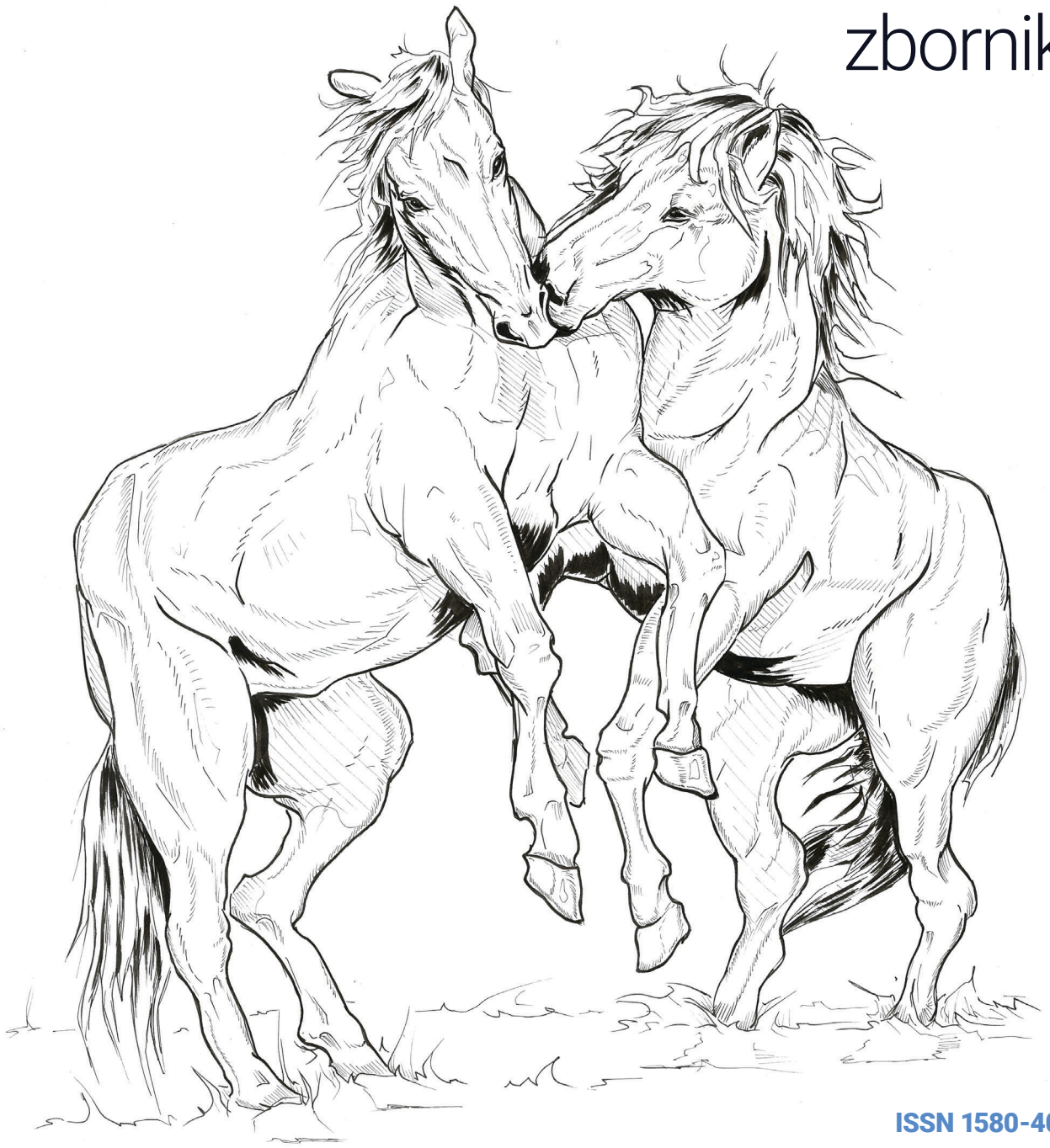
Slovenian Veterinary Research

Slovenski veterinarski zbornik

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA



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The cover illustration by Pšenica Kovačič, represents the Slovenian indigenous Posavje Horse breed.

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Editorial

Veterinary Illustration: *Science* and *Art* Telling a Story Together

Ilustracija v veterini: *Znanost* in *Umetnost* skupaj pripovedujeta zgodbo

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At first glance, *Science* and *Art* seem to be very different, as *Science* is supposed to be objective and guided by data, while *Art* is subjective and strongly influenced by feelings and opinions. While the results of *Science* and *Art* are quite different, both processes have undeniable similarities. Both put ideas about the world into a form that allows the viewer to connect with the idea. Observing a cell, a snake, or human nature is what natural sciences and art depend on, albeit for different purposes. Veterinary and medical illustrations capture visual representations of the animal or human body or individual parts for documentation purposes, for teaching, or for veterinary/medical practice. They are closely related to historical, social, and technological developments that have influenced and have been influenced by the content of illustrations, the artists themselves, and the techniques used to produce, reproduce, and disseminate them from prehistoric and historical times to the present.

Since prehistoric times, people have illustrated the natural world through art. The extensive collection of Paleolithic cave paintings found around the world testifies to this deeply rooted habit. One of the best-preserved examples, which I would like to point out because I was fascinated by them last year in Marseille, France, are the Paleolithic depictions of nature and animals, dated between 33,000 to 30,000 years ago. They were discovered in 1985 by Henri Cosqueri, a professional diver in Parc National les Canaques. Since the entrance to the Cosquer Cave is now below sea level,

Na prvi pogled se zdi, da sta si *Znanost* in *Umetnost* zelo raznoliki, saj naj bi bila *Znanost* objektivne narave ter podprta z pridobljenimi rezultati, medtem ko naj bi bila *Umetnost* bolj subjektivne narave ter močno pod vplivom občutkov in mnenj. Medtem, ko so rezultati, ki jih prinašata *Znanost* in *Umetnost* precej različni, sta hkrati po drugi strani obe zelo podobni, saj predstavita svoje ideje na način, da se opazovalec z njimi poveže. *Znanost* in *Umetnost* temeljita na opazovanju, bodisi celice, kače ali človeške narave, pa čeprav z različnim namenom. Veterinarske in medicinske ilustracije so bistvenega pomena za vizualno predstavljane zgradbe živalskega ter človeškega telesa oz. njegovih posameznih delov za namene dokumentiranja, poučevanja ali za namene veterinarske medicine/medicine v praksi. Tesno so povezane z zgodovinskim, družbenim in tehnološkim razvojem, na katerega je vplivala vsebina ilustracij, umetniki sami in tehnike, ki so jih uporabili za njihovo izdelavo, reprodukcijo in širjenje od prazgodovine do danes.

Že od prazgodovine naprej so ljudje skozi oči umetnosti upodabljali naravni svet. O tej globoko zakoreninjeni navadi priča obsežna zbirka paleolitskih jamskih slikarij, najdenih po vsem svetu. Eden najbolje ohranjenih primerkov, ki bi jih želela izpostaviti, ker so me lani navdušili v Marseillu v Franciji, so paleolitske upodobitve narave in živali, ki segajo v obdobje med 33,000 do 30,000 let pred našim štetjem. Leta 1985 jih je med potapljanjem odkril Henri Cosqueri v nacionalnem parku les Canaques. Ker je vhod v jamo

the images are very well preserved. Today we can all share this great experience by exploring the ancient art in the artificial cave in the Villa Mediterannea at the port of Marseille.

Throughout history, various forms of visual media have played an important role in communicating and explaining scientific concepts. The use of illustrations and other visual methods to convey scientific information is called scientific illustration. The main purpose of scientific illustration is to help the target audience better understand scientific concepts, whether they are researchers, students, or the general public. Therefore, scientific illustration is an important aspect of science communication at all levels. It tells a story. The distinctive feature of scientific illustration is the demand for accuracy and objectivity in presenting the concept as much as possible. A scientific illustration is certainly a form of an art, but art with the specific goal of communicating science.

Veterinary science illustrations are valuable visual representations for student and client education, publications, teaching, presentations, and many other purposes. Artists collaborate in all areas, whether illustrating histology textbooks, creating prosthetics, or designing artwork for pharmaceutical companies. Collaboration is key; researchers and clinicians, from veterinary to human medicine, microbiology, and pharmaceutical sciences, work with artists. Science is a language, and veterinary and medical illustrators translate that language for a wide audience using a visual language. Veterinary professionals can use veterinary science illustration to communicate complex and important information in clearly and concisely, in a way that is easy to understand. For example, illustrations can be used to show an animal's internal structures, such as its organs and bones, which can be difficult to visualize from the outside. They can also be used to demonstrate the progression of a disease or injury, helping clients understand what is happening to their animals and what treatment options are available. In addition, illustrations can be used to help veterinarians plan surgical procedures, by providing a detailed look at the anatomy of the affected area. They can also be used to document the progress of a treatment or procedure, allowing for more accurate tracking of changes over time.

Today, there are careers in veterinary or human medical illustration that combine scientific knowledge with artistic skill. A medical illustrator is a professional artist with advanced training in both life sciences and visual communication, who translates complex information into visual images, often in collaboration with scientists, physicians, veterinarians, and other experts. However, medical illustration is a small field in which there are not many trained professionals worldwide. Therefore, collaboration with artists and illustrators who share the same interest in this field is of great importance.

Cosquer pod morsko gladino, so slike zelo dobro ohranjene. Danes lahko vsi delimo to veliko izkušnjo z raziskovanjem starodavne umetnosti v umetno ustvarjeni jami v vili Mediterannea v pristanišču Marseilla.

Skozi zgodovino so imele različne oblike vizualnih medijev pomembno vlogo pri sporočanju in razlagi znanstvenih konceptov. Znanstvena ilustracija se nanaša na uporabo ilustracij in drugih vizualnih načinov z namenom posredovanja znanstvenih informacij različnim javnostim. Njen glavni namen je pomagati ciljnemu občinstvu bolje razumeti znanstvene koncepte, ne glede na to, ali so raziskovalci, študenti ali splošna javnost. Zato je znanstvena ilustracija pomembna na vseh ravneh v komunikaciji znanosti, saj pripoveduje njeno zgodbo. Posebnost znanstvene ilustracije je čim večja natančnost in objektivnost pri predstavitvi zamisli. Znanstvena ilustracija je vsekakor oblika umetnosti, vendar umetnosti s posebnim ciljem komuniciranja znanosti.

Veterinarske znanstvene ilustracije so dragoceni vizualni pripomočki za izobraževanje študentov in strank, pripravo raznovrstnih publikacij, poučevanje, predstavitve in številne druge namene. Umetniki lahko sodelujejo z veterinarsko stroko na vseh področjih, bodisi pri ilustriranju histoloških učbenikov, ustvarjanju protetičnih pripomočkov ali pri oblikovanju umetniških del za farmacevtska podjetja. Sodelovanje je pri tem ključnega pomena; med raziskovalci in kliniki, od veterinarske do humane medicine, mikrobiologije in farmacevtskih ved. Vsem je skupno sodelovanje z umetniki. Znanost je jezik, ki ga ilustratorji na področju veterinarske medicine in medicine prevajajo v za širše občinstvo razumljiv jezik in pri tem uporabijo svoj vizualni jezik. Veterinarski strokovnjaki lahko uporabljajo ilustracije s področja veterinarske znanosti za sporočanje zapletenih in pomembnih informacij na jasen in jedrnat način, ki omogoča lažje razumevanje vsebine. Ilustracije lahko na primer uporabimo za prikaz notranjih struktur živali, kot so njeni organi in kosti, ki si jih je od zunaj težko predstavljati. Uporabijo se lahko tudi za prikaz napredovanja bolezni ali poškodbe, kar strankam pomaga razumeti, kaj se dogaja z njihovimi živalmi in kakšne možnosti zdravljenja so na voljo. Poleg tega so lahko ilustracije uporabne za pomoč veterinarjem pri načrtovanju kirurških posegov, tako, da jim omogočijo podrobnejši vpogled v anatomijo prizadetega območja. Uporabljajo se lahko tudi za dokumentiranje napredka zdravljenja ali postopka, kar omogoča natančnejše sledenje spremembam skozi čas.

Danes celo obstajajo na področju veterinarske ali humane medicinske ilustracije poklici, ki združujejo oboje - določeno znanstveno znanje in umetniške spretnosti. Eden od takšnih je poklic medicinskega (znanstvenega) ilustratorja, ki je poklicni umetnik z daljšim usposabljanjem na področju znanosti in vizualne komunikacije, ki interpretira kompleksne informacije znanosti v vizualne podobe, pogosto v sodelovanju z znanstveniki, zdravniki, veterinarji in drugimi strokovnjaki. Vendar je medicinska (znanstvena) ilustracija

With this issue, in the light of *Science* and *Art* we welcome our first artistic collaboration with renewed artist Pšenica Kovačič, who has been working with the Veterinary faculty in Ljubljana for some time, enabling various researchers and academics to tell their stories to clients, students, and the scientific audience. She will be part of our team as an Art editor, designing and producing visual content such as illustrations for the cover of the journal and helping us communicate our stories to a wider audience.

This year's first issue contains four interesting and various peer-reviewed articles, a review article on alternatives to rectal temperature measurement in rats, two original scientific articles, on the hematological profile of the Posavje horse breed and on the influence of diet on oxidative stress parameters in cats and a case report on magnetic imaging of the crested porcupine.

