it lies caudal to the liver. Since the animals were not fasted, the food and the gas in the stomach was making a complete examination challenging.

In the stomach, the saccus caecus, fundus, and pylorus could be easily recognized in transverse scans (Figure 15). The rugae in the stomach were hard to visualize due to the fullness of the stomach in neither scan planes. The ultrasonographic layering of the stomach wall was similar to that described in dogs and cats: serosa, submucosa, and interface with the lumen (reflection on the mucosal surface) were hyperechoic, whereas a hypoechoic layer was formed by the mucosa and the muscularis mucosae. The mucosa was always the thickest ultrasonographic layer in the stomach wall.

Small intestines

The duodenum was ultrasonographically visible when the right portion of the liver was imaged in a transverse scan. As for the stomach, the layering of duodenal wall was composed by a hyperechoic layer (serosa, submucosa and interface with the lumen) and a hypoechoic layer (mucosa and muscularis mucosae). The mucosa was the thickest ultrasonographic layer in the duodenum wall (Figure 16).

The ileum and jejunum were located in the umbilical region. The ileum differed from jejunum with its thicker mucosa (Figure 17).

Large intestines

The cecum was visible as a large, food-gas mixture filled structure. In some cases it was located in the left emiabdomen occasionally occupying the caudal portion of the abdomen (Figure 17). The cecum could not be systematically examined as its contents strongly reflect the ultrasound waves. Only a small portion of the cecum wall was clearly visualized likely because the wall is thin. Imaging the cecal wall resulted in a hyperechoic curved line adjacent to the transducer from which an acoustic shadow extended. 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.

The ascending colon, with its lumen filled with a food-gas mixture, caused similar ultrasonographic difficulties as the cecum during the examination. The surface of the fecal material closest to the transducer gave rise to a hyperechoic line of reflection; behind this line, there was an acoustic shadow (Figure 17d).

Ultrasonographic reference values for wall thickness of saccus caecus, fundus and pylorus of the stomach, wall thickness of the duodenum and of the cecum were determined and are shown in Table 4.

- **Adrenal glands and hypogastric lymph node**

Adrenal glands

The location of the adrenal glands is very similar in all species of small exotic mammals. The left adrenal gland lies lateral to the aorta and cranial to the left renal vein. The right adrenal gland is located in a more cranial direction than the left: dorsolateral to the caudal vena cava, directly caudal to the liver and cranial to the right renal vein (Figure 18). Relative to the size of the animal, the adrenal glands are larger in small size species. For this reason, to image the adrenal glands using ultrasonography in the majority of small exotic mammal species results easier than in dogs and cats (Reese 2011c).

Ultrasonographic imaging of adrenal glands was carried out initiating by imaging the renal tissue in the parasagittal dorsal plane. The adrenal glands were located immediately craniomedial to the cranial pole of the corresponding kidney. The left adrenal gland was visible only in 32 of the 40 examinated animals because of the overlying of the stomach. Both adrenal glands were ovoid and their texture imaged homogeneous from mildly echoic to almost anechoic, consequently a differentiation of the medulla and cortex was not possible (Figure 18).

The hypogastric lymph node was visible immediately caudal to the aortic bifurcation, between the two internal iliac arteries and veins, and was visible as a poorly echoic, ovoid-shaped structure (Figure 19).

Table 5 summarizes the ultrasonographic reference values for the dorsoventral dimensions of the right and left adrenal glands, measured in a transverse scanning plane (Figure 7a.), and the length and width of the hypogastric lymph node (Figure 7b.). Regardless the effect of gender, there was a significant difference between right and left adrenal gland height, whereas right adrenal gland height differed significantly between males and females (Table 5).

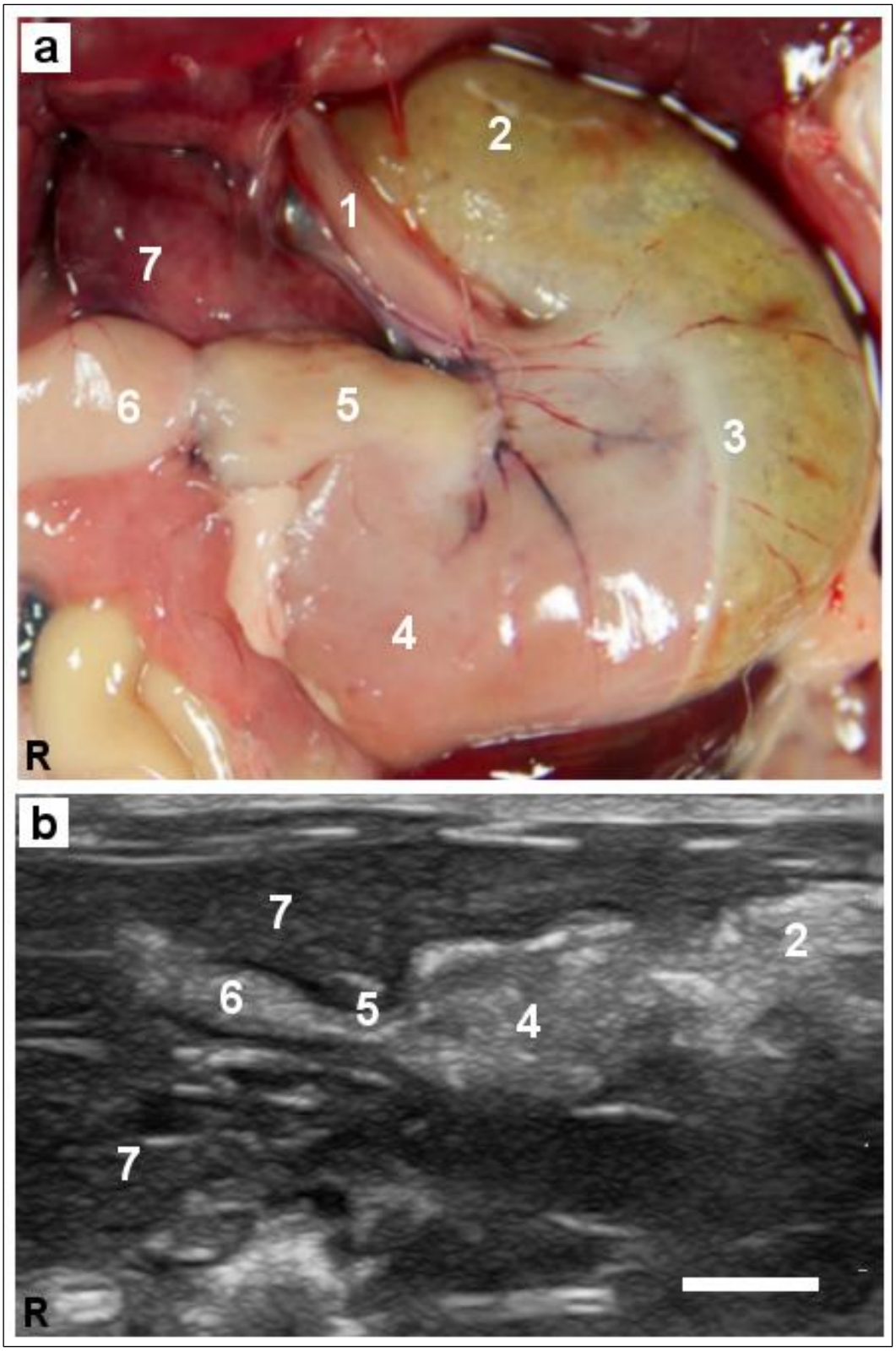

Figure 15 Photograph and ultrasonographic image of the stomach in a rat. (a) In the photograph, notice the gross anatomy of the esophagus, stomach, and duodenum (the ventral portion of the liver was withdrawn cranially to reveal the underlying structures. (b) The ultrasonographic image of the stomach (saccus cecus, body, fundus, and pylorus) was obtained in a transverse scanning plane. 1: esophagus, 2: saccus cecus, 3: body of the stomach, 4: fundus, 5: pylorus, 6: duodenum, 7: liver, R: right. Bar, 5 mm.

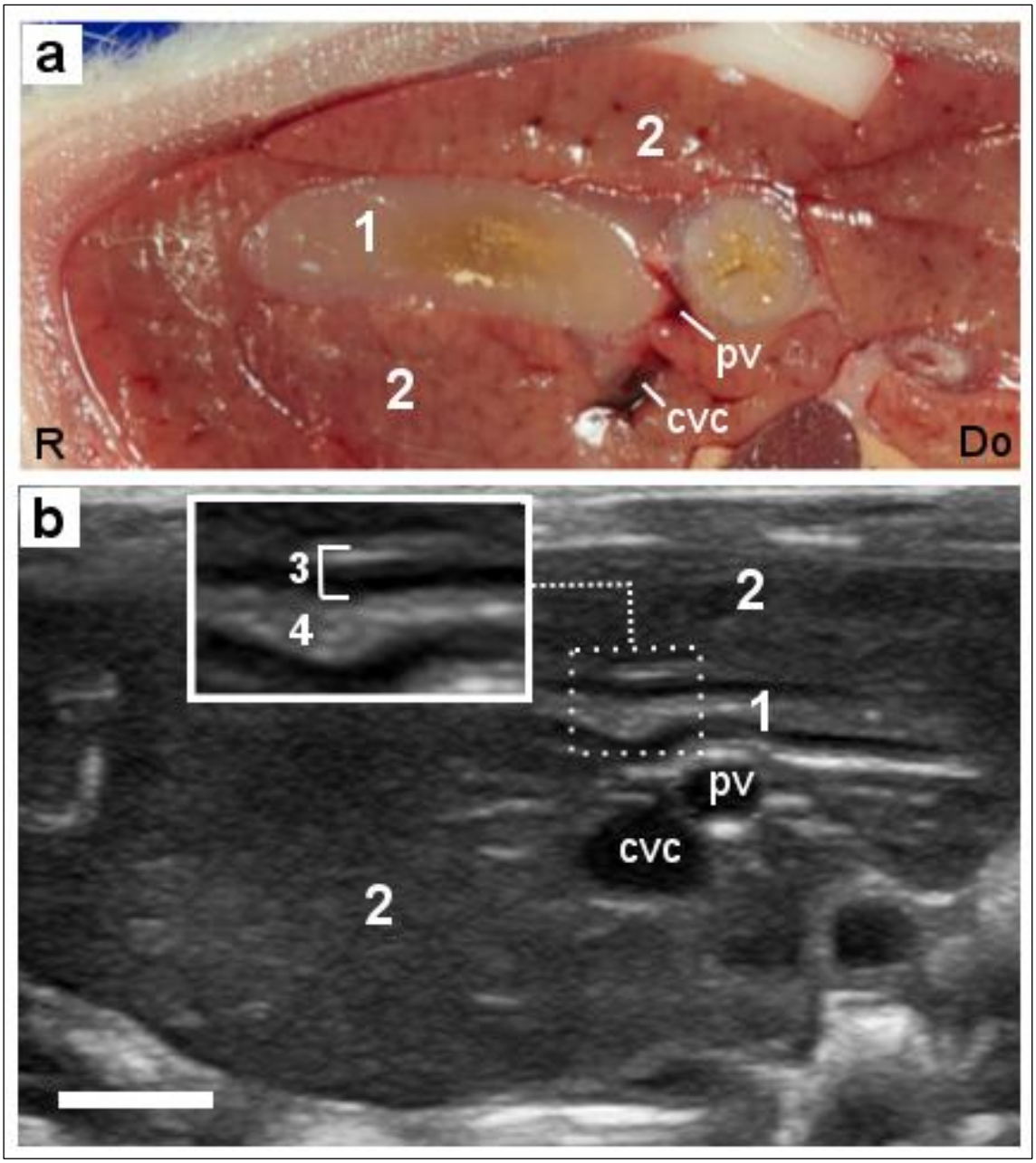

Figure 16 Photograph, an anatomic cross section of the duodenum (a) and ultrasonographic image of the duodenum (b) in a rat. The ultrasonographic image was obtained in a transverse scanning plane. Inset: notice layering of the duodenum wall (magnified detail). 1: duodenum, 2: liver, 3: the layers of the duodenum ventral wall. Notice the hyperechoic layer which comprises the submucosa and serosa and the hypoechoic layer which comprises the mucosa and muscularis mucosae., 4: an inner reflection from the mucosal surface, Pv: hepatic portal vein, cvc: caudal vena cava, Do: dorsal, R: right. Bar, 5 mm.