Since the Posavje horse is an autochthonous "cold-blooded" horse typical of the southeastern part of Slovenia and Croatia, it was an easy decision to feature it on the first illustrated cover of the Slovenian Veterinary Research journal. The breed originated from unintentional crosses with various horse breeds, especially Belgian cold-blooded horses. It was selected mainly for heavy draft work, especially in steep forest areas. Today these horses are the smallest coldblooded breed in Europe and their pedigree was introduced in 1993. This breed is characterized by a small, thin head, a straight profile, a medium-length neck with a short back, and a short, very broad, and moderately restrained lower back. They have relatively large and strong hooves. The legs are covered with a protective coat. They have a robust constitution, are very fertile and their sexual dimorphism is pronounced. The breed is known to be good-natured and peaceful.

I would like to thank our new Editor-in-Chief Dr. Klementina Fon Tacer for inviting me to write this Editorial and for paving the way to combine *Art* and *Science* in the field of veterinary illustration in our journal. During the preparation of this Editorial, a lot of material and ideas were collected on this topic, so there will be more to report in one of the future issues of the Journal.

I would like to conclude with a quote from American surgeon and medical illustrator Frank H. Netter.

"Draw what can't be seen, watch what's never been done, and tell thousands about it without saying a word." Frank H. Netter, M.D.

zelo specifično in ne preveč razširjeno področje ustvarjanja, z le nekaj izobraženimi strokovnjaki po vsem svetu. Zato je zelo pomembno sodelovanje z umetniki in ilustratorji, ki jih to področje zanima.

V prvi letošnji številki v luči *Znanosti* in *Umetnosti* pozdravljamo naše prvo umetniško sodelovanje s priznano umetnico Pšenico Kovačič, ki že nekaj časa sodeluje z Veterinarsko fakulteto v Ljubljani in omogoča večim raziskovalcem in akademikom, da pripovedujejo svoje zgodbe naročnikom, študentom, kakor tudi znanstvenem občinstvu. Postala je del naše uredniške ekipe kot umetniška urednica, ki bo oblikovala in pripravljala vizualne vsebine, kot so ilustracije za naslovnico revije, ter nam pomagala posredovati naše zgodbe širšemu občinstvu.

Letošnja prva številka vsebuje štiri zanimive in raznolike recenzirane članke, in sicer pregledni članek o alternativah rektalnemu merjenju temperature pri podganah, dva originalna znanstvena članka o hematološkem profilu avtohtone slovenske pasme posavskega konja oz. posavca in vplivu prehrane na parametre oksidativnega stresa pri mačkah ter študijo primera o magnetnem slikanju afriškega ježevca.

Ker je posavski konj kot avtohtoni »hladnokrvni« konj, značilen za jugovzhodni del Slovenije in Hrvaške, je bila odločitev, da prav to pasmo upodobimo na prvi ilustrirani naslovnici revije Slovenskega Veterinarskega Zbornika, hitro sprejeta. Pasma je nastala z naključnimi križanji različnih pasmah konj, predvsem belgijskimi hladnokrvnimi konji. Selekcioniran pa je bil zlasti za težka vlečna dela, predvsem v strmih gozdnih predelih. Danes pasma velja za najmanjšo hladnokrvno pasmo konj v Evropi. Rodovnik so uvedli leta 1993. Za pasmo je značilna majhna, tanka glava, z ravnim profilom, srednje dolgim vratom, kratkim hrbtom ter kratkim, zelo širokim in zmerno omejenim spodnjim delom hrbta. Njihova kopita so relativno velika in močna. Noge so pokrite z zaščitno dlako. Imajo robustno konstitucijo, so zelo plodni in imajo izrazit spolni dimorfizem. Pasma je znana kot dobrodušna in miroljubna.

Naši novi glavni urednici dr. Klementini Fon Tacer se zahvaljujem za povabilo k pisanju tega uvodnika in za tlakovanje poti združevanja *Umetnosti* in *Znanosti* na področju veterinarske ilustracije v naši reviji. Med pripravo uvodnika se je nabralo veliko gradiva in idej na to tematiko, zato bo sledilo podrobnejše nadaljevanje v eni od prihodnjih številk revije.

Zaključujem pa s citatom ameriškega kirurga in medicinskega ilustratorja Franka H. Netterja.

"Rišite, česar se ne vidi, glejte, kar še ni bilo narejeno, in na tisočim povejte o tem, ne da bi rekli besedo." Dr. Frank H. Netter

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Review Article

Suitability of Alternatives to Rectal Temperature Measurements in Pet Rodents, Rabbits and Ferrets: A Literature Review

Key words

rectal temperature,
rodents,
rabbits,
ferrets,
alternatives

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Abstract: Body temperature is a vital parameter to assess the health of exotic animals. Rectal thermometry is a common way to measure body temperature in rodents, rabbits and ferrets and often considered the gold standard. However, taking a rectal temperature often involves restraint and can lead to stress in these animals. To avoid the stress of rectal temperature measurements, alternative (often less invasive) techniques have been utilized in several species. These methods include tympanic thermometry, axillary thermometry and infrared thermography. It is however important to establish whether these strategies yield comparable readings to the gold standard. Therefore, a literature review was performed using the MedLine and Google Scholar databases. Base terms referring to rectal temperature and thermometry were combined with species-specific search terms. Relatively few studies were identified about alternatives to rectal temperature measurements in rodents, rabbits and ferrets. In general, it can be noted that only transponder measurements have repeatedly been described to be a valid alternative to rectal temperature measurement. Further research should be conducted.

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Introduction

Body temperature is a vital parameter to assess the health of exotic animals (1). An elevated body temperature can signal an infection or systemic inflammation (2) while hypothermia may, for example, arise as a complication as a complication of general anaesthesia and surgery (3). In rodents and rabbits, body temperature was shown to be of prognostic value in a clinical setting (4, 5).

Rectal thermometry is a common way to measure body temperature in rodents (6), rabbits (7) and ferrets (8) and often considered the gold standard. However, taking a rectal temperature often involves restraint and can lead to stress in these animals (9, 10). This can, amongst others, impact the readings (11).

To avoid the stress of rectal temperature measurements, alternative (often less invasive) techniques have been utilized in several species. These methods include tympanic thermometry (12), axillary thermometry (13) and infrared thermography (14). It is however important to establish whether these strategies yield comparable readings to the gold standard. In cats and dogs, such studies have been conducted suggesting that alternative methods are not always a good replacement for rectal thermometry (14, 15). In rodents, rabbits and ferrets, the body of literature comparing temperature measurement seems to be limited while this is a particularly important topic because of the stress-inducing consequences of rectal thermometry.

Therefore, the aim of the current review article is to summarize the non-invasive temperate measure methods investigated in pet rodents, rabbits and ferrets and assess whether they are a suitable alternative to rectal temperature measurement. Additionally, suggestions for further research are formulated.

Search strategy and inclusion

Between the 3rd and 5th of September 2022, the MedLine database was searched through Pubmed. The base term "("rect*") AND (("temp*") OR ("therm*"))" was combined with species-specific terms: "AND (("guinea pig*") OR ("cavy") OR ("cavies"))", "AND (("mouse*") OR ("mice"))", "AND (("rat") OR ("rats"))", "AND ("hamster*")", "AND ("gerbil*")", "AND ("degu*")", "AND ("chinchilla*")", "AND ("rabbit*")" and "AND ("ferret*")". Google Scholar was searched during the same period with the base term "rectal temperature" combined with the following species-specific terms: "guinea pig*", "mice", "rat*", "hamster*", "gerbil*", "degu*", "chinchilla*", "rabbit*" and "ferret*".

The titles of the publications in the search results were screened for papers that could be eligible for inclusion. The abstracts of potentially eligible publications were read and included if (1) they described a comparison between rectal temperature measurement and at least one non-invasive method, (2) described agreement between rectal measurement and a non-invasive method and (3) the study was conducted in at least one eligible species (rodent, ferret or rabbit).

Characteristics of included studies

The searches yielded eligible studies for guinea pigs (3 studies, 16-18), mice (5 studies; 16, 19-22), rats (4 studies; 9, 19, 23-24), chinchillas (1 study, 25), rabbits (3 studies; 7, 16, 26) and ferrets (3 studies; 8, 27, 28). No eligible studies were identified for hamsters, gerbils and degus. The included studies and important characteristics are shown in table 1. All publications described prospective studies of multiple animals with sample sizes ranging from 6 to 48. Studies were conducted in both healthy animals and patients. In selected studies, there was a focus on laboratory animals. Publication dates ranged from 1997 and 2021, but it is clear that a significant number was published over 15 years ago.

Suitability of alternative methods per species

In Guinea pigs, two studies (16,17) investigated the use of transponders to measure body temperature, with mixed results. Both studies were conducted in an experimental setting using animals raised as laboratory animals. One study mentioned it to be a valid alternative to rectal temperature (17) while the other mentioned it was not (16). Other methods compared to rectal temperatures were tympanic, laser, axillary and inguinal thermometry (4, 17, 18). None of these methods were mentioned to be a valid alternative for rectal temperature measurement. The authors of the second study (17) mentioned that the transponder system they used made sounds that may be disturbing to the guinea pigs. Additionally, they stated that due to the hand-held nature of non-contact thermometers, it is difficult to obtain measurements from a comparable distance.

In mice, microchip transponders were also investigated, both subcutaneously and intraperitoneally (16, 19). One of these studies reported subcutaneously and intraperitoneal transponders to be a valid alternative to rectal temperature measurement (19). One study also mentioned infrared thermometry of the ear and back skin to be a valid alternative (20). They also stated that this technique allows skin temperature to be measured easily at these sites. Other strategies were not deemed a suitable alternative in all cases (20, 21, 22). All of these studies were performed in laboratory animals.

In rats, microchip transponders (intraperitoneally and subcutaneously) and (temperature-sensitive) telemetry were deemed to be usable alternatives to rectal thermometry (9,19, 23, 24). However, as stated above, it is important to assess whether the specific detection method does not disturb the animals. Additionally, the telemetry was only investigated in a research setting (9, 23). This means it should be assessed whether these results can be translated to the clinic. Finally, all of these studies were performed in laboratory rats.

Human and veterinary thermometers were investigated as an alternative to rectal temperature measurement in chinchillas (25). Unfortunately, both methods were deemed unsatisfactory. In this case, the studies were also conducted in an experimental setting. The animals were sourced from breeding facilities. Thermography was assessed in the eye, inner ear, external ear and nose of rabbits (26). The publication mentioned that this was an effective tool to measure the temperature of several regions. However, this is not the same as being a reliable alternative for body temperature measurement in a clinical setting.

Implantable microchip transponders were mentioned as a suitable alternative (7). Noncontact infrared thermometer (ear and thigh) and tympanic thermometer (human and veterinary) were not a replacement for rectal temperature measurement (7). All of these studies were performed in a research setting with laboratory animals.

In ferrets, microchip transponder thermometry was mentioned as an alternative to rectal temperature measurement (8). Paediatric and veterinary auricular, axillary, dorsal skin, inguinal, noncontact infrared and tympanic thermometry were not deemed to be alternatives (27, 28). One study (27) was conducted in animals presented within a clinical setting. The other studies were conducted in an experimental setting in laboratory animals.

Discussion

Relatively few studies have been published about alternatives to rectal temperature measurements in rodents, rabbits and ferrets. The internal and external validity of the published studies also leaves room for improvement. In general, it can be noted that only transponder measurements have

Table 1: Study design of includes studies

Reference	Title	Study design	Sample size	Database	
	Guinea pigs				
Hartinger et al., 2003	Suitability of temperature-sensitive transponders to measure body temperature during animal experiments required for regulatory tests	Prospective	10	PubMed	
Devalle, 2005	Comparison of tympanic, transponder, and noncontact infrared laser thermometry with rectal thermometry with rectal thermometry in strain 13 Guinea pigs (Cavia porcellus)	Prospective	28	PubMed	Google Scholar
Levy et al., 2020	Comparison of axillary and inguinal body temperature to rectal temperature in healthy guinea pigs (Cavia porcellus)	Prospective	40		Google Scholar
	Mice				
Kort et al., 1997	A microchip implant system as a method to determine body temperature of terminally ill rats and mice	Prospective	10	PubMed	Google Scholar
Hartinger et al., 2003	Suitability of temperature-sensitive transponders to measure body temperature during animal experiments required for regulatory tests	Prospective	12	PubMed	
Saegusa and Tabata, 2003	Usefulness of infrared thermometry in determining body temperature in mice	Prospective	6	PubMed	
Newsom et al., 2004	Comparison of body surface temperature measurement and conventional methods for measuring temperature in the mouse	Prospective	12	PubMed	Google Scholar
Fiebig et al., 2018	Evaluation of Infrared thermography for temperature measurement for temperature measurement in adult male NMRI nude mice	Prospective	10	PubMed	
	Rats				
Dilsaver et al., 1992	Measurement of temperature in the rat by rectal probe and telemetry yields compatible results	Prospective	12	PubMed	Google Scholar
Kort et al., 1997	A microchip implant system as a method to determine body temperature of terminally ill rats and mice	Prospective	30	PubMed	Google Scholar
Eshraghi et al., 2005	Cochlear temperature correlates with both temporalis muscle and rectal temperatures. Application for testing the otoprotective effect of hypothermia	Prospective	6	PubMed	Google Scholar
Dangarembizi et al., 2017	Measurement of body temperature in normothermic and febrile rats: Limitations of using rectal thermometry	Prospective	31	PubMed	Google Scholar
	Hamsters				
	Gerbils				
	Degus				
	Chinchillas				
Ozawa et al., 2017	Comparison of rectal and tympanic thermometry in chinchillas (Chinchilla lanigera)	Prospective	47	PubMed	Google Scholar
	Rabbits				
Hartinger et al., 2003	Suitability of temperature-sensitive transponders to measure body temperature during animal experiments required for regulatory tests	Prospective	10	PubMed	
Chen and White, 2006	Comparison of rectal, microchip transponder, and infrared thermometry techniques for obtaining body temperature in the laboratory rabbit (Oryctolagus cuniculus)	Prospective	46	PubMed	Google Scholar
Jaén-Téllez et al., 2021	Relationship between rectal temperature measured with a conventional thermometer and the temperature of several body regions measured by infrared thermography in fattening rabbits. Influence of different environmental factors	Prospective	48		Google Scholar
	Ferrets				
Maxwell et al., 2016	Comparison of digital rectal and microchip transponder thermometry in ferrets (Mustela putorius furo)	Prospective	16	PubMed	Google Scholar
Aguilar et al., 2018	Comparison of body temperature acquired via auricular and rectal methods in ferrets	Prospective	27		Google Scholar
Keeney et al., 2020	Comparison of body temperature using digital, infrared, and tympanic thermometry in healthy ferrets (Mustela putorius furo)	Prospective	20		Google Scholar

repeatedly been described to be a valid alternative to rectal temperature measurement.

It is clear that there is a difference in number of studies per species. One potential reason for the higher number of studies in mice and rats is that they are often used as laboratory animals. Temperature measurements are often performed in animal experiments (6) and need to be reliable and not be impacted by stress-induced responses. This may stimulate research into this area, which can be translated into clinical practice. Further research is needed to address the lack of studies in hamsters, gerbils and degus.

Table 2: Data extracted from included studies

Reference	Alternative temperature measurement method	Agreement metric with rectal measurement	Valid alternative for rectal measurement according to publication?
		Guinea pigs	
Hartinger et al., 2003	Implanted temperature-sensitive transponders	Only graphically	No
Devalle, 2005	Tympanic thermometer	0.3956 intraclass correlation coefficient	No
Devalle, 2005	Laser	0.1229 intraclass correlation coefficient	No
Devalle, 2005	Transponder	0.5880 intraclass correlation coefficient	Yes
Levy et al., 2020	Axillary	difference of mean -0.39 (95% CI -0.54 - -0.23)	No
Levy et al., 2021	Inguinal	difference of mean was -0.73 (95% CI -0.94 - -0.52)	No
		Mice	
Kort et al., 1997	Microship transponder (subcutaneous)	differences within ± 0.5°C	Yes
Kort et al., 1997	Microship transponder (intraperitoneally)	differences within ± 0.5°C	Yes
Hartinger et al., 2003	Implanted temperature-sensitive transponders	Only graphically	No
Saegusa and Tabata, 2003	Infrared thermometry (ear)	correlation r = 0.95	Yes
Saegusa and Tabata, 2003	Infrared thermometry (back skin)	correlation r = 0.96	Yes
Saegusa and Tabata, 2003	Infrared thermometry (tail skin)	correlation r = 0.59	No
Saegusa and Tabata, 2003	Infrared thermometry (sole skin)	correlation r = 0.59	No
Newsom et al., 2004	Surface temperature measurements	correlation r = 0.9773	No
Newsom et al., 2004	Telemetry	correlation r = 0.9699	No
Fiebig et al., 2018	Infrared Thermography/Camera	mean difference of 0.56 °C	Yes* (in nude mice)
		Rats	
Dangarembizi et al., 2017	Temperature-sensitive radiotelemeters (intraperitoneally)	rectal 0.5°C lower or 0.7°C greater than radiotelemeter	Yes* (but investigated for research setting)
Eshraghi et al., 2005	Cochlear temperature	Correlation r = 0.959	No
Kort et al., 1997	Microship transponder (subcutaneous)	differences within ± 0.5°C	Yes
Kort et al., 1997	Microship transponder (intraperitoneally)	differences within ± 0.5°C	Yes
Dilsaver et al., 1992	Telemetry	after salicylate r = +0.83, after oxotremorine r = +0.93	Yes* (but investigated for research setting)
		Hamsters	
		Gerbils	
		Degus	
		Chinchillas	
Ozawa et al., 2017	Human tympanic thermometer	margin of error (combined human/veterinary) 1.7°C	No
Ozawa et al., 2017	Veterinary tympanic thermometer	margin of error (combined human/veterinary) 1.7°C	No
		Rabbits	
Hartinger et al., 2003	Implanted temperature-sensitive transponders	Only graphically	No
Chen and White, 2006	Implantable microchip transponder	95% agreement limit: ±1.48	Yes
Chen and White, 2006	Noncontact infrared thermometer (ear)	Not calculated due to systematic deviations from avg temp	No
Chen and White, 2006	Noncontact infrared thermometer (thigh)	Not calculated due to systematic deviations from avg temp	No
Chen and White, 2006	Human tympanic thermometer	Not calculated due to systematic deviations from avg temp	No
Chen and White, 2006	Animal tympanic thermometer	Not calculated due to systematic deviations from avg temp	No
Jaén-Téllez et al., 2021	Thermography (eye)	Coefficient of determination: 0.15	Unclear
Jaén-Téllez et al., 2021	Thermography (inner ear)	Coefficient of determination: 0.22	"Best"
Jaén-Téllez et al., 2021	Thermography (external ear)	Coefficient of determination: 0.24	"Inefficient"
Jaén-Téllez et al., 2021	Thermography (nose)	Coefficient of determination: 0.20	Unclear
		Ferrets	
Maxwell et al., 2016	Microchip transponder thermometry	95% Agreement limits (°F): −1.82 to +1.96 (comp. to calibrated rectal) −2.19 to +0.84 (comp. to common rectal)	Yes
Aguilar et al., 2018	Pediatric auricular thermometers	correlation thermometer 1: 0.4726 correlation thermometer 2: 0.5388	No
Aguilar et al., 2018	Veterinary auricular thermometers	correlation 0.6311	No
Keeney et al., 2020	Axillary	95% CI of Mean Difference -1.32, -0.67	No
Keeney et al., 2020	Dorsal Skin	95% CI of Mean Difference -0.14, 0.24	No* (possibly after further studies)
Keeney et al., 2020	Inguinal	95% CI of Mean Difference -1.15, -0.63	No
Keeney et al., 2020	Noncontact Infrared	95% CI of Mean Difference -8.37, -6.43	No
Keeney et al., 2020	Tympanic	95% CI of Mean Difference -0.78, -0.38	No

A further limitation is that most of the studies were conducted in an experimental setting and may therefore not fully resemble the clinical setting. Additionally, only one study was performed with animal patients while the rest were conducted with laboratory animals. These animals may have different relevant characteristics than patients, including different stress sensitivity.

For now, it seems that rectal temperature measurement should remain the golden standard until further research has been performed.

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Ustreznost alternativnih tehnik rektalnemu merjenju temperature pri hišnih glodavcih, kuncih in belih dihurjih - pregled literature

J. Stans

Izvleček: Telesna temperatura je pomemben parameter za oceno zdravja eksotičnih živali. Rektalno merjenje temperature je običajen način merjenja telesne temperature pri glodavcih, kuncih in belih dihurjih in pogosto velja za zlati standard. Vendar je merjenje rektalne temperature pri teh živalih pogosto povezano z omejevanjem gibanja in povzročanjem stresa. Da bi se izognili stresu pri merjenju rektalne temperature, so bile pri več vrstah živali uporabljene alternativne (pogosto manj invazivne) tehnike. Te metode vključujejo infrardečo termografijo ter merjenje temperature timpanično in aksilarno. Vendar pa je pomembno ugotoviti, ali te strategije dajejo primerljive rezultate z zlatim standardom. Zato smo opravili pregled literature z uporabo podatkovnih zbirk MedLine in Google Scholar. Osnovni izrazi, ki se nanašajo na rektalno temperaturo in merjenje temperature, so bili združeni z iskalnimi izrazi, značilnimi za posamezne vrste. Pri glodavcih, kuncih in belih dihurjih je bilo najdenih razmeroma malo študij o alternativah rektalnim meritvam temperature. Na splošno lahko ugotovimo, da so bile le meritve s transponderjem večkrat opisane kot veljavna alternativa rektalnemu merjenju temperature. Potrebne so nadaljnje raziskave.

Ključne besede: rektalna temperatura; glodavci; kunci; beli dihurji; alternative

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Original Research Article

Characterisation of the Haematological Profile in the Posavje Horse Breed

Key words

autochthonous breeds,
Posavje horse,
haematology,
age,
sex

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Abstract: The aim of this study was to investigate the influences of sex and age on haematological values in the Posavje Horse breed. A total of 163 healthy Posavje horses (30 foals, 94 mares and 39 stallions) were used in this study; their complete blood counts and a leucogram were obtained with a haematological analyser. The horses were classified into five groups: foals (1 to 6 months, n = 30), 3 to 6 years (n = 8 stallions/21 mares), 7 to 9 years (n = 9 stallions/22 mares), 10 to 13 years (n = 8 stallions/20 mares), 14 to 15 years (n = 6 stallions/10 mares) and 16 and over (n = 8 stallions/21 mares). The results obtained show an influence of sex on haematological parameters, with red blood cell count (RBC), haematocrit (HCT) and haemoglobin concentration (HGB) being higher in stallions ($P < 0.001$) and white blood cell count (WBC) being higher in mares. Differences between the age groups of the Posavje horses examined indicate a decrease in RBC and HGB with a compensatory increase in mean corpuscular volume and mean corpuscular haemoglobin, a decrease in WBC and platelet counts (PLT) and proportion of lymphocytes, and an increase of neutrophil to lymphocyte ratio (N/L) with age ($P < 0.001$). Although the Posavje horse is classified as a draft horse breed, its haematological parameters show characteristics common to warm-blooded breeds, with the exception of the N/L ratio. One of the most important findings of this study is a higher neutrophil count in reproductively active breeding stallions. Higher levels of RBC, HGB, HCT and neutrophil count in the Posavje stallions suggest an effect of androgens (testosterone), which may be an effective mechanism to prevent infections, that can affect the survival of the stallions and thus the evolution of the species.

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Introduction

Intensive artificial and natural selection have shaped substantial variation among horse breeds, which are reflected in the differences of haematological and biochemical parameters. In addition to geographical origin, horse breeds can be divided into phenotypic or performance categories. Due to the great diversity of breeds, horses are most often classified as "warm-blooded" breeds including light horses of Arabian descent (such as Arabians, Thoroughbreds, Standardbreds and Quarter Horses), and "cold-blooded" breeds essentially including heavy draft horses (such as the Belgian Horse, the Slovenian Cold Blooded Horse and the Posavje Horse). Several differences in haematological parameters were found between these two groups, such as a lower haematocrit in cold-blooded horses and higher erythrogram values in warm-blooded horses, which should be considered when determining reference values of blood parameters and interpreting blood tests (1-4). In contrast, light horse breeds have a higher red blood cell count (RBC), haemoglobin concentration (HGB), haematocrit value (HCT) and blood volume than draft horses (2, 5, 6). In addition to horse type, haematological parameters may also vary due to numerous internal and external factors, including breed, sex, age, reproductive status, fitness and training status, exercise load, feeding and, circadian variations.

Moreover, handling procedures during blood withdrawal, operating conditions, criteria for selecting healthy subjects, preparation of the subjects for the procedures, level of excitement and health status are also important factors that affect haematological values in horses (1, 6, 7, 8).

Differences in the cellular constituents of the blood are the result of specific changes in an organ or organ system, or a general response of the individual to certain physiological or pathological conditions (6). For example, the total leukocyte count (WBC) and differential leukocyte count in healthy horses are dependent on age, which is associated with a steady decline in leukocyte counts (9-11) while the absolute and relative leucocyte counts, especially neutrophils and lymphocytes, vary considerably (11) to reach a neutrophil to lymphocyte (N/L) ratio of 2:1 in older horses (2). Minor differences in leukocyte counts have been found between the different breeds of horses, with warm-blooded horses having higher WBC counts than to cold-blooded horses (6, 12).

The horse population investigated in this study was the autochthonous Posavje Horse breed, originating from the Lower Sava River flatlands of the southeastern part of Slovenia (especially in the districts of Krsko and Brezice) and in Croatia, this breed resulted from the crossing of local warmblood mares with Norik stallions. In addition, Ardene stallions were used to improve the Posavje horses' abilities for heavy draft work (13). The Posavje horse is the smallest cold-blooded breed in Europe, characterised by a good-natured temperament and a pronounced sexual dimorphism. It was mainly selected for heavy draft work, especially in steep forest areas, but it has also been used for meat production (13). In 1993, a Slovenian breeding and conservation programme was established for this horse breed. Since then, the breed has been bred according to the principles of conservation genetics: narrow relation of breeding stallions (sires) to mares with balanced breeding using different sires and moderate selection (13). Currently, reference values of haematological parameters are widely available for horses in general and for the most common and popular breeds (2, 5, 14). However, literature data for endemic breeds are sparse and only a few reports address haematology in autochthonous draft horse breeds (15-17). The aim of this study was therefore to investigate the characteristics of the haematological parameters in the Posavje Horse breed and to test the hypothesis that age, sex and reproductive status cause some haematological changes in horses. In addition to variations in haematological parameters in the Slovene Posavje Horse breed, the study was particularly focused on the characteristics of differential leucocyte counts and their variations with age gain. The measured haematological values and their variations could serve as guideline values for further haematological investigations and as a basis for the development of an approach to determine haematological reference values for the Posavje breed (1,2, 6, 18).