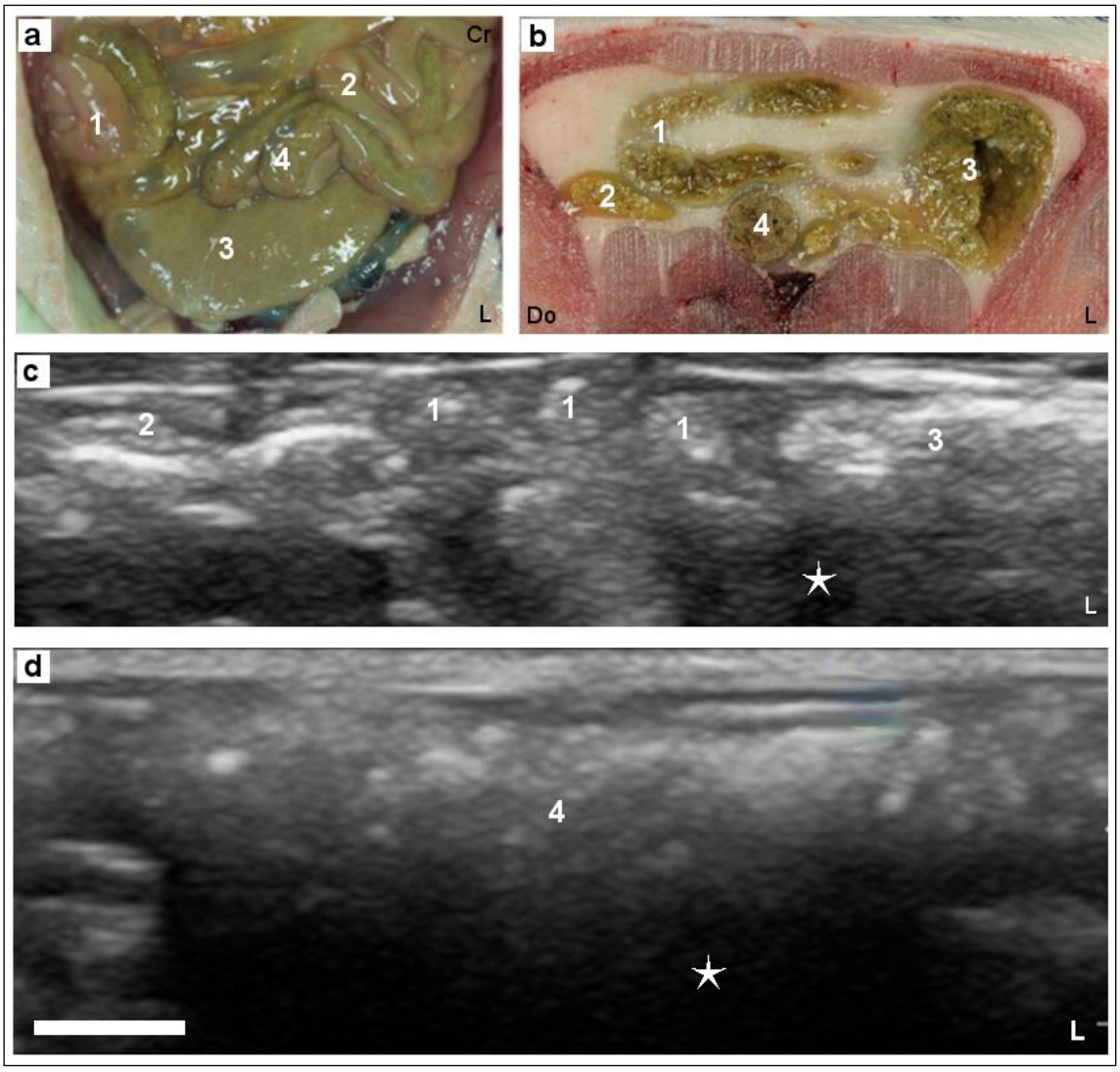

Figure 17 Photographs and ultrasonographic images of the jejenum, ileum, cecum and colon in a rat. In the photographs, notice the gross anatomy of the jejunum, ileum, cecum and colon (a) and an anatomic cross section at the level of the cecum (b). The ultrasonographic images of the jejunum, ileum, cecum (c) and colon (b) were obtained in transverse scanning planes. 1: jejenum, 2: ileum, 3: cecum, 4: colon, *Star: notice the hyperechoic lines of reflection and the following acoustic shadow caused by fecal plus gas material in intestines. L: left, Do: dorsal. Bar, 5 mm.

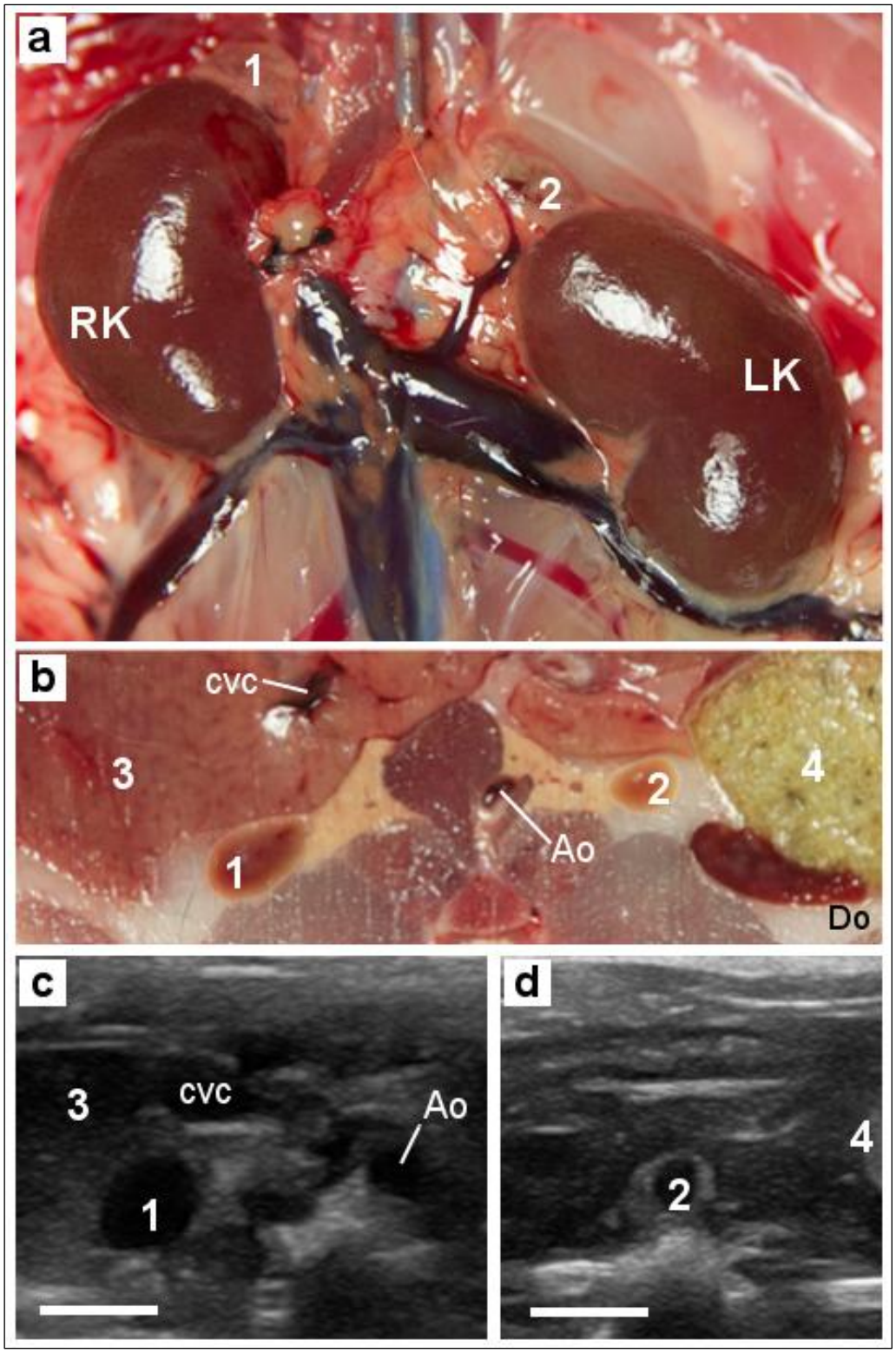

Figure 18 Photographs and ultrasonographic images of the right and left adrenal glands in a rat. (a) Gross section image of adrenal glands at the level of kidneys. (b) Cross section image at the level of adrenal glands. (c) Ultrasonographic image of the right adrenal gland. (d) Ultrasonographic image of the left adrenal gland. The ultrasonographic images were obtained in a transverse scanning plane. 1: right adrenal gland, 2: left adrenal gland, RK: right kidney, LK: left kidney, 3: liver, 4: stomach, cvc: caudal vena cava, Ao: aorta, Do: dorsal. Bars, 5 mm.

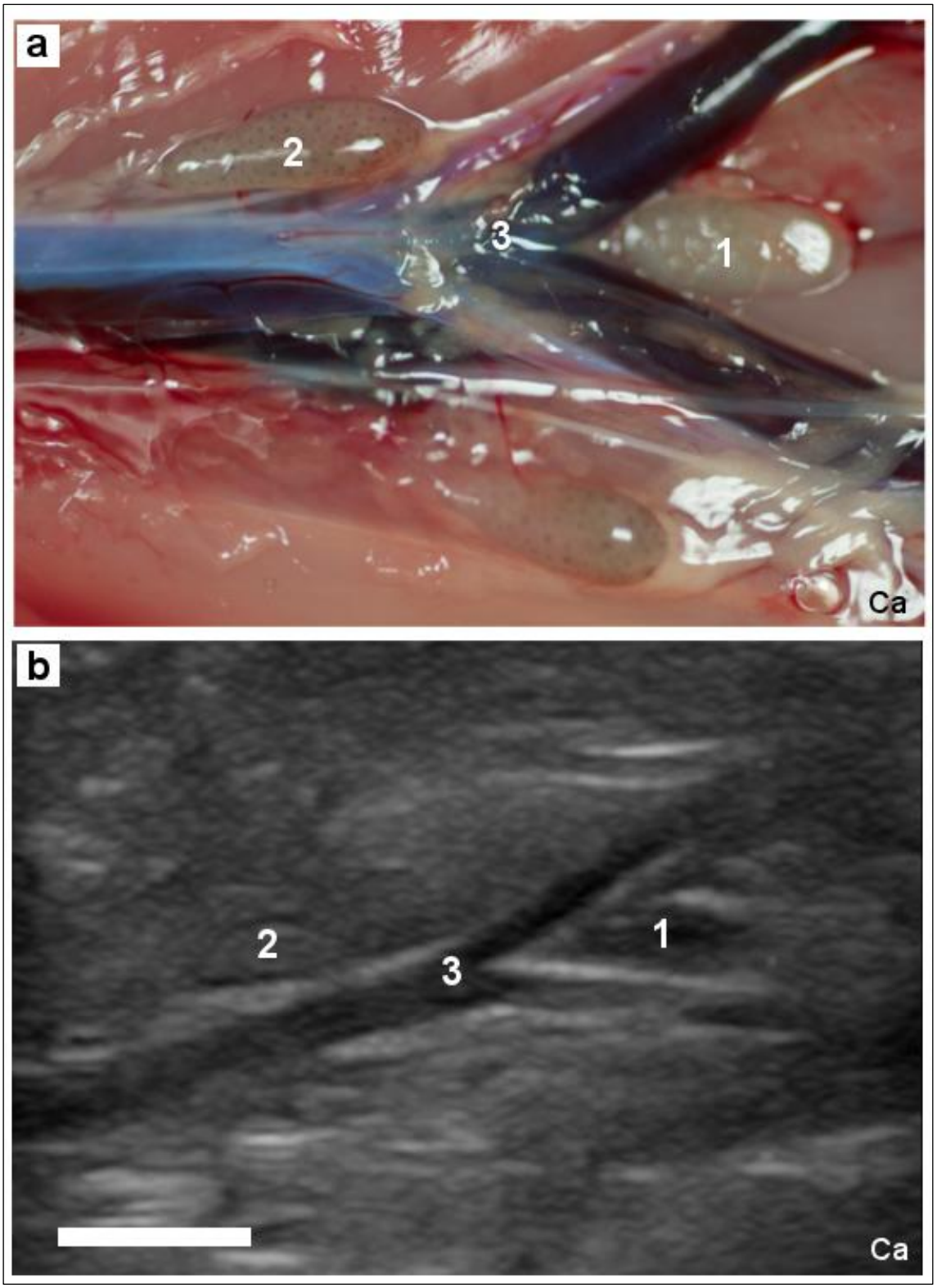

Figure 19 Photograph (a) and sagittal ultrasonographic image (b) of the hypogastric lymph node. 1: hypogastric lymph node, 2: medial iliac lymph node, 3: aortic bifurcation, Ca: caudal. Bar, 5 mm.

Table 4. Ultrasonographic measurements (mm) of the gastrointestinal tract of rats.

Variable	No	Mean±*SD*	Males	Females	BWrc	RI
Saccus cecus of the stomach	35	1.09 ± 0.20	1.04 (0.05)	1.15 (0.05)	NS	0.70–1.49
Fundus wall	34[a]	1.07 ± 0.25	1.01 (0.97–1.06)[b]	1.14 (1.09–1.19)[b]	NS	0.76–1.59[NGR]
Pylorus wall	36	2.20 ± 0.41	2.15 (0.10)	2.25 (0.10)	NS	1.40–3.00
Duodenum wall	38	1.54 ± 0.21	1.47 (1.43–1.51)[b]	1.59 (1.54–1.64)[b]	NS	1.13–1.98[NGR]
Cecum wall	38	0.89 ± 0.13	0.89 (0.03)	0.89 (0.03)	NS	0.62–1.15

Values for males and females are estimated least squares means (SE or 95% confidence intervals for normally or not normally distributed data, respectively).

No significant effect of sex on the values was found($P > 0.05$).

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.

[a]One outlier was removed from analysis. [b]Back transformed after logarithmic transformation.

Table 5. Ultrasonographic measurements (mm) of the adrenal glands and hypogastric lymph node in rats.

Variable	No	Mean±*SD*	Males	Females	BWrc	RI
Right adrenal gland DV size*	37	3.41 ± 0.49	3.2 (0.11)	3.6 (0.11)	NS	2.45–4.36
Left adrenal gland DV size	32	3.07 ± 0.36	3.0 (0.08)	3.1 (0.09)	NS	2.37–3.77
Lymph node length	31	4.19 ± 1.26	4.01 (3.71–4.34)[a]	4.03 (3.73–4.35)[a]	NS	2.23–7.55[NGR]
Lymph node width	31	1.53 ± 0.75	1.32 (1.19–1.47)[a]	1.47 (1.33–1.63)[a]	NS	0.48–2.98[NGR]

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.

[a]Back transformed after logarithmic transformation. DV: ventrodorsal

- **Liver, spleen, and pancreas**

Liver

The ultrasonographic investigation of the liver was performed systematically from left to right in the sagittal scanning plane. Then, using a transverse scan, the liver was examined directly behind the costal arch performing a fan-like craniodorsal movement of the scanning plane.

The depth of penetration was set in order to completely visualize the diaphragmatic reflection on the ultrasonographic image. The hepatic parenchyma had a homogeneous but relatively coarse echoic texture with a low degree of echogenicity. Individual liver lobes were impossible to distinguish. The abdominal aorta, caudal vena cava, and hepatic portal vein with thin hyperechoic wall were clearly identified in the liver parenchyma as hypoechoic round structures in transverse plan (Figure 20).

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 21a). The renal cortex of the right kidney resulted more echoic than the liver parenchyma.

Spleen

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 spleen and liver was not possible because the stomach was interposed between them. For this reason, the comparison was performed by coupling the images of the spleen and the liver in the same screenshot. Through this indirect method it was possible to reveal a lower echogenicity of the liver than the spleen. Likewise, the direct comparison of echogenicity of spleen and cortex of the left kidney showed a lower echogenicity of the spleen than the cortex of the left kidney (Figure 21). Blood vessels could not be imaged in the spleen in the B-mode ultrasound examination.

Pancreas

The body, the left and the right lobes of the pancreas could be imaged in all studied subjects (Figure 22). 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 when compared with that of the liver or the renal cortex.

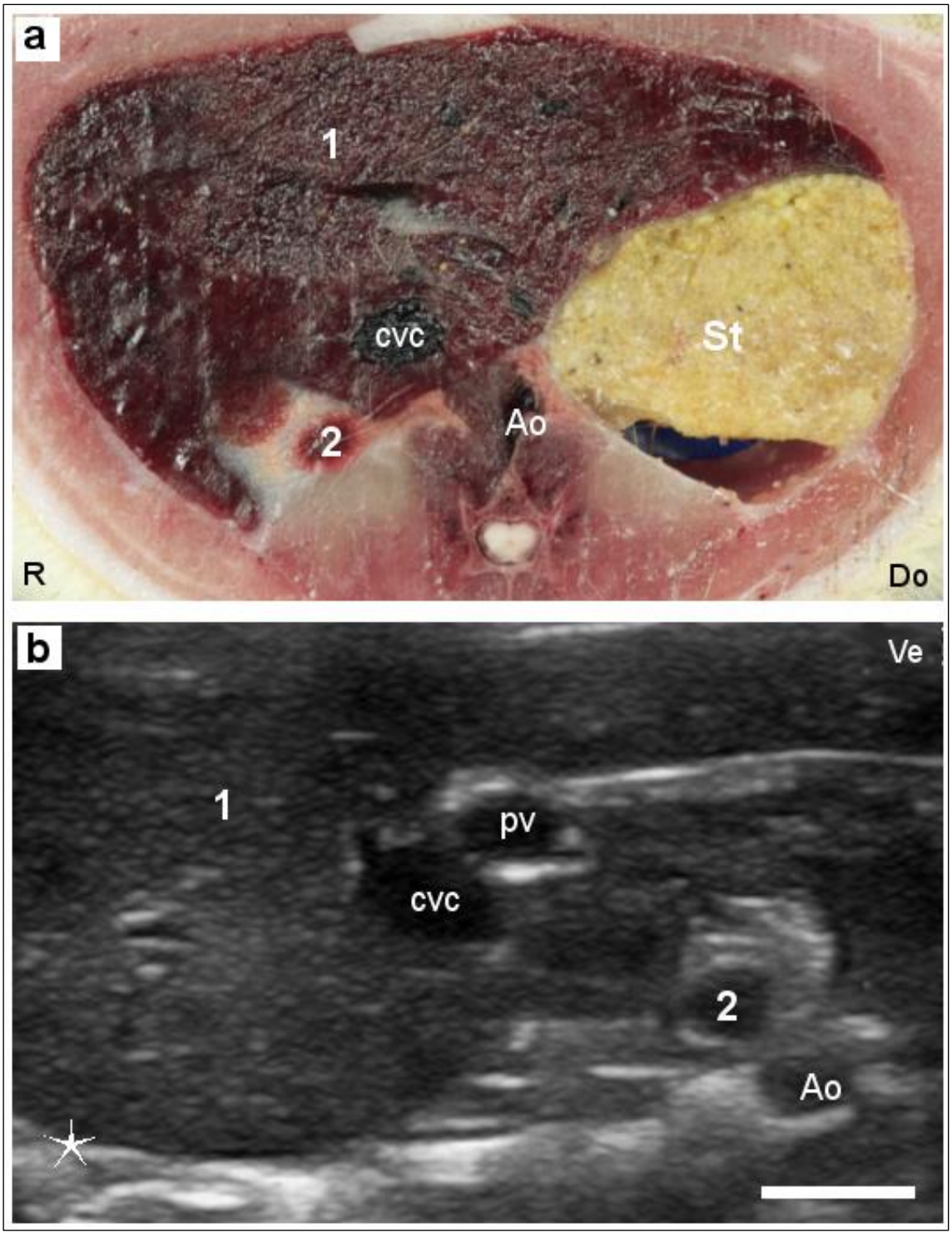

Figure 20 Photograph and ultrasonographic image of the liver in a rat. (a) Cross section of the liver at the level of right adrenal gland. (b)The ultrasonographic image was obtained in a transverse scanning plane. The liver parenchyma had a homogeneous but relatively coarse echoic texture with a low degree of echogenicity. *Star: notice the depth of penetration was set in order to visualize the diaphragmatic reflection on the ultrasonographic image. 1: liver, 2: right adrenal gland, cvc: caudal vena cava, Ao: aorta, Pv: hepatic portal vein, St: stomach, Do:dorsal, Ve: ventral. Bar, 5 mm.

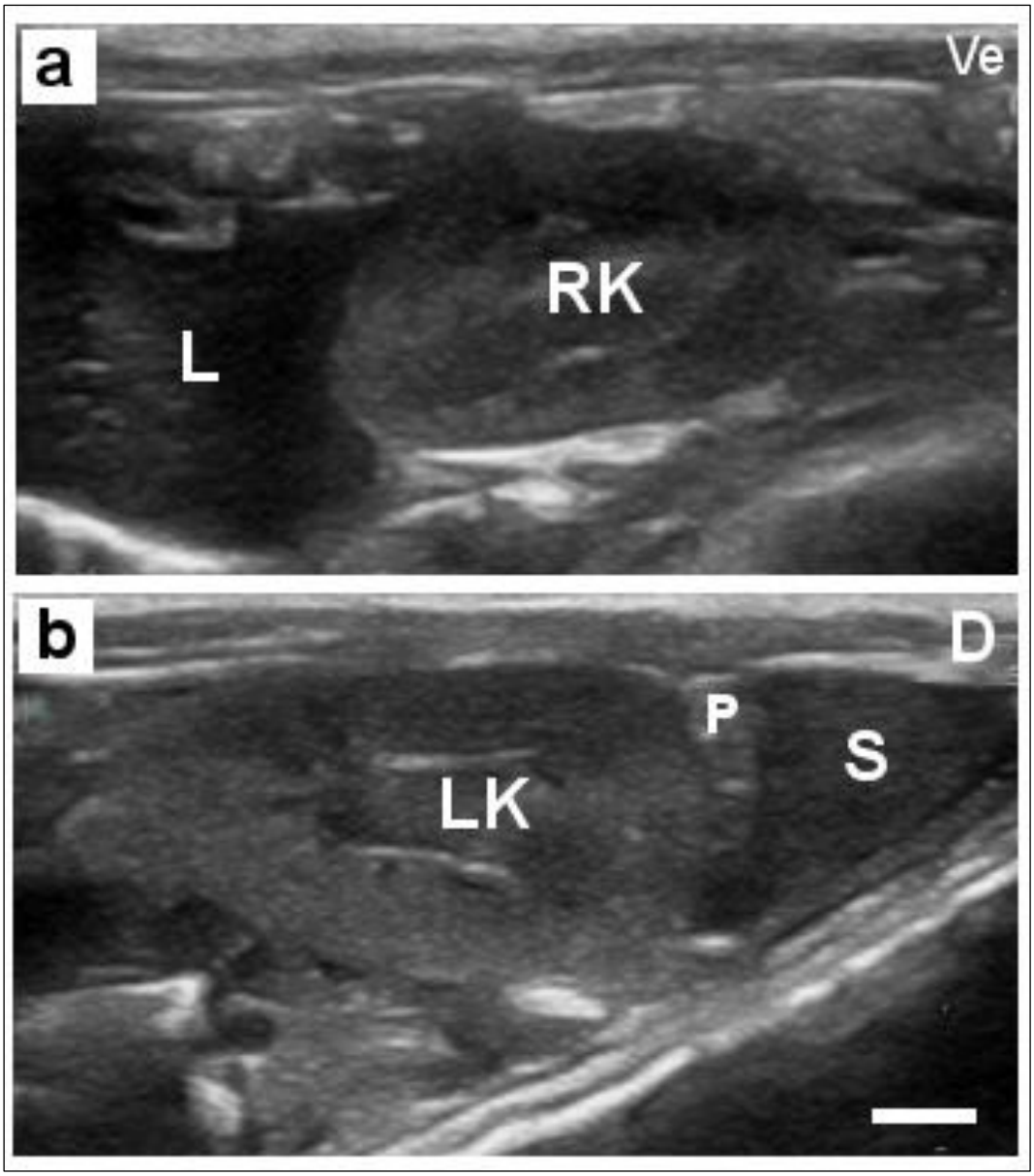

Figure 21 Liver, kidney and spleen relative echogenicities in a rat. (a) Sagittal ultrasonographic image obtained in the right cranial abdomen shows the relative echogenicity between liver and right kidney cortex. The liver is more hypoechoic than the renal cortex. (b) Sagittal ultrasonographic image obtained in the left cranial abdomen shows the relative echogenicity between the left kidney cortex and spleen parenchyma. The left kidney cortex appears relatively hyperechoic than the spleen parenchyma. The left kidney cortex appears more echoic than the right renal cortex. Through this indirect method of echogenicity comparison, the liver results less echoic than the spleen parenchyma in the rat. L: liver, RK: right kidney, LK: left kidney, P: pancreas, S: spleen, Ve: ventral, D: dorsal. Bar, 5 mm.

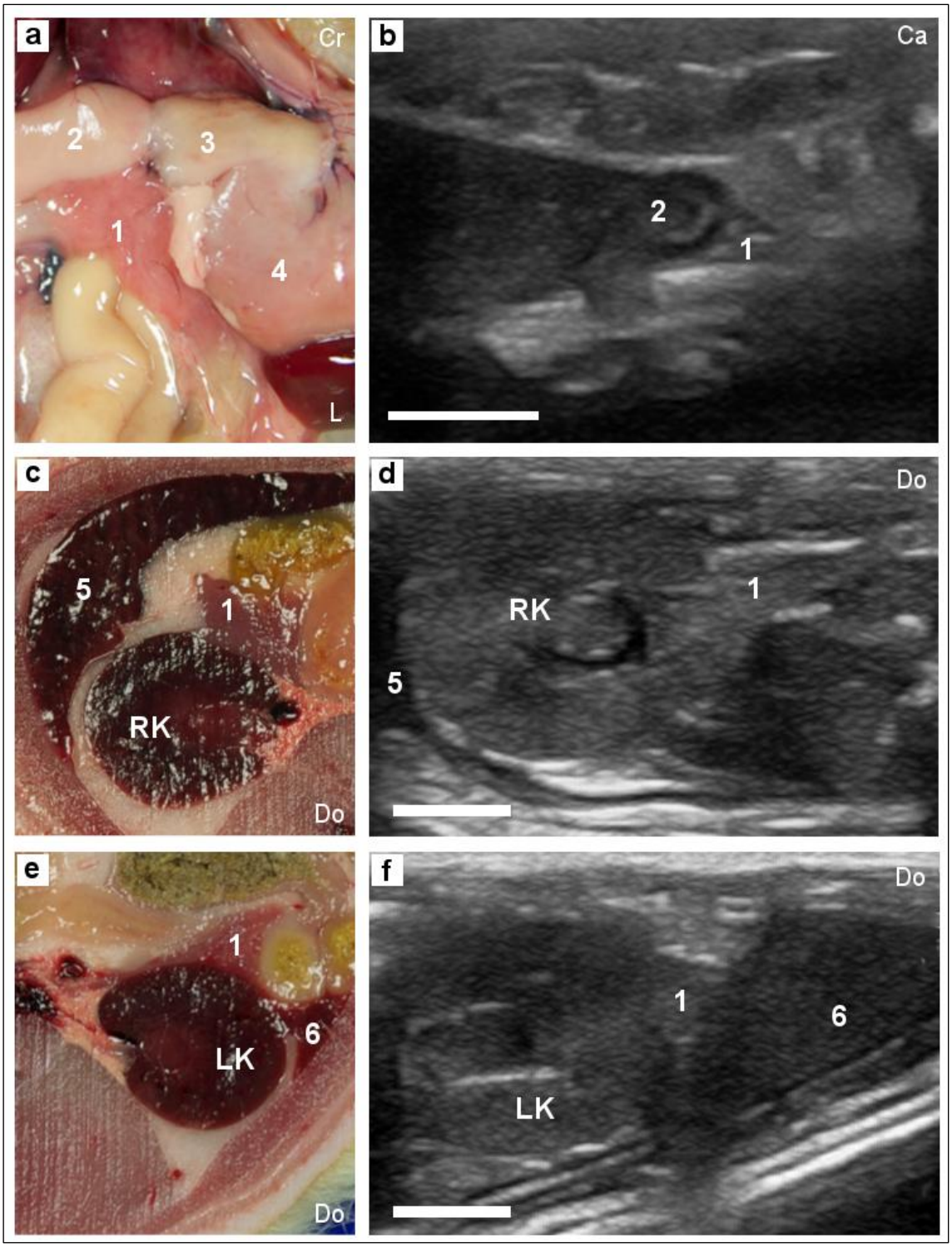

Figure 22 Photographs and ultrasonographic images of the pancreas in a rat. In the photographs, notice the gross anatomy of the pancreas at the level of the pylorus (a); a cross section at the level of the right kidney, the right lobe of the liver, and the right lobe of the pancreas (c); a cross section at the level of the left kidney, the spleen, and the left lobe of the pancreas (e). The ultrasonographic images of the body (b), the right (d) and left lobes of the pancreas (f) were obtained in a transverse scanning plane. 1: pancreas, 2: duodenum, 3: pylorus, 4: fundus, 5: liver, 6: spleen, RK: right kidney, LK: left kidney, L: left, Do: dorsal. Bars, 5 mm.