Materials and methods

The study was conducted as a part of the routine annual breeding and registration procedures of the Slovenian breeding and conservation programme for the autochthonous Posavje horse breed at different locations in the region of south-eastern Slovenia during July and August. The stallions were located as sires in breeding stations and separated from the mares and foals kept on the farms of local breeders. Regardless of category and location, the horses were kept on pasture in natural environmental conditions during the day and stabled in individual boxes during the night. While stabled, they were fed hay, considering the needs of each category, and had free access to water. They were dewormed regularly, clinically sound on the day of sampling and did not receive medication in the last 3 months before blood sampling. The mares included in the study were not pregnant. As the horses were familiar with humans and accustomed to different handling procedures, no restraint was required during sampling.

The study included 39 stallions aged 3 to 22 years (average 11.3 years), 94 mares aged 4 to 22 years (average 11.1 years) and 30 foals aged 30-180 days (average 102 days). The grouping of horses by age and sex is presented in Table 1.

Blood samples were collected from the jugular vein with double-ended needles and evacuated tubes containing K2EDTA as an anticoagulant (Vaccuette; Greiner Labortechnik GmbH, Kreimsmünster, Austria) and stored at 4 °C for haematological analyses (6, 18), which were performed within the next 6 hours at the Laboratory for Clinical Pathology of the Clinic for Reproduction and Large Animals at Veterinary Faculty, University of Ljubljana. Routine haematological analyses included the following: Red blood cell tests (red blood cell count (RBC), haematocrit (HCT), haemoglobin concentration (HGB), red blood cell indices (mean cell haemoglobin concentration (MCHC), mean cell

Table 1: Arrangement of horses to age groups

Age group	Age	Sex		Total (n)
		Mares (n)	Stallions (n)	
Foals	30–180 days	/	/	30
Adults	3–22 years	94	39	134
Group A	3–6 years	21	8	29
Group B	7–9 years	22	9	31
Group C	10–13 years	20	8	28
Group D	14–15 years	10	6	16
Group E	16 and more	21	8	29

Table 2: Haematological parameters in Posavje horse foals, stallions and mares in total ($\bar{x}$ ± SD)

Variable (unit)	Foals (*n*=30)	Stallions (*n*=39)	Mares (*n*=94)
RBC (× 10^{12}/L)	9.84 ± 1.26[a,b]	8.79 ± 0.99[c]	7.45 ± 1.01
HGB (g/L)	124.27 ± 12.82 [a,b]	138.41 ± 14.93[c]	117.08 ± 12.82
HCT (L/L)	0.35 ± 0.04[a,b]	0.40 ± 0.05[c]	0.33 ± 0.04
MCV (fL)	36 ± 1.86[a,b]	45.18 ± 2.76	44.79 ± 3.35
MCH (pg)	12.68 ± 0.66[a,b]	15.78 ± 1.01	15.83 ± 1.25
MCHC (g/L)	351.93 ± 12.49	349.54 ± 8.26	353.52 ± 15.16
PLT (× 10^{9}/L)	350.93 ± 101.08[a,b]	229.54 ± 67.12	242.65 ± 67.62
MPV (fL)	7.48 ± 0.79[a,b]	6.36 ± 0.38[c]	6.61 ± 0.37
RDW (%)	17.86 ± 1.11	17.94 ± 0.74	17.99 ± 0.71

Legend: RBC - red blood cell count; HGB - haemoglobin concentration; HCT - haematocrit; MCV - mean cell volume; MCH - mean cell haemoglobin; MCHC - mean cell haemoglobin concentration; PLT – platelet count; MPV – mean platelet volume; RDW – red cell distribution width. Values in a row with the same superscript show significant differences ([a]differences between foals and mares; [b]differences between foals and stallions; [c]differences between mares and stallions; P < 0.05)

volume (MCV), mean cell haemoglobin (MCH)) and red blood cell distribution width (RDW)), white blood cell tests (total white blood cell count (WBC), absolute differential leukocyte count), platelet count (PLT) and mean platelet volume (MPV) were performed using an automated veterinary haematology analyser (Scil Vet abc Plus+, Horiba, Japan), validated for equine samples and following original instructions for use. The relative differential leukocyte count (neutrophils - NEU, eosinophils - EOS, basophils - BAS, monocytes - MON, lymphocytes - LYM) was measured under the microscope using blood smears stained with the commercial staining kit Hemacolor (Merck Cat. No. 1.11661, Merck KGaA, Darmstadt, Germany). The neutrophil/lymphocyte ratio (N/L ratio) was calculated by dividing the neutrophil proportion by the lymphocyte proportion.

Statistical calculations were performed using the Statistical Package for Social Sciences (SPSS for Windows, release 8.0.0). The normality of the data distribution was assessed using a Shapiro–Wilk test and significance was determined using all pairwise multiple comparisons (Tukey's test). Differences between values calculated for horses grouped by age or sex were statistically analysed by one-way analysis ANOVA. When significant differences were found, a posthoc analysis was performed (Bonferroni–Holm test) to clarify the groups between which these differences existed. Differences were considered significant at P≤0.05. The values measured are presented as the mean ± standard deviation in the text ($\bar{x}$ ± SD) and as mean ± error of the mean ($\bar{x}$ ± SE) in the figures.

Results

Red blood cell tests

The mean values of haematological parameters for all examined foals, stallions and mares of Posavje horses are shown in Table 2. Statistically significant differences between mares and stallions were found for RBC, HCT and haemoglobin concentrations (P < 0.001). In foals, RBC levels were significantly higher (P < 0.001) than in stallions and mares, but HCT, MCV and MCH levels were significantly lower (P < 0.001). The mean HGB concentration was significantly lower in foals than in stallions (P < 0.001) and higher than in mares (P < 0.01). The differences between foals, mares and stallions were not significant for MCHC and RDW (Table 2).

Age-dependent variations in RBC and indices in mares and stallions of the Posavje breed are presented in Fig. 1. The RBC values (Fig.1A) were highest in foals and significantly decreased thereafter in both sexes with age gain (P < 0.001). With the exception from foals and 3 to 4-year-olds, the RBC values in mares were lower than those in stallions (P < 0.001).

The lowest MCV (Fig.1 B) and MCH (Fig.1 D) were measured in foals and increased significantly with age in both sexes (P < 0.001 for both parameters). MCV was significantly lower in mares aged 3 to 6 years than in older animals (P < 0.001). The differences between stallions and mares of all ages were insignificant for MCV and MCH. HCT (Fig. 1C) and HGB (Fig. 1E) values in mares decreased with age (P < 0.001), reaching the lowest values in the 14- to 15-year-old

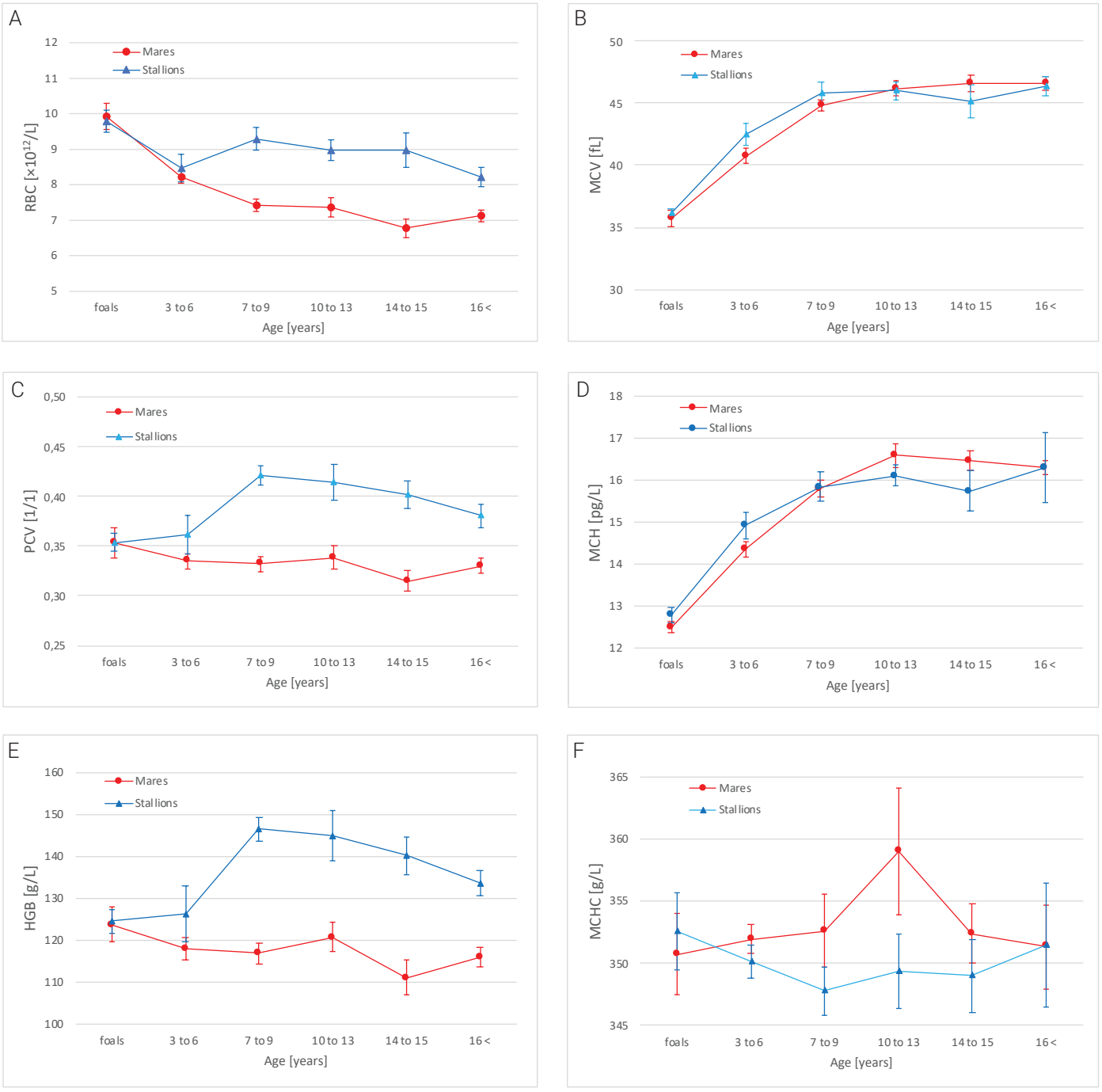

Figure 1: Age-dependent changes of red blood cell number (RBC; Panel A), mean cell volume (MCV; Panel B), Haematocrit (HCT; Panel C), mean cell haemoglobin (MCH; Panel D), haemoglobin concentration (HGB; Panel E) and mean cell haemoglobin concentration (MCHC; Panel F) in Posavje mares and stallions (mean ± SE)

group, while in stallions, a decrease was observed with peak values for HCT and HGB in the 7- to 9-year-old age group, followed by a gradual decrease in both values with age. Stallions aged 7 to 9 years or more had significantly higher HCT and HGB values than mares in the same age group ($P < 0.001$, respectively). The MCHC value (Fig. 1F) in stallions decreased slightly and reached the lowest values at the age of 7 to 9 years and increased thereafter. In mares, MCHC increased with age, peaking at 10 to 13 years of age and decreasing thereafter; the differences in MCHC between mares and stallions of all ages were not significant ($P > 0.05$).

RDW values remained stable with age in both mares and stallions and differences between sexes were not significant (hence, the changes are not shown graphically). The PLT count and MPV were significantly higher in foals than in stallions and mares ($P < 0.001$). The mean platelet volume (MPV) was higher in mares than in stallions ($P < 0.001$).

Table 3: Total WBC count and relative/absolute differential leukocyte count ($\overline{X}$ ± SD) in foals, stallions and mares

Variable	Unit	Foals (*n*=30)	Stallions (*n*=39)	Mares (*n*=94)
WBC	×10⁹/L	11.20 ± 2.03[a,b]	8.76 ± 1.81	9.14 ± 2.03
NEU	%	39 ± 11.42	56.26 ± 12.93	51.9 ± 11.65
	×10⁹/L	4.55 ± 1.57	4.99 ± 1.84	4.71 ± 1.45
LYM	%	52.67 ± 10.39[a,b]	37.26 ± 12.38	39.2 ± 11.62
	×10⁹/L	5.78 ± 1.66[a,b]	3.20 ± 1.11	3.63 ± 1.54
MON	%	0.93 ± 1.27	1.23 ± 1.37	0.85 ± 0.97
	×10⁹/L	0.12 ± 0.15	0.11 ± 0.13	0.08 ± 0.10
EOS	%	3.52 ± 3.20	4.10 ± 3.14	5.66 ± 3.62
	×10⁹/L	0.37 ± 0.25	0.36 ± 0.29	0.50 ± 0.32
BASO	%	0.11 ± 0.42[a]	0.38 ± 0.63	0.79 ± 1.07
	×10⁹/L	0.01 ± 0.04[a]	0.03 ± 0.06	0.07 ± 0.09
Ratio N/L	1/1	0.88 ± 0.54[a,b]	1.86 ± 1.23	1.55 ± 0.84

Legend: WBC – white blood cell count; NEU – neutrophil; LYM – lymphocyte, MON –monocyte; EOS – eosinophil; BAS – basophil; N/L –neutrophil/lymphocyte ratio. Values in a row with the same superscript indicate significant differences ([a]differences between foals and mares; [b]differences between foals and stallions; [c]differences between mares and stallions; P < 0.05)

White blood cell tests

The mean total WBC and the relative and absolute differential leucocyte counts in foals, stallions and mares are shown in Table 3. The mean total WBC and LYM counts (Table 3) were significantly (P < 0.001) higher in foals than in mares and stallions, while the NEU, MON, EOS and BAS counts were significantly lower in foals than in adults (P < 0.05).