2.3.3 Kidney measures

- **Ultrasonographic kidney measurements**

Both kidneys were ultrasonographically evaluated in all studied subjects. The ultrasonographic reference values for kidney size (length, width, and height) and renal pelvis height were recorded (Table 3). The reference intervals for left kidney were 14.97-23.97 mm for length, 8.56-12.93 mm for width and 8.68-13.23 mm for height. For the right kidney the reference intervals were 14.40-23.51 mm for lenght, 8.85-12.63 mm for width and 8.93- 13.12 mm for height. Spearman correlation coefficient test results showed significant positive correlations between body weight and ultrasonographic kidney size (length, height, and width). Pelvis height of the right kidney was significantly different in males and females (Table 3).

- **Radiographic kidney measurements**

Radiographic measurements were recorded for the left kidney in 24/40 and for the right kidney in 19/40 animals. The data are summarized in Table 6. The reference intervals for left kidney were 16.95-24.79 mm and 8.63-16.44 mm for length and width, respectively. For the right kidney the reference intervals were 14.06-27.05 mm and 7.16-17.18 mm for length and width, respectively.

In addition, paired t-test was applied to see whether there was a significant difference between left and right kidney measurements. Based on the results, there was no significant difference in the radiographic measures between right and left kidneys for length or width (P=0.35 and P=0.82, respectively).

The mean length of the second lumbar vertebra (L2) was 7 mm for both genders with standard deviations of 0.48 and 0.67 for females and males, respectively.Table 7 summarizes ratio values for both kidneys (renal length/width : L2 body length). The reference intervals for left kidney ratios were 2.39-3.85 and 1.21-2.51 for length and width, respectively. For the right kidney ratios the reference intervals were 2.13-3.98 and 1.16-2.44 for length and width, respectively.

Table 6. Direct kidney length and width measurements* from ventrodorsal survey radiographs in rats.

Variable		No	Mean (mm)	SD[a] (mm)	95% CL[b] (mm)	Range (min-max) (mm)	RI[c] (mm)
LEFT							
	Length	24	21.04	1.85	20.25-21.82	18-25	16.95-24.79
	Width	23	12.06	1.80	11.82-13.38	9-17	8.63-16.44
RIGHT							
	Length	19	20.52	2.96	19.09-21.95	14-27	14.06-27.05
	Width	19	12.78	2.32	11.66-13.90	10-18	7.16-17.18

SD[a]= Standard deviation, CL[b]= Confidence limit. RI[c]= Reference interval, RI estimated with robust method.

*Data presented in millimeters**.**

Table 7. Ratio of kidney length and width to the length of the body of second lumbar vertebra (L2) in rats.

Variable		No	Median	Interquartile range	Range (min-max)	RI
LEFT						
	Length:L2	24	3.14	0.45	2.57-4.2	2.39-3.85
	Width:L2	23	1.85	0.28	1.28-2.8	1.21-2.51
RIGHT						
	Length:L2	19	3.0	0.57	2.0-3.86	2.13-3.98
	Width:L2	19	1.85	0.33	1.43-2.57	1.16-2.44

RI= Reference interval, estimated with robust method.

2.3.4 Comparison between ultrasonographic and radiographic kidney measures

As a result of the paired t- test, for the left kidney, there was a significant difference between radiographic and ultrasonographic measures for length and width (P=0.0036 and P<0.001, respectively). For the right kidney, there was a significant difference between radiographic and ultrasosonographic measures for width (P=0.0017) whereas there was no significant difference for length (P=0.22) (Table 8).

For the left kidney, there was a significant correlation between radiographic and ultrasonographic measures for length and width (r=0.516, P=0.015; r=0.716, P<0.001, respectively). However, for the right kidney there was no significant correlation between radiographic and ultrasonographic measures for length or width (r=0.235, P=0.345; r=0.388, P=0.112).

Spearman correlation coefficient test was performed to determine correlation between measurements and body weight. L2 body length was significantly correlated with body weight (r=0.518, P=0.001). There was also a strong positive correlation between body weight and left kidney length (P<0.001), and a weak but statistically significant correlation between body weight and right kidney width (0.01<P<0.05). However, there was no significant correlation between body weight and left kidney width, left renal length ratio, left renal width ratio, right kidney length, right renal length ratio and right renal width ratio (for all P>0.05).

There was weak but statistically significant effect of gender on left kidney length, right kidney width, left kidney length ratio and right kidney width ratio (for all 0.01<P<0.05). However, no significant effect of gender was found on left kidney width or left kidney width ratio (P>0.05) (Table 9; Figure 23).

Similarly, there was no effect of gender on right kidney length or right kidney length ratio (P>0.05) (Table 9, Figure 23). Compared to females, the male rats had bigger values for the length of left kidney and for the width of right kidney. Similarly, also the ratios of the left kidney length and right kidney width resulted higher in male than in female rats (0.01<P<0.05) (Table 9).

Table 8. Results of paired Student's t-test between radiographic and ultrasonographic kidney measurements* in rats.

Measurements (mm)		No	Mean Difference (mm)	SDD[a] (mm)	95% CL[b] (mm)	T-value	P-value**
LEFT							
	Length	22	1.27	1.82	0.46-2.08	3.28	0.0036
	Width	23	1.76	1.33	1.18-2.33	6.34	0.0000
RIGHT							
	Length	18	1.13	3.77	-0.74-3.01	1.27	0.2202
	Width	18	1.83	2.09	0.79-2.88	3.72	0.0017

SDD[a]= Standard deviation of the difference.
95% CL[b]= Confidence limit of mean difference.
*Data presented in millimeters.
** P<0.05 significant.

Table 9. Results of ANCOVA: F-values, R^2 index and significance of sex and body weight for radiographic kidney dimensions in rats.

		F- values		R^{2a}	RMSE[b]	LS[c]means±SE[d]			BWrc[e]
LEFT		Sex	Weight			Females	Males	Sem[f]	
	Length	7.68*	26.36***	0.62	1.19	20.37	21.71	0.34	0.021
	Width	2.84	3.42	0.23	1.65	12	13.16	0.49	0.01
	Ratiolength	7.54*	0.97	0.26	0.31	2.98	3.33	0.09	-0.00003
	Ratiowidth	4.4	0.02	0.17	0.47	1.61	2.01	0.13	0.0002
RIGHT									
	Length	2.02	0.84	0.17	2.87	19.52	21.42	0.96	0.011
	Width	4.82*	7.33*	0.46	1.8	11.82	13.65	0.6	0.02
	Ratiolength	2.36	0.06	0.12	0.42	2.86	3.17	0.14	-0.0004
	Ratiowidth	5.97*	3.18	0.39	0.25	1.73	2.01	0.08	0.001

* 0.01<P<0.05; ** 0.001<P<0.01; *** P<0.001
R^{2a} = Variance explained by the model (% of total variance)
RMSE[b] =Root means squares error
LS[c] =Least squares
SE[d] =Standard error
BWrc[e] =Body weight regression coefficient
Sem[f] = mean standard error

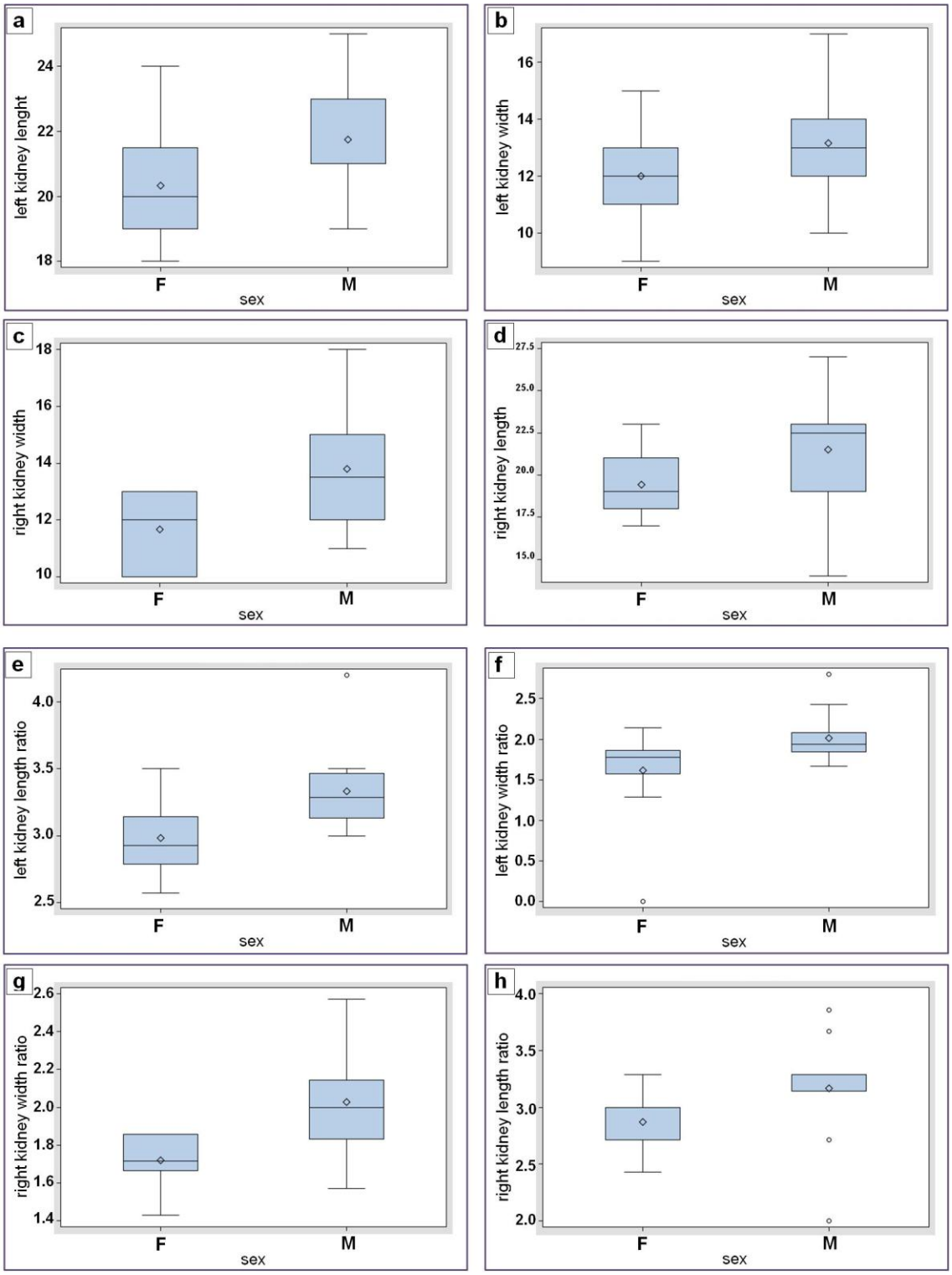

Figure 23 Comparison of radiographic kidney linear (mm) and ratio measurements based on sex in 40 rats. There was statistically significant effect of sex on left kidney length (a), left kidney length ratio (e), right kidney width (c) and right kidney width ratio (g) (for all 0.01<P<0.05). Note that male rats had bigger values for above mentioned measurements than female rats. However, no significant effect of sex was observed on left kidney width (b), left kidney width ratio (f), right kidney length (d) and right kidney length ratio (h) (for all P>0.05). Whiskers represent range, boxes represent interquartile range, diamonds within circles represent mean and crossline represents median value.

2.4 DISCUSSION

2.4.1 Radiography of the thorax and abdomen in rat: general considerations

In the present study, we followed the standard radiographic examinations by providing right lateral view with the second plane being ventrodorsal projection. Despite the small size of rats, whole body radiographic examinations are generally not recommended and it is emphasized to take the radiographs of the thorax and abdomen separately (Reese and Hein 2011b). However, since collimation and particularly the positioning were difficult due to the dominance of the abdomen and the small size of the rats, the whole body radiographs were carried out in this project.

Another difficulty we met during the radiographic examinations was to take the radiographs during full inspiration, which is desirable for thorax evaluation (Nautrup 2011). Due to the rapid respiratory rate in rat (70-115 breaths per minute), it was not feasible to obtain the radiographs at the correct time and thus, the images were obtained at a coincidental phase. However, the digital image processing system along with windows level/brightness/contrast adjustments was valuable to evaluate the thorax and abdomen in rats.

2.4.2 Radiographic anatomy of the thorax in rat

- **Relevant skeletal system**

Variation in number of the vertebrae was not found among the rats evaluated in this study. All the rats had 7 cervical vertebrae, 13 thoracic vertebrae, 6 lumbar vertebrae and sacrum consisted of 4 fused segments. This was consistent with (O'Malley 2005b; Reese 2011b; Sharp and Villano 2012). The most of the subjects herein had 2 floating ribs, this was inconsistent with reported value of 3 floating ribs (O'Malley 2005b), however 8 of the rats had 3 floating ribs.

- **Thorax**

It has been noted that the radiopacity of the lungs might differ significantly between the radiographs imaged during inspiratory and expiratory reaspiratory phases (Reese and Hein 2011b). As stated above, the respiratory phase at the time of obtaining the radiographic image was coincidental in the studied subjects. Nevertheless, the thoracic

contrast was adequate to discern the heart, the pulmonary vasculature, caudal vena cava and aorta. In some instances it was difficult to discern the cranial border of the heart. This was likely due to the remnant thymus which is responsible for the higher degree of radiopacity found in the precardial lung field in rats (Reese and Hein 2011c).

Heart size assessment

Cardiac measurements are helpful for inexperienced observers as a starting point in evaluating heart size (Buchanan and Bücheler 1995). The present study reports the radiographic heart size, the T5-8 skeletal size and the effect of body weight and gender on these measures in domestic rat.

As described in the results, weight and skeletal size (T5-8) were highly correlated, and measures of cardiac silhouette size were similarly correlated with T5-8 measurements and body weight. However, there was no significant positive correlation between body weight and the ratio obtained from the sum of cardiac long and short axes on the T5-8 vertebral bodies length, or body weight and the vertebral heart size (VHS). Application of measure systems that are independent of patient size, such as the ratio and VHS, may provide more useful information than absolute measures in rats.

Results obtained in the present study revealed that there were no significant differences in body weight, T5-8 body lengths, absolute measures of cardiac size (long axis, short axis and sum of the two), ratio (sum of cardiac axes/T5-8 lenght) and VHS between male and female rats. Thus, the reference ranges provided in this study could be used to evaluate cardiac size in male and female rats indifferently. Stepien et al. (1999) published a study on ferrets suggesting the use of the ratio method in evaluating cardiac size. In their study, the ratio appeared to result in less measurement variation than the modified VHS method. However, in their VHS method the length was estimated to the nearest 0.25 vertebrae (Stepien, Benson, and Forrest 1999) instead of 0.1 vertebrae, the one that was suggested by Buchanan and Bücheler (1995). In our study, the ratio of cardiac long axis plus short axis to T5-8 vertebral bodies length in right lateral view appeared to produce the least variable results, but it is more labor than the use of VHS method as it has been also confirmed by Stepien et al. (1999).

In addition, in the initial assessment of heart size, the VHS method has been suggested to be useful in monitoring the progression of heart enlargement over the time. Moreover, recording the heart size in vertebral scale appears to encourage objectivity and to be a convenient way of recording changes in heart size in response to treatment or progression of cardiomegaly (Buchanan and Bücheler 1995).

The cardiac measurements in this study were made on right lateral (RL) thoracic views because the ventro dorsal (VD) view was challenging to evaluate cardiac size due to the remnant thymus in adult rats (Bivin, Crawford, and Brewer 1979). However, the images obtained on lateral view were found to be less variable than those derived from VD view in ferrets (Stepien, Benson, and Forrest 1999) and dogs (Buchanan and Bücheler 1995), whereas in cats there was no statistical difference between lateral view and VD view (Ghadiri et al. 2008).

The magnification effect on cardiac size on radiographs should be considered as minimal in rats. This is likely due to the rat's relatively small body size and the use of table-top radiography which reduces the amount of magnification on the radiographs (Kealy, McAllister, and Graham 2010).

The VHS and the ratio reference values in right lateral radiographs provided here could be useful parameters for assessing the cardiac size in healthy rats. However, the normal range we identified in this study for the ratio (1.72 to 2.26) is sufficiently narrow than the VHS range (6.3-8.5 vertebral units) to allow detection of cardiomegaly in rats. Therefore, we may suggest to use the ratio along with VHS in rats although the VHS is considered a more objective assessment of the heart size and has been proven to be particularly useful for assessing the size of the heart in the dog (Buchanan and Bücheler 1995; Nautrup et al. 2011).

However, it is important to note that normal heart size does not rule out heart disease, because substantial hypertrophy may exist without an increase in external heart size. Therefore, the cardiac silhouette always must be examined for subtle changes in contour that may develop in rats, as in dogs, with concentric hypertrophy without dilatation (Buchanan and Bücheler 1995).

The main limitation of this project was the impossibility to perform the echocardiographic examinations in the studied animals in order to exclude subclinical and pre-existing heart disease. An interesting further study might be to develope in rats with known heart disease in order to ascertain the best method of detecting abnormal cardiac size in this species.

2.4.3 Radiographic anatomy of the abdomen in rat

- **Abdomen**

In the present study the radiographic abdomen of the rat was divided in cranial, middle and caudal sections to facilitate the identification and evaluation of abdominal organs. The stomach and intestines have been easily localized owing to the gas present in the lumen of these organs. Conversely, the liver was easy to identify due to the lack of gas in the area. Additionally, the liver could be identified by its homogeneous appearance which was contrasted by the surrounding small and large intestines even when its exact caudal margin on ventrodorsal view could not clearly be determined.

Liver

In the present study, the right lobe of the liver was extended beyond the costal arch and had sharp caudoventral tip in most rats (34/40) on right lateral view. In general, the typical radiographic sign of hepatomegaly in the small exotic mammal patient is when the ventral contour of the liver extends clearly outside of the costal arch in the lateral projection and has rounded edges (Reese and Hein 2011a). Interestingly, in rats the caudoventral extension of the liver beyond the costal arch is physiological (Reese and Hein 2011d) and thus it is important to determine the limit of caudoventral extension in clinically normal rats. In the present study, the length of caudoventral liver extension was provided on right lateral view and compared with L2 vertebral length. Based on this study results, the maximal length of the caudoventral extension of the liver in normal rats was 3.23 times of the L2 vertebral length. Since a number of diseases may result in an enlarged liver in rats, the range of caudoventral extension limit may be useful to identify hepatomegaly in this species even if the definitive etiological diagnosis can only be achieved through the use of an ultrasound-guided aspirate or biopsy of the organ (Reese and Hein 2011d).

Based on ANCOVA test results, there was a significant effect of gender on caudoventral extension length of liver. However, no significant effect of body weight was found on caudoventral extension length of liver (linear dimension or ratio). This could be likely, because the length of the caudoventral extension of liver is not reflective of the entire liver volume, but simply a measurement that can be made on the lateral radiograph.

Stomach

In the present study, the stomach was located intrathoracic in most animals (26/40) while in 14/40 rats the stomach was beyond the costal arch. This variability might be due to the

fact that the distension of stomach beyond the costal arch depends on its degree of filling (Reese and Hein 2011d). In pet rats, whole body radiographs may help in the diagnosis of conditions such as gastrointestinal stasis (Hawkins and Graham 2007), overloaded or severely tympanic stomach (Reese and Hein 2011a). In the latter case, it is noted that up to two-thirds of the stomach may lie beyond the costal arch and may also extend to the full height of the thorax. When the stomach is overfilled, the intestines are caudally displaced. However, if the stomach is round and large but the intestinal tract can still be recognized behind the stomach, the degree of filling may not be so critical (Reese and Hein 2011a). In all the animals examined in this study the caudal extension of the stomach was related to the degree of filling.

Spleen

In the present study it was difficult to discern radiographically the spleen. In general, with the exception of ferrets, in rats and the other small mammals, splenomegaly is not associated with any specific radiographic signs and thus, the diagnosis of splenomegaly in these species requires an ultrasonographic examination (Reese and Hein 2011a).

Kidneys

The kidneys of the rat are in some cases difficult to discern in plain radiographs, therefore some authors suggested alternative techniques such as using contrast media (Knotek, Wildnerová, and Jekl 2004) or compression method using a prism from a non-contrast material (Knotek, Wildnerová, and Jekl 2004; Wildnerova 2003). In the present study, the kidneys were visualized by means of digital images which might provide better contrast resolution and by the ability to manipulate images (magnification, windows/level adjustments) (Eshar, Briscoe, and Mai 2013). Furthermore, by using low kVp and high mAs it is possible to obtain an image with a high degree of contrast (Reese and Hein 2011b). Left kidney was more frequently visible than the right. As it is observed in dogs and ferrets (Burk and Feeney 2003; Eshar, Briscoe, and Mai 2013), this is likely the result of the right kidney being more cranially positioned and firmly attached below the inferior right lobe of the liver which can cause the cranial pole of the right kidney to be obscured by the hepatic parenchyma (Chen et al. 2012).

Intestines

The location of small and large intestines and cecum described in this study may help veterinarians to identify displacement of these structures in particular when they are dilated. Moreover, space-occupying lesions of other organs, strictures, adhesions, or tumors of the small intestines can all lead to a partial narrowing or complete occlusion of

the intestines. One of the radiographic signs of an obstructive ileus is a dilatation of the intestines proximal to the ileus due to the accumulation of gas and/or fluid. However, a subsequent ultrasonographic examination can help determine the cause of obstructive ileus, especially in cases with invagination or tumors. The definitive diagnosis of an obstructive ileus can be achieved by performing a contrast study, which can reliably indicate the position of the intestinal blockage (Reese and Hein 2011a).

Urinary bladder

The urinary bladder was discernible only in RL radiographs. Diseases of the urinary bladder occur quite rarely in small exotic rodent patients. However, the diagnosis of urinary bladder and urethral stones in rats can be achieved using standard radiographic imaging techniques whereas the thickening of the bladder wall, which can only be diagnosed in the filled bladder, can be diagnosed using ultrasonography (Reese and Hein 2011a).

Considering the poor abdominal contrast, the benefit of radiology as a worthwhile diagnostic tool for abdominal pathology in rats may be at issue. However, it should be useful in many instances such as, hepatomegaly, gastric stasis, gastric overloading, severe tympany, foreign bodies, renal and uretral calculi, dystrophic mineralization, obstructive ileus and masses. As a complementary to radiography, an abdominal ultrasonographic examination should be considered. Other diagnostic imaging techniques such as contrast studies of the gastrointestinal tract and urogenital system could also be helpful to reach a correct and complete diagnosis. The description and anatomical landmarks along with some useful measurement ranges of the abdominal organs provided in this study might be a helpful platform for practitioners.

2.4.4 Ultrasonographic anatomy of the abdomen

Most of the clinically relevant organs and structures of the abdomen were identified in all studied subjects. Although some authors (Reese 2011c) 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 (8.5 MHz) may also be applied for diagnostic imaging in this species. However, it is likely 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 obtained in this study were consistent with the gross anatomic measurements described for laboratory rats (Hebel and Stromberg 1986), suggesting that neither the different strain analyzed nor the sedation procedures affected organ size or shape. However, all the studied subjects were sedated with a combination of medetomidine and butorphanol. Medetomidine may cause a 3-fold increase of urine output in rats (Harada et al. 1992), 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. It is also reported that the sedation may influence the size and echogenicity of the spleen in dogs (O'Brien, Walker, and Osgood 2004). However, similar data regarding the rat spleen are not available and thus veterinarian clinicians should be aware that the data and images of the spleen could have been affected by sedation.

The ultrasonographic reference measurements and the thorough description of the abdominal organ ultrasonography patterns described in this study may enable a deeper insight into the imaging and medical knowledge of domestic rat.

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 (Hecht 2008). This difference should be considered in the clinical examination and the veterinarian should 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 (Reese 2011c). Based on the this study results, it could be hypothesized that the lack of mediastinum visualization could be or a specific characteristic of the rat testicle, or caused by the low-frequency transducer, or both. Reproductive system tumors are among the most common neoplasias in both male and female rats (Greenacre 2004). The matched anatomic and ultrasonographic images provide in this study may help to guide the clinician in evaluating the genitourinary system in rats.

Significant relationships between body weight and kidney height, width and length were observed. Chronic interstitial nephritis is a common, naturally occurring disease in rats and is usually associated with reduced kidney size (Reese and Hein 2011a), whereas

infiltrative neoplasms such as lymphoma or leukemia often cause kidney enlargement (Car, Eng, and Everds 2005; Boorman and Everitt 2005). The matched anatomic and ultrasonographic images, the reference intervals and the body weight regression coefficients may be useful to clinicians in detecting and interpreting different pathological features in the kidneys in rats.

The ultrasonographic wall layering of both the stomach and the duodenum was similar to that described in dogs and cats (Penninck 2008). Reference values for gastrointestinal tract wall thicknesses have been reported for dogs and cats and were also related to body weight in dogs (Delaney, O'Brien, and Waller 2003). Furthermore, ultrasonography is currently considered a reliable tool to diagnose and differentiate enteritis from infiltrative intestinal diseases in pets (Penninck et al. 2003). 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 (Boorman and Everitt 2005; Zwicker et al. 1992) and several infectious diseases (Orr 2002) occur in rats, the data reported herein may help clinicians in the ultrasonographic assessment of the gastrointestinal tract of rat.

The normal ultrasonographic pattern of the liver parenchyma and its similarity in echogenicity with that of the right kidney cortex were apparent in the studied animals. 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 (Lessa et al. 2010). Moreover, several proliferative and nonproliferative diseases of the hepatobiliary system of rats have been described (Thoolen et al. 2010).

Pituitary tumors that cause hyperadrenocorticism, adrenal gland hyperplasia (Kaspareit-Rittinghausen, Hense, and Deerberg 1989), pheochromocytomas (Martinez and Mog 2001) and adrenal gland adenomas ("Rat Health Guide") have been described as spontaneous diseases in rats. The reference values reported here may provide useful information for veterinarians to detect any changes to the size of the adrenal glands in rats. The right adrenal gland was larger than the left in both males and females. In addition, the right adrenal gland was 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, regardless of gender.

The figures and the data reported in the present study may provide veterinary clinicians and researchers with useful reference material for ultrasonographic evaluation of common

pet rats. However, further studies on higher frequency transducer images and reference data may deepen the information regarding the normal ultrasonographic appearance of this species.

2.4.5 Kidney measures and comparison between ultrasonographic and radiographic kidney measures

This study reports radiographic measurements of the kidneys and L2 vertebra in pet rats and the comparison between kidney radiographic and ultrasonographic measurements. L2 vertebral body length in rats was significantly correlated with body weight. However, there was no significant positive correlation between kidney ratios (kidney measures/L2 length) and body weight. For this reason, ratios can be considered independent of patient size and likely to provide more useful information than simple linear dimension (Feeney and Johnston 2007; Ackerman 1983).