The highest WBC value (Fig. 2A) was found in foals of both sexes and then gradually decreased in mares to reach the lowest value in the age group of 16 years and over (P < 0.001). A significant decrease in WBCs was observed in stallions at 3 to 6 years of age (P < 0.01), followed by an increase in WBCs at 10 to 13 years of age and a gradual decrease thereafter. WBC counts were also significantly lower in stallions aged 3 to 6 years (P < 0.01) than in mares of the same age. In mares, WBC counts were significantly higher in the 3- to 6-year-old group than in the older group (P < 0.001). The NEU count increased and the LYM count decreased significantly (P < 0.001 and P < 0.001, respectively) with increasing age in stallions and mares (Fig. 2B). Mares aged 3 to 6 years had a significantly higher NEU count (4.61) compared to stallions of the same age (3.36, P < 0.05; Fig.2B).

The N/L ratio was significantly lower in the foals than in Groups B (7 to 9 years), D (14 to 15 years) and E (16 and more years) (P < 0.001) (Table 4). The interactions between age and sex of NEU and LYM are shown in Fig. 2.

MON did not significantly differ between Posavje foals, stallions and mares (Table 3). The BAS counts in foals were statistically lower than those in mares and stallions (P < 0.001 and P < 0.05, respectively).

Discussion

Red blood cell tests

The mean RBC of Posavje stallions and mares was at the lower end of the normal range for warm-blooded horses (19) but at the upper end of the normal range for draft horses (16, 17) and slightly lower than in warmbloods (9, 20). In general, erythrogram values in this study were mostly comparable to those for warmblooded horses (5, 14, 21), which is surprising considering that the Posavje horse is a cold-blooded breed, although some warmblood characteristics are still present. In Posavje foals, the mean RBC was higher than in adult horses, consistent with the literature (2, 9). However, MCV and MCH were lower than in adult horses, as in warm-blooded foals (19).

The erytrogram values determined in our study were also related to the age of horses as described recently (14, 5, 22), with the RBC decreasing with age, followed by a

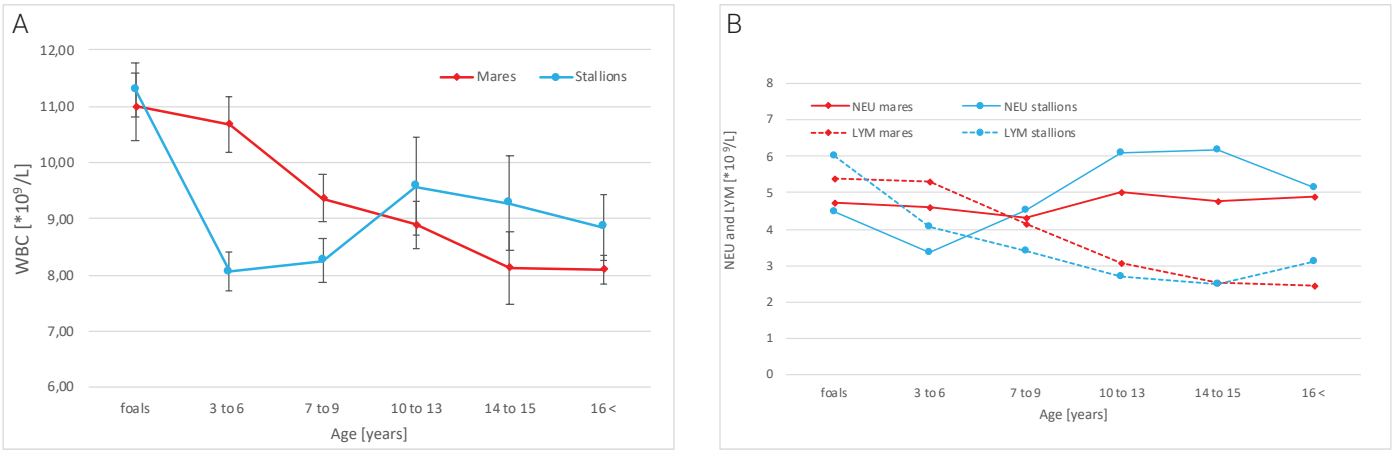

Figure 2: Changes of total white blood cell number ($\bar{x}$ ± SE) (A), and neutrophil (NEU) and lymphocyte (LYM) counts (B) in Posavje mares and stallions with age

compensatory increase of MCV and MCH in both sexes (5, 14, 18, 21, 22, 23, 25, 26). A gradual increase in MCV appears to be a common finding associated with equine ageing (22-24) causing changes in the dynamics of erythrocyte maturation (24). The MCV values of the Posavje horses studied were lower than those in the Przewalski and Kathiawari horse breeds (27, 28) but higher than those in the Zemaitukai horses (21) of comparable ages, while in foals they were consistent with those reported in many other breeds (29, 30).

HGB concentrations differed between sex and age groups of Posavje horses in the present study and were consistent with (15, 31) or higher (32) literature data. In contrast to Lahora working horses (33) and Lipizzans (5), a significant increase in HCT and HGB was observed only in Posavje stallions up to the age of 7 to 9 years, followed by a gradual decrease in older groups, while the values in mares decreased with age. In general, the mean total RBC, HCT and HGB levels were significantly higher in Posavje stallions than in mares, as common for horses (15, 33, 34, 35). This trend is most likely due to the effect of testosterone, which is also known to increase circulating HGB, HCT and RBC in humans (36) stimulating haematopoietic tissue and erythropoiesis in men more than in women (37). The role of testosterone in haematopoiesis was also supported by the study in castrated goats (38).

The mean RDWs of Posavje horses did not differ between age groups and sexes and were lower than (7, 39, 40) or similar to reported values (17, 19, 41, 42, 43, 44). A significant decrease in RDW was previously observed in stallions after exercise (7), but this decrease was not observed in our study. Platelet count in Posavje horses decreased with age, which is consistent with literature data (21, 22, 23), although no differences between age groups were reported (24). MPV was higher in Posavje mares than in stallions; in both sexes, the values were higher than in Shetland ponies (41) and lower than in Holstein horses (7, 44). Elevated MPV has been proposed as an indicator of platelet activation in humans, but the lack of defined limits to distinguish between activated and nonactivated platelets and the failure of platelet aggregation inhibitors to reverse a high MPV limit its utility as a platelet activation marker in human medicine (45).

White blood cell test

In the present study of horses of the Posavje breed, the highest mean WBC value was found in foals, followed by mares, and the lowest in stallions, with the later exhibiting the lowest value (14, 21, 25). In contrast, the WBC levels of Thoroughbred (9) and Lipizzan (46) stallions were higher in than in mares, and some studies failed to find significant differences between the sexes (47). In all age groups of Posavje horses WBC values in mares were higher than those in stallions, although this difference was significant

Table 4: Neutrophil/lymphocyte ratio (N/L) ($\bar{X}$ ± SD) in mares and stallions of various age groups (A: 3–6, B: 7–9, C: 10–13, D: 14–15, E: 16 and more years old)

Sex	Age group					
	Foals	Group A	Group B	Group C	Group D	Group E
Mares	0.84 ± 0.54	0.94 ±0.35	1.14 ±0.53	1.78 ±1.08	1.91 ±0.55	2.20 ±0.82
Stallions	0.75 ± 0.55	0.96 ±0.57	1.43 ±0.47	2.50 ±1.47	2.84 ±1.82	1.86 ± 0.81

only at the age of 3 to 6 years. The in WBC count of Posavje horses decreased with age gain (11, 24, 44) which could be attributed to the gradual decline in immunocompetence and cannot be considered as leukopenia (24, 44).

In Posavje horses, slightly higher NEU and lower LYM counts were measured in stallions than in mares (48). A significant age-related decrease in LYM count, proportional to the decrease in WBC, was observed in both sexes of the Posavje horse, as also reported in other horse breeds (2, 15, 16, 46). This decrease may be the reason for the decreased immunocompetence in older horses (10). The absolute NEU count has been reported to be higher in foals than in adult horses and remains stable with age gain (9, 25, 46), whereas it increased significantly in Posavje horses of both sexes. This increase was also the reason for a steady increase in N/L ratio with age, ranging from 0.84 to 2.01 in mares and from 0.75 to 1.68 in stallions. Similar changes in the N/L ratio in Andalusian horses (11) indicate a natural state reflecting a decreased bone marrow response. The predominance of NEU in the Posavje horses studied reflects the cold-blooded origin of this breeds (12).

In stallions aged 3 to 6 years the NEU count decreased significantly in parallel to the WBC count; however, it remained within physiological limits. In all other age groups, NEU counts were higher in stallions than in mares, as also reported for other horse breeds (32, 48, 49, 50); this difference could be attributed to increased testosterone production in reproductively active stallions. The plasma levels of testosterone are an important regulator of NEU function and the associated inflammatory response in humans (51, 52) which represents the first line of defence against invading pathogens and tissue injury (53, 54). Therefore, the physiological increase in NEU in the blood of the stallions studied could be an evolutionary adaptation to prevent infections caused by injuries of stallions, fighting for mares within a harem. Surprisingly, that the described changes in the NEU counts of stallions have thus far gone unnoticed. Modest increases in NEU counts within the normal range may have been ignored and the stallions in studies that addressed this issue (14, 21, 34, 46) were not sufficiently old or were reproductively inactive. Another reason for leucocytosis in horses with increased NEU and decreased LYM numbers could be increased plasma cortisol levels under stress (53). We can exclude this cause in the Posavje stallions, as all age groups of the examined stallions were housed under similar environmental conditions and treated in the same manner.

Neither age nor sex affected the EOS and MON, confirming the results of previous studies in horses (46, 49). Higher BAS values in older Posavje stallions were likely the result of altered immunological load (14).

Conclusions

In conclusion, our study indicates breed-related differences in haematological parameters of horses, and we have shown that haematological parameters vary with age and sex in the Posavje breed. The haematological traits identified in our study represent interesting breed-, age- and sex-specific adaptations/responses but are of limited diagnostic value. In general, the values of the haematological parameters in our study most closely matched those of warm-blooded horses, although the Posavje horse is a cold-blooded breed. The leucogram values and the N/L ratio determined in our study corresponded to those of cold-blooded horses. Furthermore, the results confirm and extend previous reports on age- and sex-related changes in haematological variables. One of the most important findings of our study is a higher NEU level in active breeding Posavje stallions, indicating an effect of androgens on the defence mechanism to prevent infections, which may influence survival and thus evolution. Further studies are needed to confirm the mechanisms underlying these differences.

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Ethics approval and consent to participate: All samples were obtained through standard breeding and registration procedures, so no approval was needed from the local animal experimentation ethics committee, in accordance with the Resolution on the Protection of Animals Used for Scientific and Educational Purposes and European Directive EU/2010/6. All owners gave their consent to the procedures and to the publication of the results.

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Karakterizacija hematološkega profila pri posavskem konju

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Izvleček: Cilj raziskave je bil proučiti vpliv spola in starosti na hematološke parametre pri pasmi posavski konj. V raziskavo je bilo vključenih 163 konj posavske pasme (30 žrebet, 94 kobil in 39 žrebcev), pri katerih smo v vzorcih krvi določali hematološke parametre s hematološkim analizatorjem. Diferencialna bela krvna slika in razmerje med nevtrofilci in limfociti (N/L) je bilo določeno na krvnih razmazih. Konje smo razdelili v pet starostnih skupin: žrebeta (od 1 do 6 mesecev, n = 30), 3 do 6 let (n = 8 žrebcev/21 kobil), 7 do 9 let (n = 9 žrebcev/22 kobil), 10 do 13 let (n = 9 žrebcev/20 kobil), 14 do 15 let (n = 6 žrebcev/10 kobil) ter 16 in več let (n = 8 žrebcev/21 kobil). Rezultati naše raziskave kažejo vpliv spola na preiskovane hematološke parametre; pri žrebcih so število rdečih krvnih celic (RBC), hematokrit (HCT) in koncentracija hemoglobina (HGB) značilno višji (P < 0,001), pri kobilah pa je višje število belih krvnih celic (WBC). Med starostnimi skupinami posavskih konj smo ugotovili zmanjšanje RBC in HGB in posledično kompenzacijo s povečanjem povprečnega volumna in hemoglobina eritrocitov, zmanjšanjem števila levkocitov, trombocitov (PLT) in limfocitov ter povečanjem razmerja med nevtrofilci in limfociti (N/L) s starostjo (P < 0,001). Posavski konj po zunanjosti spada med hladnokrvne konje, v raziskavi ugotovljeni hematološki profil pa kaže značilnosti, ki so skupne toplokrvnim pasmam konj, z izjemo razmerja N/L. Ena od pomembnejših ugotovitev te študije je večje število nevtrofilcev pri aktivnih plemenskih žrebcih. Višje vrednosti RBC, HGB, HCT in števila nevtrofilcev pri posavskih žrebcih kažejo učinek androgenov (testosterona), kar bi lahko bil učinkovit mehanizem za preprečevanje okužb, ki lahko vplivajo na preživetje žrebcev in s tem na evolucijo vrste.