The present study revealed that there was a statistical difference between means of left kidney length measured by radiography and ultrasonography. However, this difference was only 1.27 mm. In a survey radiographic study conducted on the ferret kidney, a similar significant difference of 2 mm was reported. However, based on the inter-observer variation in the radiographic measurement of the kidneys, the authors concluded that 2 mm of difference would not have any meaningful impact in a clinical setting (Eshar, Briscoe, and Mai 2013). In our study, as apparent differences between ultrasound and radiographs were minor and less than 2 mm, these measures could be treated interchangeably and one reference range can be used for both.

Radiographic kidney dimensions were larger than ultrasonographic ones in this study. Kang *et al.* found that ultrasonography has the tendency to underestimate the actual kidney size and the plain radiography to underestimate or overestimate the kidney size in human (Kang et al. 2007). The actual kidney size was not estimated in our study, therefore further studies comparing radiographic methods with actual kidney size in rats might provide useful information regarding normal kidney size of this species. In radiographs of rats the effect of magnification on kidney size should be minimal because these animals have relatively small body size and a table-top radiography was used which reduces the amount of magnification on the radiographs (Kealy, McAllister, and Graham 2010).

Variability for the kidney width was higher than the kidney length as a percentage of radiographically obtained linear renal dimensions. This is likely explained by greater

difficulty in defining the kidney width than the length (Eshar, Briscoe, and Mai 2013). This has also been observed in ferrets, cats and humans and suggests that kidney length is a good indicator of kidney size and a better radiographic criterion than width (Shiroma et al. 1999; Eshar, Briscoe, and Mai 2013; Huntington, Hill, and Hill 1991).

In the present study, statistically significant correlation was found between sex and left kidney length whereas no correlation between right kidney length and sex was found. However, strong correlation was found between body weight and kidney length. Kidney size differs by sex in humans, rats, mice, hamsters, ferrets, dogs and lizards with males commonly having larger kidneys than females. Differences in kidney size between genders are likely due to hormonal influence on the kidneys (Broulik 1983; Lane 2008).

The limitations of this study included the inability to rule out subclinical urinary tract disease, which in some cases may result in an alteration in kidney size. In addition, as animals were not fasted, the volume of the gastrointestinal tract overlying the kidneys made visualization challenging.

To conclude, this study provides useful information on the radiographic measurements of the rat kidney, which may be helpful for the veterinary clinician practice. Moreover, an interesting result came out from this project was that both radiography and ultrasonography appear valuable options for measurement of kidneys in this species. In addition, the obtained result supports the clinicians in the use of kidney/L2 ratios when evaluating kidney size, because the linear measurements seem to be correlated and dependent to body size in rats.

2.5 References of CHAPTER 2

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CHAPTER 3

Radiographic Anatomy of Dwarf Rabbit Abdomen with Normal Measurements

This chapter was adapted from

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3.1 INTRODUCTION

3.1.1 Diagnostic imaging in rabbits

Rabbits are widely kept as pets and their importance as veterinary patients continues to grow with specific requests for imaging (Reese 2011a). Radiographic images not only provide valuable information about various areas of dental and skeletal disease, but also help in the diagnosis of respiratory, gastrointestinal and urinary diseases in rabbits. Moreover, radiography plays an essential role in treatment decisions and prognosis determination in this species (Reese 2011a). Since large areas of the body can be imaged with radiography, size, shape, opacity, position and relationship of one organ to another can be determined (Redrobe 2001).

In literature, there are some introductory book chapters on normal radiographic anatomy (Girling 2002; Silverman and Tell 2005; Reese and Hein 2011; Fischetti 2012; Capello and Lennox 2013) or specific works on the head, thorax, vertebral column (Gibbs and Hinton 1981) and abdomen along with radiological features of some disorders of rabbits (Hinton and Gibbs 1982). In the latter study, the authors briefly described the normal radiologic examination of the abdominal viscera along with the location of stomach, large and small bowels and their contents, and the position of liver and kidneys (Hinton and Gibbs 1982). Several authors have focused particularly on the normal urinary tract of the rabbit using contrast-enhanced radiographic examination to assess the kidneys, ureters and urinary bladder (Dimitrov and Chaprazov 2012). Another two contrast studies on the normal kidney in the rabbit have been published (Porzio, Pharr, and Allen 2001; Sağlam et al. 2004). Urograms obtained by two routes (intravenous and intraosseus) were evaluated for renal opacification, delineation of the parts of the kidneys, ureteral opacification, urinary bladder opacification and filling (Porzio, Pharr, and Allen 2001; Sağlam et al. 2004).

Radiologic anatomy of the rabbit liver in terms of vasculature and biliary anatomy was studied by using hepatic venograms, aortograms, hepatic arteriograms, cholangiograms, and portograms (Seo et al. 2001). In addition, rabbit liver anatomy was studied using CT along with comparison with frozen crossectional cuts (Yovcheva et al. 2013). Moreover, a cross-sectional anatomy of the rabbit head (Van Caelenberg et al. 2010), neck and trunk (Zotti, Banzato, and Cozzi 2009) was studied using computed tomography (CT). The authors compared the computed tomographic results of the head (Van Caelenberg et al. 2010), neck, thorax and abdomen of the rabbit with the cadaver anatomy to provide an atlas of normal cross-sectional gross and CT anatomy (Zotti, Banzato, and Cozzi 2009). A comprehensive description of the normal ultrasonographic features, including some reference measurements, of the abdomen in mixed-breed dwarf rabbits has also been reported (Banzato et al. 2015).

3.1.2 Kidney measurements and comparison between radiographic and ultrasonographic kidney dimensions

A variety of diseases can affect the rabbit kidneys. Chronic interstitial nephritis, often associated with subclinical infections caused by Encephalitozoon cuniculi, is reported to cause a reduction in kidney size (Harcourt-Brown 2003a; Reese 2011b), whereas different spontaneous neoplasms of the kidney, including lymphoma may cause kidney enlargement in rabbits (Hatley and Smith 2004). Radiology can be a valuable part of the diagnostic workup of rabbits with suspected renal disease and in detecting renomegaly in rabbits (Harcourt-Brown 2007a).

The second lumbar vertebra (L2) is commonly used as a radiological Landmark to calculate kidney/L2 ratio in several species including rabbits (Hinton and Gibbs 1982; Dimitrov and Chaprazov 2012). Fifth lumbar vertebra (L5) has also been reported as a useful parameter for assessing kidney size in dogs (Barella et al. 2012) and as a reference for colon diameter assessment in cats (Trevail et al. 2011).

Diagnostic ultrasound and radiology are complementary and valuable diagnostic imaging techniques in rabbits and both represent non-invasive, minimally stressful and rapid diagnostic imaging tools (Redrobe 2001). Although ultrasound is the imaging tool of choice for soft tissue evaluation and renal size determination (Banzato et al. 2015), however, ultrasonographic equipment may not be available in all veterinary practices. Therefore, it could be interesting to investigate if accurate radiological methods could be used as an alternative in the evaluation of kidney size in rabbits.

3.1.3 Aims

To the author's knowledge, there is no comprehensive survey radiologic description, along with reference resources, for normal measurements of abdominal viscera in the dwarf rabbit and there is no calculated kidney/ L5 ratio in rabbits. Moreover, there is no comparison study in which kidney measures obtained by radiography and ultrasonography were compaired. Therefore, the aims of this study were to:

I. provide a comprehensive description of the abdominal radiographic features, including the most common abdominal reference measurements (kidneys, liver, colon diameter, ventral displacement of kidneys and colon from vertebral column) in clinically healthy dwarf rabbits;

II. compare kidney/L2 ratio with kidney/L5 ratio in healthy domestic dwarf rabbits to determine whether one ratio could be used instead of another in view of anatomical or pathological changes that could affect L2;

III. compare the linear dimensions of the kidney measured by ultrasonographic and radiographic methods.

3.2 MATERIALS AND METHODS

3.2.1 Animals

The study used 19 healthy adult pet dwarf rabbits (11 males and 8 females), with a mean age of 30 month (range 6-60 months) and a mean body weight of 1698.42±567.6 grams. The rabbits were client-owned and were enrolled to partecipate in the study on a voluntary basis. The rabbits were considered healthy on the basis of individual history and clinical examination. Moreover, complete abdominal ultrasound scan (Banzato et al. 2015), and whole-body radiographic analysis were obtained for each subject. The study was approved by the Padua University Ethical Committee (CEASA): Protocol No. 41060; July 12, 2010.

3.2.2 Imaging procedures

Radiographic imaging procedures were held for all animals in Department of Animal Medicine, Production and Health, University of Padua.

The animals were not fasted prior to the examination. Animals were sedated for radiographic examination with a combination of dexmedetomidine (25-50 μg/kg, Dexdomitor, Orion Corp, Espoo, Finland), midazolam (0.2-0.6 mg/kg, Midazolam- hameln, Hospira Italia, Naples, Italy) and ketamine (30 mg/kg, Ketavet 100, Intervet, Milan, Italy) injected intramuscularly in order to: cause less stress for the patient; provide exactly positioned radiographic images that can be reliably evaluated; repeat fewer radiographs are necessary and avoid manual fixation (radiation safety) just as we aimed for rats.

3.2.3 Radiographic examinations

a) General principles and equipment

Ventrodorsal (VD) and right lateral (RL) whole-body survey radiographs were obtained for all 19 rabbits using 6mAs (200-mA; 0.03 s) and 46-kVp with a focal film distance of 100 cm. No grid was used. A computed radiography system was used (Kodak point-of-care CR-360 system Carestream Health, Inc-Rochester, USA). Digital images were assessed on a DICOM workstation. Measurements were performed using electronic callipers.

b) Evaluation of radiographs

The radiological evaluation and technique used in this study was derived from (Lee and Leowijuk 1982) and adapted for the rabbit to efficiently assess the abdomen in this species. The ventrodorsal and lateral views were divided into segments using landmarks illustrated on Figure 24. The location of each organ was described in relation to these segments. The number of lumbar vertebrae for each

animal was recorded. Furthermore, the length of each lumbar vertebral body was measured from cranial end-plate to caudal end-plate and recorded.

The length and width of both kidneys were measured (Figure 25a.) and the position of the kidneys was determined in relation to the vertebrae on VD view. Moreover, the distance between the ventral surface of the lumbar vertebrae and kidneys was recorded (Figure 25b). Two ratios were calculated for kidneys by dividing the length or width by the length of the L2 and L5 vertebral bodies, respectively. The length of the liver was measured from the most cranial to the apex of the hepatic caudal border (Figure 26).

The maximum diameter of the descending colon at the level of the last three lumbar vertebrae was measured (Figure 27a). In addition, the distance between the ventral surface of L5 and the colon was recorded (Figure 27b).

The sizes of organs had been compared to the length of the L2 body in order to have a practical evaluation of the studied organs and to avoid the body weight effect when assessing normal size ranges.

For both kidneys and lumbar vertebral bodies measurements were performed on the VD view, whereas the RL view was used to perform measurements for the liver size, diameter of colon, ventral displacement of kidneys and colon from vertebral column.

All the anatomical terms were labelled according to the nomenclature proposed by Barone et al. (1973); Barone (2001).

3.2.4 Kidney measures

- **Ultrasonographic kidney measurements**

The ultrasonographic kidney measurements of the studied animals herein were obtained in a parallel research project developed by my group (Banzato et al. 2015).

- **Radiographic kidney measurements**

The kidney length and width were calculated using electronic callipers. The radiographic kidney length was considered as the maximum distance between cranial and caudal poles of the kidney and the kidney width as a perpendicular axis to the length of the kidney from the most medial margin to the most lateral margin (Figure 25a).

- **Comparison between radiographic and ultrasonographic kidney measurements**

The comparison between radiographic and ultrasongraphic kidney measurements was carried out using statistical tests which are explained in detail below.

3.2.5 Statistical analysis

- **Statistical analysis for radiographic data**

The descriptive statistics used were mean, standard deviation (SD), 95% confidence interval (CI) of the mean and the range (min-max). A t-test analysis was used to compare effect of sex on body weight, left/right kidney linear dimensions and kidney/L2 or kidney/L5 ratios. A paired t-test was used to determine whether there was significant difference between kidney/L2 ratio and correspondent kidney/L5 ratio. Furthermore, Spearman's rank correlation was used to assess the relationships between body weight and kidney, liver dimensions, distance between the vertebrae and kidney or colon. In addition, correlation between body weight and L2 or L5 body length was investigated. One way ANOVA variance analysis was run to compare the lumbar vertebral lengths in different positions (L1 vs L2 vs L3 vs L4 vs L5 vs L6 and vs L7).

Statistical analysis was performed with SAS 9.1 (SAS Institute Inc, Cary, NC). For all tests a P value < 0.05 was considered significant.

- **Statistical analysis for comparison of kidney measures**

Radiographic kidney data were tested for normal distribution using Shapiro-Wilk test and graphic methods such as histogram and Q-Q plot. Radiographic and ultrasonographic kidney measurements were compared using paired t- test to see whether one imaging modality could be used instead another. Moreover, Pearson Correlation coefficient test was used to assess the relationships between kidney dimensions measured using ultrasound and radiography.