Ključne besede: avtohtone pasme; posavski konj; hematologija; starost; spol

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Original Research Article

The Effect of a Specific Chicken Based Renal Diet as Monotherapy on Clinical, Biochemical, Urinary and Serum Oxidative Stress Parameters in Cats With CKD Stage 1 and 2

Key words

clinical parameters,
symmetric dimethylarginine,
oxidative stress,
renal diet,
cats,
chronic kidney disease,
urinary protein electrophoresis

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Abstract: The aim of the study was to investigate the effect of a therapeutic renal diet on selected clinical, biochemical, and urinary parameters and on selected parameters of oxidative stress in cats with early stages of chronic kidney disease (CKD). A prospective study of a 3-month duration was conducted to evaluate the effect of renal diet on selected clinical and laboratory parameters in client-owned cats with early stages of CKD. Of a total of 29 enrolled client-owned cats, nineteen (19) cats completed the study, ten receiving renal diet and nine receiving a diet of the owner's choice. A clinical examination was performed, and blood and urine samples were collected on the day of presentation and at regular check-ups after 3-4, 7-8, and 10-12 weeks. Serum creatinine and symmetric dimethylarginine (SDMA) concentrations and selected parameters of oxidative stress (plasma glutathione peroxidase (GPX) activity and plasma malondialdehyde (MDA) and serum selenium concentrations), were measured and electrophoresis of urinary proteins was performed. At inclusion, a significant positive correlation ($p < 0.001$) was found between serum selenium concentration and plasma GPX activity (Pearson correlation coefficient 0.83 (95% CI: [0.65 - 0.92]) and a significant negative correlation ($p < 0.001$) between serum SDMA and urine specific gravity (Pearson correlation coefficient -0.70 (95% CI: [-0.87 - (-0.38)]). At the end of the 3-month feeding trial no significant difference was found in SDMA and creatinine concentrations.

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Introduction

Chronic kidney disease (CKD) in cats has an overall prevalence of 2-3% of the feline population (1, 2). The prevalence is higher in older cats: 10% in cats older than ten years (3) and 28% in cats older than 12 years (4). A prevalence of 28% and a 67% is reported in the overall population of cats and in those older than 18 years old, respectively, based on the serum concentration of symmetric dimethylarginine (SDMA) (5). Chronic kidney disease is characterized by progressive loss of functional renal tissue, leading to renal fibrosis, which can cause uremic crisis and death (6). Early recognition of CKD is essential for prompt management of such patients, leading to a better long-term prognosis (7,8).

Proteinuria is an important and independent predictor of worsening of CKD (9,10,11). In contrast to dogs, chronic interstitial nephritis is predominantly found in cats. Therefore, tubular proteinuria is more common than glomerular proteinuria (12).

Oxidative stress aids progression of CKD in human patients (13). Plasma glutathione peroxidase (GPX), synthesized by renal tubular cells, is the major reactive oxygen species scavenger in the kidneys (14). In human CKD patients, plasma GPX activity decreases as the disease progresses and its activity is already reduced in patients with mild chronic uremia (15,16). On the other hand, a significantly higher plasma GPX activity was found in cats with CKD IRIS stage 4 compared with healthy cats (17).

Plasma malondialdehyde (MDA) is one of the most popular and reliable markers of the extent of lipid peroxidation and thus oxidative stress (18). Selenium is an integral part of selenoproteins, one of which is plasma GPX. This microelement is present in protein-rich foods, and its excess is excreted via the kidneys (19). Unlike human uremic patients, uremic cats do not have a selenium deficiency (17).

According to evidence-based veterinary medicine, renal diet is the therapy of choice in both feline and canine CKD patients from the International Renal Interest Society (IRIS) stage 2 (20,21,22). Hall and colleagues (23) reported that cats with IRIS CKD stage 1 and 2 benefit from a diet with increased caloric density and enhanced concentrations of carnitine and essential amino acids. The biomarkers of kidney function, body weight and lean muscle mass were stable in cats consuming such a diet. However, a recent study reported that feeding a highly phosphorus-restricted diet to cats with early-stage CKD may lead to hypercalcemia and urolithiasis, while a diet moderately restricted in protein and phosphorus may be beneficial (24).

The authors are aware of only a few studies that deal with oxidative stress in feline CKD (17, 25, 26, 27, 28, 29, 30). However, the results of these studies are inconclusive about the role of oxidative stress in the pathophysiology of CKD. Furthermore, there is a lack of data on the effects of renal diet on oxidative stress parameters in CKD cats.

The aim of the present study was to investigate/explore the effect of a therapeutic renal diet on selected clinical, biochemical, and urinary parameters and on selected parameters of oxidative stress in cats with early stages of CKD and to provide credible insight with monitoring of clinical and laboratory parameters.

Materials and methods

This prospective study was conducted on client-owned cats with early stages of CKD and lasted for three months. The inclusion criterion was CKD stage 1 or 2, according to the IRIS guidelines (31), with no previous treatment recorded.

Cats with acute kidney injury, prerenal or postrenal azotemia, nephropathy of toxic or infectious origin within the last 28 days, urinary tract obstruction, acute systemic inflammation, liver disease, chronic heart failure, cancer or serologically positive for feline leukemia or feline immunodeficiency were excluded from the study.

All owners signed a consent form before enrolling the cats in the study. All procedures complied with the relevant Slovenian governmental regulations (Animal Protection Act, Official Gazette of the Republic of Slovenia, No. 43/2007).

The cats were randomly divided into two groups: cats in the control group, which received a regular diet and cats in the experimental group, which received a renal diet. Simple randomization method with sequentially numbered sealed envelopes was used to group the patients (32).

The cats in both groups received their diet *ad libitum* according to their habitual regime. Clinical examination including body weight monitoring, blood pressure measurement, routine hematological and biochemical analyses, and urinalysis with UPC (urine protein to creatinine ratio) were performed on the day of presentation and regular check-ups after 3-4, 7-8, and 10-12 weeks.

In addition to routine laboratory parameters, measurements of SDMA concentration, and selected parameters of oxidative stress (GPX, MDA, selenium) were also performed at each check-up and will be described below.

Composition of the diet

The cats in the experimental group were fed Vet Life Feline Renal Formula (Farmina Pet Foods, Naples, Italy). The composition of the renal diet is shown in Table 1. The renal diet used in the study had the same lot number for all cats. The cats in the control group continued to receive the maintenance diet to which they were accustomed to prior to participation in the study.

Blood and urine sample collection, processing, and analysis

Blood samples were taken from the jugular vein and transferred into serum separator tubes (Vacuette, Greiner Bio-One, Kremsmunster, Austria) for the determination of serum biochemical profiles, including SDMA, and antigen detection of feline leukemia virus (FeLV) and specific antibody against feline immunodeficiency virus (FIV). The tubes were stored for 30 minutes at room temperature to clot and then centrifuged at 1300 × g for 10 minutes at room temperature to separate the serum. Serum samples for the determination of routine biochemical parameters (urea, creatinine, alanine aminotransferase, alkaline phosphatase, total proteins, albumins, total calcium, inorganic phosphate, electrolytes (sodium, potassium, chloride)) were analysed on the day of blood collection. For measurement of SDMA concentration in serum, an aliquot of the serum sample was prepared and immediately stored at -80°C until analysed in batch.

Table 1: The composition of the renal diet

Raw protein	26.00%
Raw oils and fats	20.00%
Raw fiber	2.40%
Raw ashes	7.30%
Calcium	0.80%
Phosphorus	0.60%
Sodium	0.35%
Potassium	0.90%
Magnesium	0.07%
Omega 3 fatty acids	0.40%
Omega 6 fatty acids	3.90%
EPA	0.10%
DHA	0.15%
Energy value	3965 kcal/kg – 16.6 MJ/kg
Nitrogen-free extract/1000 kcal	11.77 g/1000 kcal
Selenomethionine	60 mg per kg corresponding to 13.5 mg selenium/kg dry matter)

Legend: EPA eicosapentaenoic acid; DHA docosahexaenoic acid

Composition: pea starch, potatoes, chicken fat, hydrolyzed fish proteins, dehydrated whole eggs, hydrolyzed chicken proteins, dehydrated chicken meat, quinoa seed extracted, dehydrated fish, fish oil, calcium carbonate, inulin, fructooligosaccharides, mannanoligosaccharides, potassium chloride, sodium chloride, glucosamine (500 mg/kg), Marigold extract (source of lutein)

Additives per kg

Nutritional additives: Vitamin A 15000 IU; Vitamin D3 600 IU; Vitamin E 550 mg; niacin 125 mg; pantothenic acid 42 mg; Vitamin B2 17 mg; Vitamin B6 7 mg; Vitamin B1 8 mg; Vitamin H 1.3 mg; folic acid 1.3 mg; Vitamin B 12 0.08 mg; choline chloride 2500 mg; beta-carotene 1.5 mg; zinc chelate of the analogous methionine hydroxylase 725 mg; manganese chelate of the analogous methionine hydroxylase 385 mg; ferrous chelate of glycine hydrate 185 mg; copper chelate of the analogous methionine hydroxylase 54 mg; selenomethionine 60 mg; calcium iodate anhydrous 2.4 mg; taurine 2000 mg; DL methionine 5000 mg; L-lysine HCl 2000 mg; L-tryptophan 2000 mg; L-carnitine 250 mg. Technological additives: potassium citrate 3000 mg.

Antioxidants: tocopherol-rich extracts of natural origin 10 mg.

Blood samples for hematological analysis were collected into 0.5 ml EDTA-containing tubes (BD Microtainer Tubes, Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA).

Urine samples were collected by cystocentesis and analyzed within 1 to 2 hours.

Biochemical profiles (urea, creatinine, alanine aminotransferase, alkaline phosphatase, total proteins, albumins, total calcium, inorganic phosphate), except electrolytes and SDMA, were determined with an automated biochemistry analyser RX Daytona (Randox, Crumlin, UK). The electrolytes were determined with an Ilyte electrolyte analyzer (Instrumentation Laboratory, Lexington, Massachusetts, USA). Hematological analyses were performed with an automated laser hematology analyzer ADVIA 120 (Siemens, Munich, Germany) using species-specific software.

ELISA for the detection of antigen against FeLV and specific antibody against FIV were carried out according to the instructions of the manufacturer (IDEXX, Lenexa, Kansas, USA) on the day of collection.

Determination of the MDA concentration

Blood samples for the determination of plasma MDA concentration were collected into 2 ml EDTA-containing tubes (Vacuette, Greiner Bio-One, Kremsmunster, Austria). All samples were immediately centrifuged at 1500 × g for 15 min at 4°C. The plasma was separated and immediately frozen at -80°C until analysis.

The total plasma concentration of MDA was determined by a gentle alkaline saponification and derivatization method (33). MDA was derivatized with 2,4-dinitrophenylhydrazine to a pyrazole derivative and determined with an Agilent 1200 series high performance liquid chromatography system (Agilent, Waldbronn, Germany). The derivatized samples were separated on an Agilent Eclipse XBD-C18 column by gradient elution with acetonitrile, water and acetic acid and the MDA derivative was detected with the diode array detector. The plasma MDA concentration was expressed as µmol per L (µmol/L).

Determination of the SDMA concentration

All serum SDMA concentrations were measured in batch at the end of the study by IDEXX Laboratories in Germany (IDEXX SDMA Test, IDEXX Laboratories INC., Leipzig, Germany).

Determination of GPX activity

Plasma GPX activity was measured spectrophotometrically with an automated biochemistry analyzer RX-Daytona (Randox, Crumlin, UK) using the commercial Ransel kit (Randox Laboratories, Crumlin, UK) which is based on the

Paglia and Valentine method (34). The activity of plasma GPX was expressed as units per L (U/mL).

Determination of the selenium concentration

The microwave digestion of serum samples was performed with a Start D Microwave Acceleration Reaction System (Milestone, Sorisole, Italy). 0.4 to 1 mL of the samples were transferred into a 100 mL Teflon vessel and 3 mL 65% nitric acid, 0.5 mL 30% hydrogen peroxide and 4.5 mL Milli-Q water were added. The samples were digested in a closed 10-vessel microwave system at 200°C for 30 min. After cooling to room temperature, the solutions were diluted with Milli-Q water, and the concentrations of selenium were determined by inductively coupled plasma mass spectrometry (Varian 820-MS, Mulgrave, Australia). Argon was used as the carrier gas, and the isotope ^{78}Se was selected as the analytical mass in ICP-MS normal sensitivity mode. For measurements of selenium, a Collision Reaction Interface (CRI) was used to reduce common polyatomic interferences.

Urinalysis

Urinalysis included the measurement of specific gravity with a refractometer, the use of a standard multitest urine dipstick (Multistix 10SG, Siemens, Munich, Germany) and microscopic examination of the urine sediment. The urine samples were centrifuged at 800 × g for 10 minutes at room temperature. Urine supernatants were used to determine protein and creatinine concentrations to calculate the UPC. Protein and creatinine concentrations were measured with an automated biochemistry analyzer RX Daytona (Randox, Crumlin, UK) using the pyrogallol red and picric acid methods, respectively. Protein concentrations were not determined if the urine samples were grossly contaminated with blood. Gel electrophoresis was performed routinely by a commercial laboratory Euregio Laboratory Services (Kerkrade, Netherlands) in batch at the end of the study.

Statistical analysis

Based on the sample size, five keynote parameters were selected for statistical analysis (body weight, creatinine, SDMA, MDA and GPX). The differences between the first and the last (4^{th}) measurement time-point were compared. The remaining parameters were presented in the form of descriptive statistics (median and interquartile range (IQR)) and with boxplots over time (Supplementary material).

Basic characteristics and baseline measurements of systolic blood pressure selected hematological, biochemical, oxidative stress, and urinalysis parameters in both groups were compared using the Fisher exact test (categorical) and the Mann-Whitney U test (numerical variables). Since a slight deviance towards older cats in the experimental group was observed, the comparison of the difference in keynote parameters was adjusted for age by the use of linear regression. The P-values for the group comparison from the linear models were corrected by Holm procedure (5%).

The Pearson product-moment correlation was used to investigate the possible correlation between the parameters (scatter plots are presented in the Supplementary material).

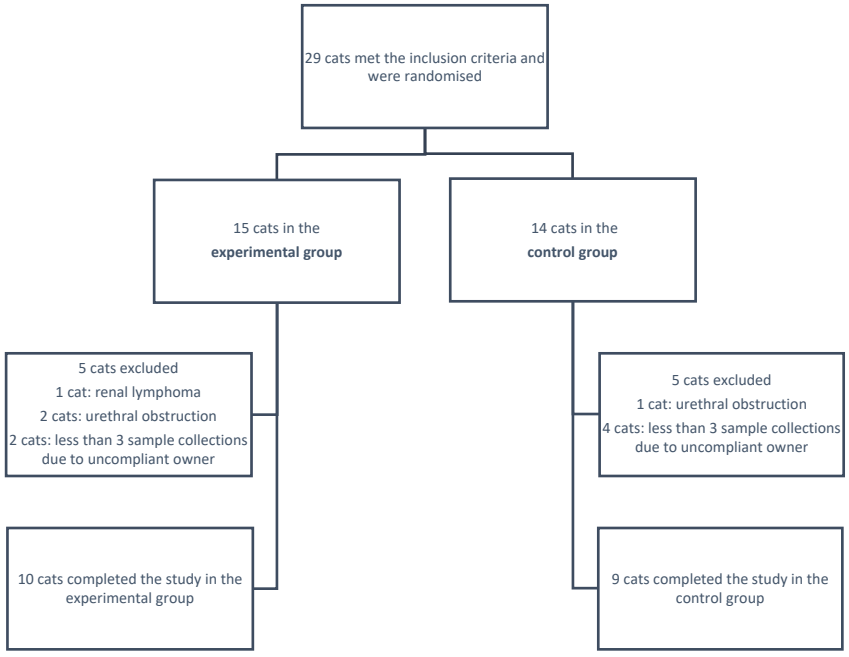

Figure 1: The flow diagram of cats with CKD during the study

Results

Patients' baseline characteristics

A total of 29 cats of different breeds were enrolled, all of which were neutered.

Of 29 cats enrolled in the study, 19 cats (ten cats in the experimental group and nine cats in the control group) completed the study. They represented the sample that we used for all analyses. The flow diagram of cats included in the study is presented in Figure 1.

Blood and urine samples were collected at all four scheduled check-ups from most cats enrolled. The missing blood and urine samples were not obtained due to problems with owners' compliance. In all animals the first and the last (10-12 weeks later) check-up were performed.

Baseline characteristics (demographic data) are shown in Table 2. At the point of randomization in terms of age, sex, and body weight, there were no significant differences between the experimental group and the control group; however, the cats in the experimental group that completed the study were slightly older.

Clinical signs and baseline clinical and laboratory parameters

Clinical signs at presentation were mild in all included cats; the owners usually reported polyuria/polydipsia. At the end of the study, most owners of cats in the experimental group reported an improvement in the clinical status of the cats with reduced vomiting. At inclusion, all cats of both groups were reported to have normal appetite, which remained unchanged during the whole observation period.

On each occasion, the owners were asked to evaluate their cats' appetite and acceptance; the information on the appetite and acceptance of the diet was recorded at each check-up. The cats were given the amount of the diet that was appropriate for their weight as recommended by the manufacturer. The new diet was accepted 100 % by all cats in the experimental group; and the diet change was achieved in two weeks.

During the first two weeks, the new diet was mixed with the usual diet and the amount of the new diet was gradually increased until the meal consisted only of the new diet. The owners reported that the entire amount of the meal was eaten by all included cats both during the transition period and during the study. Furthermore, the amount of the diet eaten during the study remained the same as before being enrolled into the study.

Medians (IQR) for baseline and final (4^{th}) measurement of laboratory parameters are presented in Supplementary material. The distributions of none of the parameters measured at baseline significantly differed between groups. Time monitoring of all measured parameters is also presented graphically in Supplementary material.

Serum creatinine concentration

During the study, median serum creatinine concentrations decreased in both the experimental and control groups (Supplementary material). The age adjusted difference in mean serum creatinine concentration decrease between both groups was not significant (Figure 2 A, Table 3). With one exception, all the included cats had stable CKD and remained at the same IRIS stage during the study. However, one cat in the experimental group was reclassified from IRIS 2 to 3 at the end of the study. The exact cause of the increase in serum creatinine concentration in that cat was not found. Seven out of 19 cats with elevated serum SDMA and creatinine concentrations, abnormal renal imaging findings and pathological urinary sediment, consistent with CKD, that were classified to IRIS stage 2, retained their urine concentration ability (Supplementary material).

Serum SDMA concentration

After the 3-month feeding trial, the median serum SDMA concentration decreased numerically in the

Table 2: Baseline demographic and laboratory characteristics of cats in the experimental and the control group

Group	Control group (n = 9)	Experimental group (n = 10)	p value
F/M	4/5	3/7	0.65
Age (months) Median (IQR)	78 (64–107)	116 (94–166)	0.066
Body weight (kg) Median (IQR)	5.9 (3.3–6.5)	4.9 (3.6–6.2)	0.842
Creatinine Median (IQR)	140.2 (130.0–178.8)	168.9 (161.1–178.8)	0.441
UPC (unitless) Median (IQR)	0.14 (0.12–0.19)	0.17 (0.11–0.26)	0.755

Legend: F-female cats; M-male cats; IQR-interquartile range

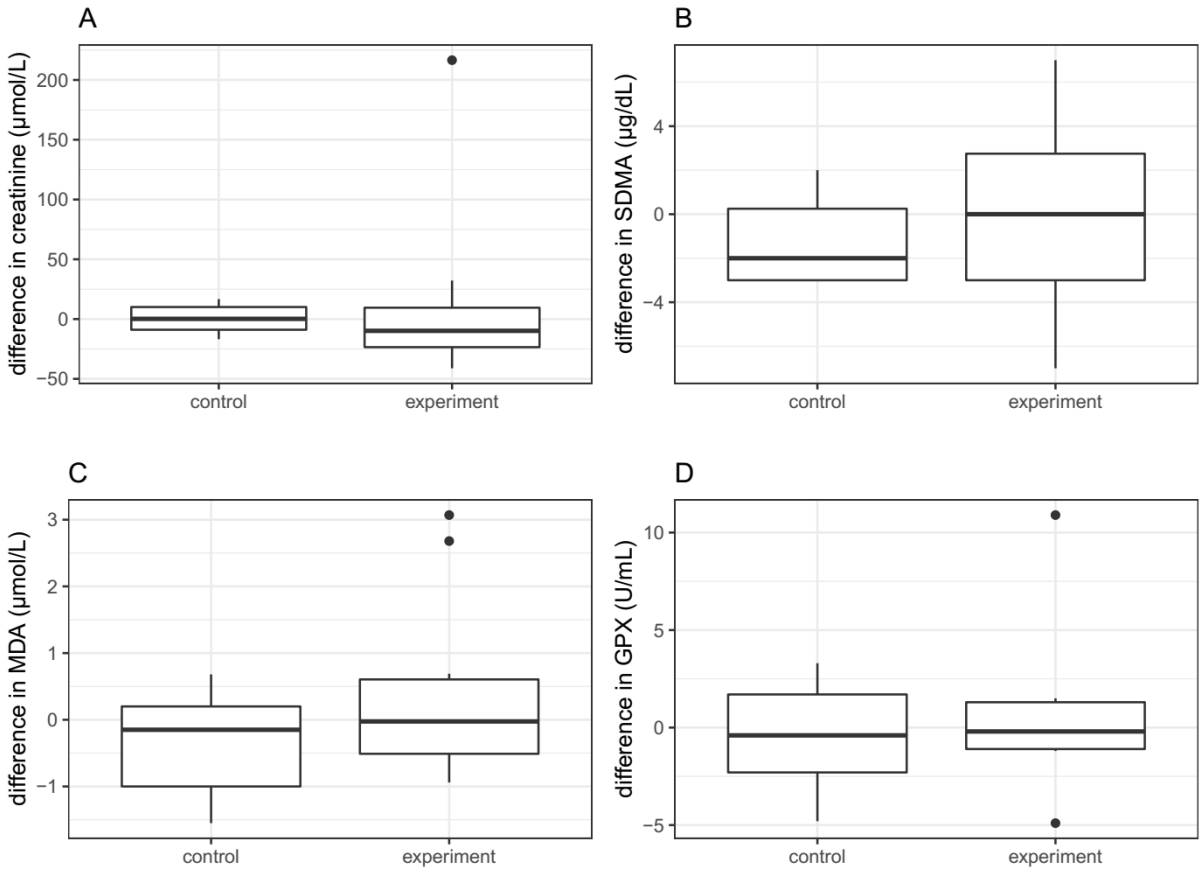

Figure 2: The changes in the concentrations of serum creatinine, serum SDMA, plasma MDA, and plasma GPX activity from the beginning to the end of the study in cats receiving renal diet in comparison to the control group

A – change in serum creatinine concentration (P = 0.92), B – change in serum SDMA (symmetric dimethylarginine) concentration (P = 0.85), C – change in plasma MDA (malondialdehyde) concentration (P = 0.18), D – change in plasma GPX (glutathione peroxidase) activity (P = 0.65); P-values stand for comparisons of age-adjusted differences

experimental group but remained the same in the control group (Supplementary material).

There was no significant difference in age-adjusted decrease of serum SDMA concentration between both groups (Table 3).

Plasma MDA concentration

At inclusion there was no significant difference in median plasma MDA concentrations between the sampled cats in the control group and those in the experimental group (Supplementary material). After the 3-month feeding trial, the mean age-adjusted difference in MDA was negligible in both groups (Table 3).

Serum selenium concentration and plasma GPX activity

In sampled cats receiving renal diet, median values of plasma GPX activity and serum selenium concentration did not differ from the median values in the control group (Supplementary material). There was a negligible mean age-adjusted difference in plasma GPX activity after 3-month feeding trial in both groups (Table 3).

A significant positive correlation (Pearson correlation coefficient 0.83 (95% CI: [0.65 - 0.92]) was found between the selenium concentration and plasma GPX activity at the beginning of the study.

Urinary Protein electrophoresis

At the beginning of the study, four cats in the control group and three cats in the experimental group had microalbuminuria. Furthermore, more cats in the experimental group had non-zero fractions of protein in the urinary sample. The leading protein fractions at the beginning of the study were alpha 1 and albumin in the control group and beta in the experimental group. At the end of the study, beta fraction predominated in both groups. The median per cent fraction of the urinary protein at the beginning of the study in comparison to the end of the study is shown in Table 4.

Moreover, at the beginning of the study, a significant negative correlation (Pearson correlation coefficient -0.70 (95%

Table 3: Results of five linear regression models, modelling difference (measurement 4 – measurement 1) between control and experimental group; the model controls for the age of cats

	Linear regression coefficient at group	P value	Adjusted P value
Weight (kg)	0.401	0.054	0.272
Creatinine (µmol/L)	-2.907	0.919	> 0.999
SDMA (µg/dL)	0.373	0.854	> 0.999
MDA (µmol/L)	0.891	0.175	0.699
GPX (U/mL)	0.875	0.654	> 0.999

Legend: Adjusted P-values are adjusted by Holm procedure for multiple comparisons; SDMA-Serum symmetric dimethylarginine; MDA-Plasma malondialdehyde; GPX-Plasma glutathione peroxidase

CI: [-0.87 - (-0.38)]) between SDMA and USG in the sampled cats were found.

Discussion

Cats with CKD IRIS stage 1 and 2 were monitored during a prospective 3-month feeding trial. All cats tolerated the diet change well in the experimental group and had normal appetite. During the study, the SDMA concentrations did not significantly change in any of the groups studied. Previously published data in cats with CKD IRIS stage 1 and 2 report a gradual increase in serum SDMA concentration regardless of the diet used (23). In the mentioned study, serum SDMA concentration increased from baseline at the first check-up after one month and continued to increase after three months in both the experimental and control groups (23). According to the published data, SDMA has a lower index of individuality than creatinine in cats (35) and dogs (36). Furthermore, consecutive measurements were performed in our study, and an individual value for each cat determined. A gradual increase in SDMA in successive measurements can therefore be due to a gradual decrease in kidney function (35,37). However, the difference in results of the mentioned studies might be ascribed to different renal pathologies of the patients that were included in both studies. Since the IRIS classification is applied to all patients suffering from CKD regardless of their cause, a variety of patients can be included. The progression of CKD and its response to treatment may be more variable at early stages than later when the majority of nephrons are lost. Although the serum SDMA concentration is correlated with the glomerular filtration rate (GFR) (38), further studies are warranted to assess the effect of the renal diet on the GFR.