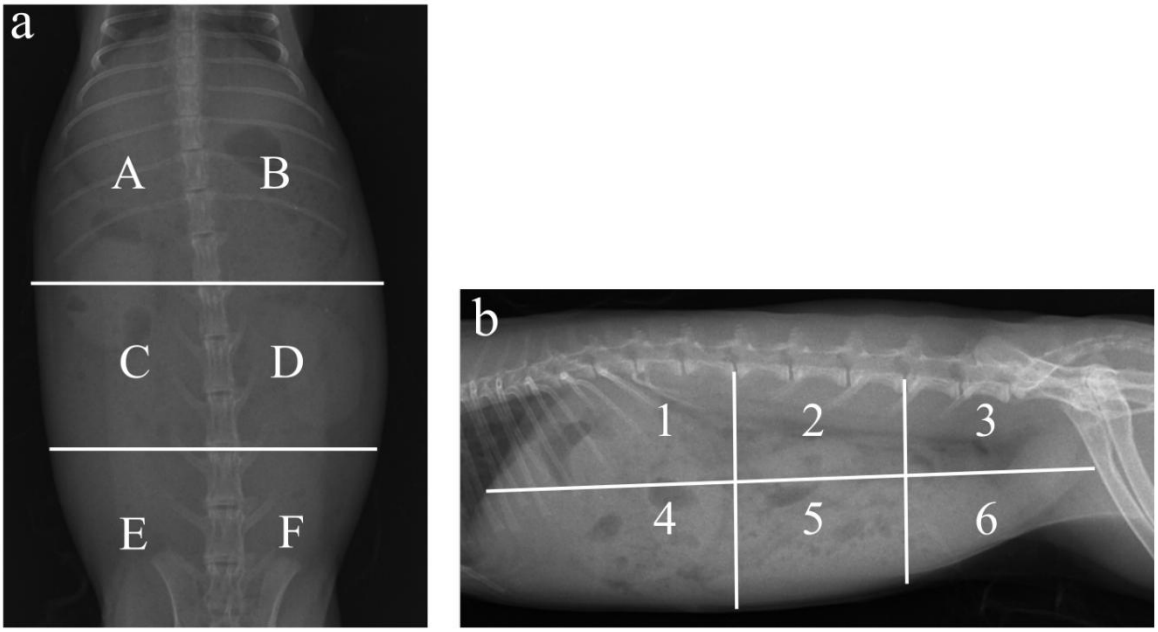

Figure 24 Schematic representation to illustrate the subdivision of the abdominal radiographs into six segments. (a) Ventrodorsal view (VD), segments A-F. (b) Right lateral (RL) view, segments 1-6.

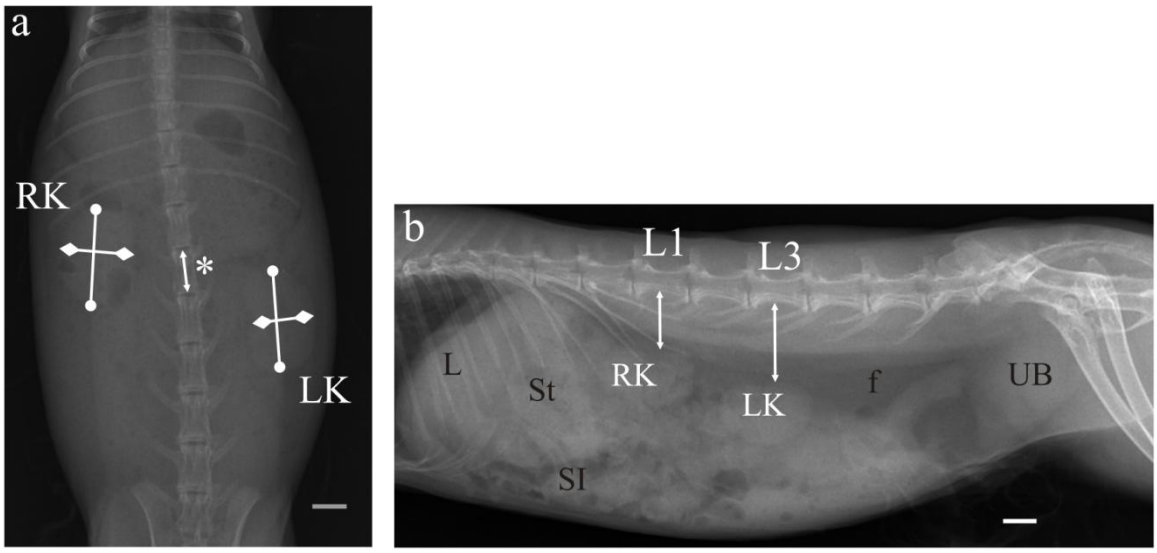

Figure 25(a) Ventrodorsal (VD) abdominal radiograph in a rabbit. The image illustrates with lines the measurements performed in the present study: L2 (star), length of L2 (arrow tip), kidney length (round tip), kidney width (diamond tip). (b) Right lateral (RL) abdominal radiograph in a rabbit. The image shows how ventral displacement of the right (RK) and left kidney (LK) was measured. L: liver, St: stomach, SI: small intestine, f: sublumbar fat, UB: urinary bladder. Bars, 10 mm.

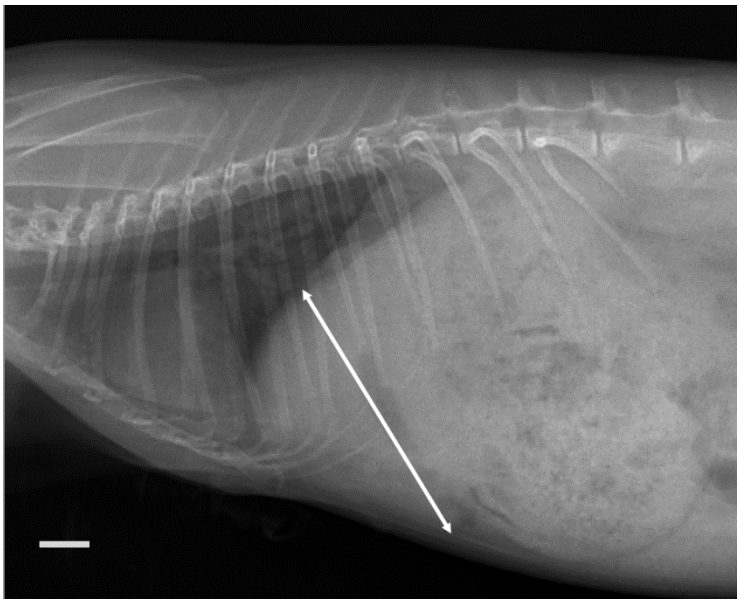

Figure 26 Right lateral radiograph in a rabbit. The image shows the measuring method of the liver length: a line drawn from the ventral border of caudal vena cava to the apex of the hepatic caudal border. Bar, 10 mm.

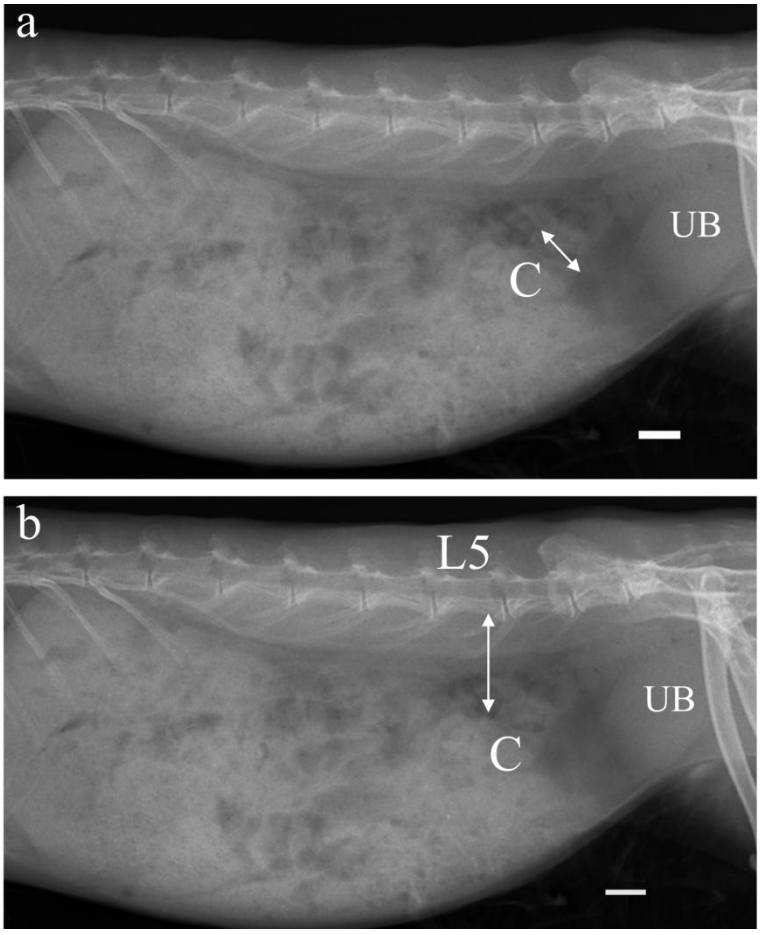

Figure 27(a) Right lateral radiograph in a rabbit. The image shows the measurement of the maximum diameter of the colon on the lateral view. C:colon, UB:urinary bladder. Arrow tip: the maximum diameter of the colon. (b) Right lateral radiograph in a rabbit. The image illustrates the measuring method of the distance between ventral surface of L5 (fifth lumbar vertebra) and colon (C). Bars, 10 mm.

3.3 RESULTS

3.3.1 Radiographic anatomy of the abdomen

- **Kidneys**

Radiographic measurements were obtained for the left kidney in 18/19 and for the right kidney in 17/19 animals and results are summarized in Table 1. The right kidney was between T13-L2 and was located in segments A-C and 1-2. On the other hand, the left kidney was between L2- 4 and was located in segments D and 2.

On average, t-test revealed no significant differences in female and male kidney linear dimensions and kidney ratios (P>0.05) (Table 10). Moreover, there were no significant gender differences in body weight and L2 body length, (P>0.05). The weight was highly correlated with right kidney length (r=0.60, P=0.009) whereas there was weak correlation between weight and left kidney length (r=0.46, P=0.05).

Paired t-test revealed a significant difference between kidney/L2 and kidney/L5 ratios for length and width, respectively (for all P<0.001 and t-values were 6.36, 6.38 for left kidney length and width; 6.19, 6.18 for right kidney length and width, respectively.)

The range (min-max) for renal displacement from the vertebral column was 13.14–16.47 (mm) and 11.31–22.80 (mm) for the right and the left kidney, respectively (Table 11). There was a significant correlation between body weight and distance between the vertebrae and left kidney (r=0.57, P=0.03). However, there was no significant correlation between body weight and distance between vertebrae and right kidney (r=–0.42, P=0.39).

- **Urinary bladder and ureters**

The bladder was seen in 13/19 RL radiographs. It could rarely be identified as a distinct organ on VD radiographs. The bladder was situated predominantly in segments E-F and 3-6. The ureters were not identified in any views.

Table 10. Direct kidney measurements and kidney ratios from ventrodorsal survey radiographs in rabbits.

Variable		No	Mean±SD[a] (total)	Males*	Females*	95% CI[b] (total)	Range (min-max)
LEFT	length(mm)	18	31.49±3.69	30.4±3.31	32.86±3.89	29.65-33.32	26.43-40.16
	width(mm)	18	17.71±1.84	17.66±2.14	17.77±1.51	16.79-18.62	14.43-21.44
	length:L2	18	2.11±0.33	2.07±0.38	2.15±0.27	1.94-2.28	1.39-2.56
	width:L2	18	1.19±0.19	1.2±0.21	1.17±0.18	1.09-1.28	0.76-1.44
	length:L5	18	2±0.29	1.97±0.34	2.05±0.23	1.86-2.15	1.33-2.47
	width:L5	18	1.13±0.17	1.14±0.2	1.11±0.15	1.04-1.22	0.72-1.37
RIGHT							
	length (mm)	17	30.34±3.5	29.66±3.22	31.11±3.86	28.54±32.15	24.49-40.01
	width (mm)	17	16.98±2.31	16.05±2.26	18.02±2	15.79±18.17	13.44-22.08
	length:L2	17	2.05±0.28	2.06±0.28	2.04±0.3	1.91-2.20	1.67-2.50
	width:L2	17	1.15±0.19	1.12±0.23	1.18±0.16	1.05-1.25	0.82-1.47
	length:L5	17	1.95±0.24	1.96±0.23	1.95±0.26	1.83-2.08	1.64-2.29
	width:L5	17	1.09±0.17	1.06±0.2	1.13±0.14	1-1.19	0.78-1.43

[a]SD: standard deviation, [b]CI: confidence interval; * Differences between genders were not statistically significant ($p>0.05$).

- **Liver**

The liver was identified as a homogeneous soft tissue shadow in both projections although the delineation of the apex of the hepatic caudal border was superior on RL view. The liver was situated mainly in the anteroventral segment (Figure 24b), segment 4) in RL view of the abdomen. In the VD view, it was restricted to segments A and B (Figure 24a). The range (min-max) for the liver length measurement and ratio (liver length/L2) was 42.18–56.54 (mm) and 2.54–3.64, respectively (Table 11). The Spearman correlation test revealed a significant relationship between body weight and liver length (r=0.61, P=0.015). The pancreas and adrenals were not identified.

- **Stomach**

The stomach was mainly distended beyond the costal arch with the pylorus positioned on the right side lying mainly at the level of T10-11 and the fundus, on the left side, was considerably caudal to the pylorus.

On the VD view, the stomach was between T10-L2. On VD projection, the stomach displayed a "J"-like shape whereas on RL view it showed a ballshape conformation. The stomach formed a heterogeneous, mottled soft tissue and gas density and was located in segments A-D and 1-4 (Figure 24).

- **Caecum**

Despite its size, the caecum was difficult to differentiate on radiographs. The cecal haustrae were not clearly identified. On VD view, the cecum was at the level of L1-6. Although the cecum was mainly located on the abdominal right side, it was slightly distended to the left side. The component parts of the cecum were not clearly identified in most studied animals. Its content was similar to that of the stomach, heterogeneous and mottled with small gas bubbles. On RL view, it resembled a non-homogeneous mass delineated with inguinal and sublumbar fat and was in contact with the ventral abdominal wall.

The cecum could be observed mainly in segments C-F and 2-4-5-6 (Figure 24).

- **Colon**

The ascending and transverse colon were difficult to identify as distinct radiographic components on both projections. The descending colon was easy to recognize in 14/19 radiographs. It was well delineated with the sublumbar fat pad and formed fecal pellets could be seen in the terminal colon although this was not consistent for all 14 rabbits. The colon could be observed in C-F and 2-5 segments. The maximum diameter and the ratio of the descending colon were 6.44-18.35 mm

(range) and 0.49-1.14 (range), respectively. The ventral displacement from the L5 vertebra was 10.45-28.80 mm and the ratio was 0.58-1.69 (Table 11). There was no significant correlation between body weight and distance between vertebra and colon (r=0.16, P=0.5).

- **Small intestines**

The components of the small intestine were not distinguishable radiographically. However, the small bowel was differentiated from large bowel with its curvilinear configuration and was located on the left, caudal to the stomach. Generally, the small intestine could be observed in segments A, C, D and 4-5 (Figure 24).

- **Spleen**

The spleen was identified in only 3/19 radiographs on VD view and in 1 radiograph on RL view. The spleen was usually located in the segment B, lying caudal to the stomach on VD view and in segment 4 on RL view, lying caudal to the liver in contact with the ventral abdominal wall.

- **Reproductive organs**

The uterus was identified in 2/8 females and was situated in segments 3-6, on RL view. On VD view, it was not identified. It was well delineated by sublumbar and inguinal fat as a contrasting radiopaque cord, running dorsal to the urinary bladder.

Nevertheless, the ovaries were not identified on neither views. The prostate and seminal vesicles could not be differentiated on any radiographs.

- **Relevant skeletal system**

One rabbit had 6 and the remaining 18 rabbits had 7 lumbar vertebra. The mean ± SD of the L1, L2, L3, L4, L5, L6 and L7 were 14.31±2.29; 15.23±2.3; 15.52±2.35; 15.76±2.25; 15.97±2.37; 14.78±2.13 and 12.27±1.86, respectively. The one way ANOVA variance analysis resulted with L7 to be the shortest lumbar vertebra and to have significant difference mean of the body length compared to the other lumbar vertebrae ($P<0.001$). However, there were no significant differences between the body length of L2, L3, L4, L5 and L6 ($P>0.05$). There was no significant difference between L1 and L7 body lengths ($P>0.05$). The Spearman correlation test revealed high linear correlation between L2 or L5 body length and body weight ($r=0.95$, $P<0.001$; $r=0.96$, $P<0.001$ respectively).

Table 11. Summarized radiographic measurements and ratios of ventral displacement of kidneys, liver length, colon maximum diameter, and ventral displacement of colon in rabbits.

Variable	No	Mean	SD	95% CI	Range (min-max)
VD-RK (mm)	6	14.8	1.34	13.38-16.21	13.14-16.47
VD-LK (mm)	13	16.82	3.22	14.87-18.77	11.31-22.80
VD-RK : L2	6	0.89	0.08	0.81-0.98	0.77-0.98
VD-LK : L2	13	1.09	0.17	0.99-1.19	0.81-1.37
LIVER (mm)	15	48.83	4.96	46.08-51.58	42.18-56.54
LIVER:L2	15	3.13	0.35	2.93-3.32	2.54-3.64
COLON (mm)	14	11.51	3.33	9.59-13.43	6.44-18.35
COLON : L2	14	0.73	0.21	0.61-0.85	0.49-1.14
VD-COLON (mm)	14	18.91	6.04	15.43-22.40	10.45-28.80
VD-COLON:L2	14	1.20	0.35	0.99-1.40	0.58-1.69

VD-LK: Ventral displacement of left kidney
VD-RK: Ventral displacement of right kidney
VD-LK: L2: Ratio of ventral displacement of left kidney to L2 (second lumbar vertebra)
VD-RK: Ratio of ventral displacement of right kidney to L2 (second lumbar vertebra)
VD-COLON: Ventral displacement of colon
VD-COLON:L2: Ratio of ventral displacement of colon to L2.

3.3.2 Comparison between ultrasonographic and radiographic kidney measures

For the left kidney, there was no significant difference between ultrasonographic and radiographic measures for width (P=0.5) whereas there was a significant difference for length (P=0.001). For the right kidney, there was no significant difference between ultrasosonographic and radiographic measures for width and length (P=0.29; P=0.06 respectively) (Table 12).

For the left kidney, there was a significant correlation between radiographic and ultrasonographic measures for length and width (r=0.72, P=0.0007; r=0.62, P=0.005 respectively). For the right kidney, there was no significant correlation between radiographic and ultrasonographic measures for width (r=0.24, P=0.35) whereas for the length there was a high significant correlation (r=0.73, P=0.0007).

Table 12. Results of paired Student's t-test between ultrasonographic and radiographic kidney measurements in dwarf rabbits.

Measurements	No	Mean Difference (mm)	SDD[a] (mm)	95% CL[b] (mm)	T-value	P-value*
LEFT						
Length	18	-2.42	2.57	(-3.7) - (-1.14)	-3.99	0.001
Width	18	-0.26	1.71	(-1.11) - (0.59)	-0.65	0.5
RIGHT						
Length	17	-1.21	2.54	(-2.52) - (0.08)	-1.98	0.06
Width	17	-0.66	2.50	(-1.94)- (0.62)	-1.09	0.29

*P <0.05 significant.

SDD[a]: Standard deviation of the difference, CL[b]: Confidence limit of the mean difference.

3.4 DISCUSSION

3.4.1 Radiographic anatomy of the abdomen

The size was measured and location described for each of the most clinically relevant and radiographically visible organs.

Kidneys

In the present study, the visibility of both kidneys was enhanced by intra-abdominal fat. The left kidney was more visible than the right. As it is observed in dogs, ferrets and rats (Burk and Feeney 2003; Eshar, Briscoe, and Mai 2013; Chen et al. 2012), this is likely the result of the right kidney being more cranially positioned and silhouetted with the liver (O'Malley 2005). In the present study, the position of the left kidney was more variable than the right and was located between L2-4, whereas the right kidney in most subjects was mainly located at the level of L1-2. As animals were not fasted, the volume and the content of soft tissue density of the gastrointestinal tract, especially cecum, may have induced this variability of the visualization and position of the kidneys. Moreover, in thin animals with lack of fat contrast to see the margin may influence this variability.

The magnification effect on radiographs should be minimal in the present study. This is likely due to the rabbit's small body size and the use of table-top radiography, which reduces the amount of magnification on the radiographs on kidney size (Kealy, McAllister, and Graham 2010).

The ratio of kidneys (kidney length/ L2 body length) recorded in this study was greater than that reported in another study (Hinton and Gibbs 1982) but smaller than that reported by (Dimitrov and Chaprazov 2012). The latter authors had carried out a contrast study of the urinary system. The greater ratios could be explained by magnification effects and the osmotic action of contrast medium (Ninan et al. 1990).

Bones of rabbits are relatively light and delicate compared to other mammals and the skeleton represents only 7% to 8% of body weight in rabbits (Vella and Donnely 2011). For this reason, lumbar fractures, vertebral spondylosis, scoliosis and lordosis are common among pet rabbits (McCullough et al. 2012; Harcourt-Brown 2003b). These conditions might affect the L2 lumbar vertebra which is used as a radiographic anatomical landmark to calculate the ratio for kidney length and width (Hinton and Gibbs 1982; Dimitrov and Chaprazov 2012). Therefore, we investigated whether L5 could be used instead of L2 to calculate kidney ratio. As it is suggested in previous studies (Barella et al. 2012; Trevail et al. 2011), L5 could be a reference for assessment of kidney size.

The present study revealed that there was a statistical difference between means of kidney/L2 ratios and kidney/L5 ratios for length and width. However, L2 and L5 were relatively similar in length

and there was no significant difference between the body length of the two vertebrae. This study confirmed that dwarf rabbits have fairly uniform lumbar vertebral length and consequently, it might be accurate to use any of those (L2 or L5) vertebrae for ratios in view of anatomical or pathological changes that might affect L2 which is commonly used as anatomical landmark in rabbits (Hinton and Gibbs 1982; Dimitrov and Chaprazov 2012). L2 and L5 body lengths in studied subjects were significantly correlated with body weight. Furthermore, there was a significant positive correlation between kidney length and body weight. For this reason, ratios can be considered independent of patient size and likely to provide more useful information than simple linear dimension. This is because proportional increase in renal measurements offsets longer lengths of L2 or L5, which correlate with patients' size. Therefore, the ratios result independent of patients' size (Eshar, Briscoe, and Mai 2013).

Pet rabbits are frequently obese and have extensive sublumbar fat bodies, which in severe cases can encompass the greater part of the abdomen, causing a cranioventral displacement of the internal organs (Reese and Hein 2011). In this study, the amount of the ventral displacement of kidneys and colon in rabbits and the relationship of the body weight on the displacement were investigated. As a result, the body weight was positively correlated with ventral displacement of the left kidney from the vertebral column. However, there was no significant correlation between body weight and right kidney ventral displacement. The latter finding might be due to small number of right kidney visualized on lateral radiographs. Nevertheless, during the evaluation of the displacement of the kidneys from their location due to pathologic changes in the abdominal cavity, these values might be helpful to determine maximum displacement of these organs.

Urinary bladder

The urinary bladder was identified in most subjects on RL view. However, the caudal extent of the bladder could not be discerned in some subjects. This might be likely due to the bladder was intrapelvic or the femur summation with the bladder.

Liver

Because radiography is considered the most basic and accessible form of diagnostic imaging that is available to the general practitioner (Meredith and Rayment 2000), the measurements of liver length and position provided in this study could be helpful to detect hepatomegaly in rabbits. However, the length of the liver, measured from the most cranial to the apex of the hepatic caudal border is not reflective of the entire liver volume, but simply a measurement that can be consistently made on the lateral radiograph. Liver length was significantly correlated with body weight in the rabbits used in this study. Similarly, L2 body length was also correlated with body weight. For a practical point,

liver length was expressed as a ratio of the L2 vertebral body. Therefore, we suggest to use the ratios rather than linear dimension when evaluating liver size in rabbits.

Stomach

Radiography continues to be the single most important diagnostic test for the evaluation of gastrointestinal tract of rabbits and to determine the response to therapy (Capello and Lennox 2008; Lichtenberger and Lennox 2010). Moreover radiographs are important for evaluation of location, size, shape and, to some extent, contents of the gastrointestinal tract (Harcourt-Brown 2007b; Oglesbee and Jenkins 2011; Lichtenberger and Lennox 2010). In the present study, the stomach was mainly distended beyond the costal arch, whereas in another study the stomach was reported to be mainly within the costal arch (Hinton and Gibbs 1982). In the case of gastrointestinal obstruction, it is reported that the stomach would be severely distended with gas, fluid or both (Harcourt-Brown 2007b; Oglesbee and Jenkins 2011). Although we had some inconsistent results regarding the position of the stomach, it is suggested that abdominal radiographs of the normal rabbit are somewhat variable and depend on the current phase of digestion (Lichtenberger and Lennox 2010).

Intestines

The caecal haustrae, the ascending and transverse colon and small intestines could not be reliably identified radiographically in this study. In obese rabbits sublumbar fat is affirmed to cause a significant (centimeters) caudoventral displacement of the descending colon (Reese and Hein 2011). In the present study, the ventral displacement of the descending colon was not correlated with body weight. Therefore, further studies using larger sample size may provide additional information regarding body weight effect on ventral displacement of colon in this species.

For the assessment of pathological conditions affecting these parts, it might be helpful to know the normal species-specific position of these structures (Reese and Hein 2011). The location of stomach, small intestines, colon, caecum and the amount of ventral displacement of colon from vertebral column reported in this study might be helpful to veterinary clinicians in this regard.

Spleen

Rabbits have a relatively small spleen which is thought to be related to the gut-associated lymphoid tissue they have (Harcourt-Brown 2003c) and normally cannot be identified on a radiographic image (Reese and Hein 2011). In this study, the spleen was identified in only few subjects on VD view superior to RL view. The landmarks provided herein to describe the location of the spleen might be helpful in pathological conditions affecting the spleen in rabbits.

Uterus

The uterus is rarely visualised in normal female rabbits unless they are obese (Reese 2011a). Although our study subjects were not overweight, in 2/8 animals the uterus was visualized. Uterine

adenocarcinoma is reported to be the most prevalent disorder, however pyometra and uterine torsion can be identified as an enlarged uterine mass in the caudal abdomen radiographically (Paul-Murphy 2007). The normal appearance and position reported in this study may help veterinary clinicians evaluate the uterus in this species.

The limitations of this study included small sample size and the inability to rule out subclinical liver disease and urinary tract disease, which in some cases may result in an alteration in kidney or liver size.

In conclusion, this study provides information on the abdominal radiographic anatomy and some useful measurements of the dwarf rabbit which may be helpful for the veterinary clinician. In addition, the clinicians should consider use of ratios, rather than linear measurements when making kidney and liver size evaluation. Both L2 and L5 body lengths appear viable options for measurement of kidney ratios in dwarf rabbits.

3.4.2 Comparison between ultrasonographic and radiographic kidney measures

The present study revealed that there was a statistical difference only between left kidney length means measured by radiography and ultrasonography. However, high correlation was found between ultrasonographic and radiographic left kidney length. Since right kidney means and left kidney width means measured by ultrasonography and radiography did not differ significantly, these measures could be treated interchangeably and one reference range can be used for both. This might particularly be helpful when the ultrasonographic equipment is not readily available in general practice or when the practitioner is unfamiliar with ultrasound machine or ultrasound anatomy of this species. So, an alternative might be to use the radiographic kidney measurements in dwarf rabbits.

3.5 References of CHAPTER 3

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