Serum selenium concentration and plasma GPX activity measured in the present study were generally consistent with previously reported values (13, 39). No cat was selenium deficient at the beginning nor at the end of the 3-month feeding trial. In addition, a significant positive correlation between serum selenium concentration and plasma GPX activity was found, which contrasts with previously published data in cats (39). The study mentioned above found a correlation between serum selenium concentration and plasma GPX activity only in the case of selenium deficiency. When selenium concentrations continued to increase, plasma GPX activity reached its plateau, which was not observed in our study. Unlike in human patients, selenium is not a limiting factor in feline CKD (16, 17, 39). In addition, the correlation between the above-mentioned parameters is inconsistent in human CKD patients (16).

The median plasma MDA concentrations measured in our study were slightly higher, but in general agreement with the previously published values in healthy and CKD cats (40). We found no significant difference in mean change (final measurement – baseline) of plasma MDA concentration or

Table 4: Median per cent fraction of the urinary protein at the beginning of the study in comparison to the end of the study (after 3 months)

		albumin	Alpha 1	Alpha 2	Beta	gamma
Beginning	Control group	4.0	5.8	0	2.4	0
	Experimental group	3.1	4.1	0	14.7	0
After 3-month diet	Control group	3.8	7.3	0	54.1	0
	Experimental group	1.9	9.4	0	12.7	0

plasma GPX activity between the experimental group and the control group. Our results suggest that the renal diet had no significant effect on the parameters of oxidative stress measured in our study.

In contrast to previously published studies (39, 41), we found that three out of ten cats staged to IRIS 1 with elevated serum SDMA concentration, abnormal renal imaging findings as well as pathological urinary sediment, consistent with CKD, had a normal urinary specific gravity. The loss of the ability to concentrate urine is one of the first clinical signs of CKD and occurs when two-thirds of the nephrons are not functional. Apparently, the serum SDMA concentration increased before the ability of the kidneys to concentrate urine was impaired and proved valuable in the clinical evaluation of feline CKD patients at risk of developing CKD.

Furthermore, it was observed that some cats (seven out of 19) with elevated serum SDMA and creatinine concentrations, abnormal renal imaging findings and pathological urinary sediment, consistent with CKD, that were classified to IRIS stage 2, retained their urine concentration ability. The USG in these cats was up to 1.070 without showing clinical signs of heart failure, dehydration or hypovolemia. The literature data on this topic are scarce; Watson (42) reported that in contrast to dogs, USG values may remain normal (up to 1.045) in some cats with CKD and azotemia and that kidney disease may therefore still be suspected in a cat if these values are accompanied by persistent azotemia. Furthermore, cats often retain some concentrating ability in IRIS stages 2 and 3 CKD (43). The authors assume that the high USG in the CKD cats included in the present study could partially be caused by eating dry food. Further research is warranted to address this topic.

Cats of both groups had early kidney disease. Most of them exhibited borderline proteinuria, some were non-proteinuric. Except for one cat in the experimental group, the kidney disease was stable and did not deteriorate during the study. Two cats in the experimental group ended the study with a marked decrease of UPC. However, in one of these cats the serum creatinine and SDMA concentration rose to such extent that the cat was restaged from IRIS 2 to 3. The reason for this progression remained unknown. As serum SDMA and creatinine concentrations are negatively correlated to glomerular filtration, it might be assumed that subclinical dehydration, lower glomerular filtration rate as well as lower glomerular pressure may have led to a decreased UPC in that cat.

Though not expected, we found a negative correlation between SDMA and USG in the sampled cats. A similar finding has already been reported in dogs with decreased glomerular filtration rate (44). Cats with CKD have and impaired GFR; SDMA in such patients is increased (38). With a concurrent impairment of urine concentration ability, a decrease in USG is observed. A negative correlation between SDMA and USG in the sampled cats that all had CKD might therefore reflect the pathogenesis of CKD or it might be a consequence of a stochastic chance.

At the beginning of the study, four cats in the control group and three cats in the experimental group had microalbuminuria. According to Giraldi and colleagues (45), microalbuminuria is found in cats at risk for developing CKD. Overall, urinary protein electrophoresis and the UPC values indicate that tubular processes rather than glomerular disease were present in the cats that were enrolled in the study. Furthermore, we observed more cats in the experimental group to have non-zero fractions of protein in the urinary sample which might be partially ascribed to a higher systolic blood pressure or to a different predominant renal pathology. At the end of the study, beta fraction predominated in cats of both groups of our sample. However, when compared to the beginning of the study, there is an increase in the median percent beta fraction in cats in the control group, while it remained similar in the experimental group. Furthermore, the per cent albumin fraction decreased in cats in the experimental group while it remained similar in the control group. The presence of a leading beta fraction at the end of our study in both groups suggests tubular kidney damage, although the cats were non-azotemic, non-proteinuric or borderline proteinuric. Thus, we may assume that the tubular inflammatory processes progressed in both groups of cats, but to a different extent, although the UPC values remained grossly unchanged (45).

Furthermore, the results of our sample show no effect of renal diet on USG and UPC. The results are similar to the study published by Hall and colleagues (23), where no change in UPC or USG are reported. Therefore, we may conjecture that the tested renal diet had no effect either on electrophoretic pattern of urinary proteins or on halting the progression or development of proteinuria. Urine protein electrophoresis seems to be a valuable tool in assessing the progression of CKD. In order to provide better insight into the dynamics of CKD, we suggest urine protein electrophoresis to be added into monitoring scheme of feline CKD. Furthermore, recent recommendations in dogs with CKD include urinary electrophoresis, especially in those where renal biopsy is not indicated or not possible to be performed. The same recommendations may also be proposed in cats (46).

The main limitation of our study was the small number of patients who completed the study. In addition, the study lasted for a relatively short period of time, which may be an additional reason for the lack of significant differences in the measured parameters between the groups. Further studies with greater number of animals and with the assessment of GFR and urinary protein typization including the LMW (low molecular weight) spectrum are needed to get a thorough insight of the effect of renal diet on renal pathology.

Another limitation of the study is the fact that the diet of the cats in the control group was not standardized. As some cats do not tolerate any changes in their feeding regime including the diet change, the data gathered in this study give insight into the natural progression of early CKD where no medical intervention is possible. Moreover, some nutritional studies in human medicine follow similar design, where only experimental group receives diet, and the control group consists of individuals who continue with their habitual diet (47).

The study was performed on client-owned cats. Due to this fact, some cats were not brought to every scheduled check-up and some samples could therefore not be collected. The compliance of the owners in clinical studies like the present one tends to be a common problem.

Conclusions

After the 3-month feeding trial, no significant change in difference of body weight, serum creatinine or serum SDMA concentrations between experimental and control group was observed. Renal diet did not significantly increase the level of lipid peroxidation and decrease the activity of GPX, indicative of increased oxidative stress. Furthermore, our study demonstrated a significant positive correlation between serum selenium concentration and plasma GPX activity and a significant negative correlation between SDMA and USG in all CKD cats at inclusion.

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Učinek monoterapevtske ledvične diete na klinične, biokemijske, urinske in serumske parametre oksidativnega stresa pri mačkah s KLB stopnje 1 in 2

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Izvleček: Namen študije je bil raziskati učinek terapevtske ledvične diete na izbrane klinične, biokemijske in urinske parametre ter parametre oksidativnega stresa pri mačkah v začetnih stopnjah kronične ledvične bolezni (KLB). Raziskava je bila zasnovana kot prospektivna, tri mesece trajajoča klinična študija, v katero je bilo vključenih 29 lastniških mačk. Devetnajst mačk je zaključilo študijo, od teh jih je deset prejemalo ledvično dieto, devet pa vzdrževalno dieto po izboru lastnika. Pri vseh mačkah smo izvedli klinični pregled in odvzem krvnih ter urinskih vzorcev na dan vključitve v študijo in pri treh kontrolnih pregledih, ki so bili izvedeni 3–4, 7–8 in 10–12 tednov kasneje. Določili smo serumsko koncentracijo kreatinina, simetričnega dimetilarginina (SDMA) in izbrane parametre oksidativnega stresa (aktivnost plazemske glutation peroksidaze (GPX) in plazemsko koncentracijo malondialdehida (MDA) ter serumsko koncentracijo selena). Poleg tega smo izvedli elektroforezo urinskih proteinov. Ob vključitvi mačk v raziskavo smo ugotovili značilno pozitivno korelacijo ($p < 0,001$) med serumsko koncentracijo selena in aktivnostjo plazemske GPX (Pearsonov korelacijski koeficient 0,83, 95 % CI: [0,65–0,92]) ter značilno negativno korealcijo ($p < 0,001$) med koncentracijo SDMA in specifično težo urina (Pearsonov korelacijski koeficient –0,70 (95 % CI: [–0,87–(–0,38)]). Trimesečno hranjenje s terapevtsko hrano ni privedlo do značilnih sprememb v serumski koncentraciji SDMA in kreatinina pri vključenih mačkah.

Ključne besede: klinični parametri; simetrični dimetilarginin; oksidativni stres; ledvična dieta; mačke; kronična ledvična bolezen; elektroforeza urinskih proteinov

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Case Report

Computed Tomography and Magnetic Resonance Imaging of a Rhinosinusitis Secondary to a Dental Abscess in a Crested Porcupine (*Hystrix cristata*)

Key words

porcupine,
dental abscess,
computed tomography,
magnetic resonance imaging

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Abstract: A captive crested porcupine (*Hystrix cristata*) adult male was imaged due to reduced food intake, anorexia, fever, nasal discharge, changes in fecal quantity and size, and respiratory difficulties. Advanced imaging diagnostic techniques such as computed tomography and magnetic resonance imaging were performed to evaluate the animal. These techniques were very helpful to delineate the dental abscess, as well as the extension of the process to other locations such as the nasal cavity and the tympanic bulla. This is the first description of rhinosinusitis secondary to a dental abscess in a crested porcupine.

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Introduction

In recent years, the introduction of modern diagnostic imaging techniques has improved the visualization of diseases in exotic mammal medicine. Traditionally, standard radiography has been used by clinicians (1). Nonetheless, results of previous works have proposed that computed tomography (CT) and magnetic resonance imaging (MRI) may provide more information to improve diagnostic accuracy, prognosis, and treatment of diseases (2, 3, 4). These techniques avoid the superimposition of adjacent anatomical structures and depict the anatomic detail of specific tissue densities more finely, which improves its interpretation (2). Moreover, the refinements in CT technology involve the application of computer software for the generation of three-dimensional (3D) reconstruction of an area of anatomic interest (5). These advantages have demonstrated great value for the diagnosis of several diseases in exotic mammal species (2 ,3, 4, 5). Some of these species, such as the crested porcupine (*Hystrix cristata*), appear in worrisome categories of the IUCN red list since are regionally or locally threatened and thus require appropriate conservation policies in regional and local contexts. It is a species of rodent in the family Hystricidae native to Italy and Sicily, and a broad central strip ranging from Senegal and west to Somalia and east to Kenya and Tanzania (6, 7).

Porcupine presents strong and pointed quills that cover their tail, sides and top of the body. Concerning its head, it is large and robust with an enlarged infraorbital foramen so that portions of the masseter extend through it and arise from the frontal side surface of the snout (8). The anatomy and physiology of their oral cavity can produce several dental disorders. The teeth of rodents grow continuously (incisors in all species and molars in some species), therefore, any disease affecting the positioning of teeth within this cavity and disrupting normal attritional movements will lead to overgrowth and malocclusion (2, 3). Nonetheless, their oral cavity is very difficult to examine because of its anatomic configuration; these features typically allow the evaluation of superficial changes (9). For these motives, new imaging modalities are of tremendous importance for

the assessment of teeth and surrounding structures (10). Therefore, the use of CT and MRI allows an accurate diagnosis of dental disorders since it is highly effective in visualizing soft tissues and, unlike conventional radiology, can be used to diagnose even small dental abscesses, as well as osteoarthritis of the temporomandibular joint and odontogenic tumours (2, 11).

Case Presentation

An adult male crested porcupine (*Hystrix cristata*) weighing 7,8 kg from Rancho Texas Lanzarote Park (Lanzarote, Canary Islands, Spain) was admitted to the Veterinary Hospital of Las Palmas de Gran Canaria University (Canary Islands, Spain) to be imaged due to medical history of weight loss, reduced activity and food intake, anorexia, fever, mucopurulent nasal discharge, reduced stool production, and respiratory difficulties. The blood collection revealed mild anemia, marked lymphopenia, hypoglycaemia, hypoalbuminaemia, slightly high serum urea concentrations, and low potassium levels.

To perform the imaging study and evaluate the animal, we sedated the porcupine using a combination of dexmedetomidine (0,25 mg/kg IM, DEXDOMITOR®, Ecuphar, Barcelona, Spain) and ketamine (25 mg/kg, Imalgene®, Boehringer Ingelheim, Barcelona, Spain). No physical abnormalities were observed when we checked the head, However, the inspection of its oral cavity revealed an abscess of the right maxillary molar tooth and halitosis. The images were obtained using a 16-slice helical CT scanner (Toshiba Astelion, Toshiba Medical System, Madrid, Spain). The animal was positioned symmetrically in sternal recumbency on the CT couch, and a standard clinical protocol (120 kVp, 80 mA, 512 X 512 acquisition matrix, 1809 x 858 field of view, a spiral pitch factor of 0,94, and a gantry rotation of 1,5 s) was used to acquire sequential transverse CT images of 1 mm thickness slice. To optimize the CT appearance of the head structures, two CT algorithms (bone/

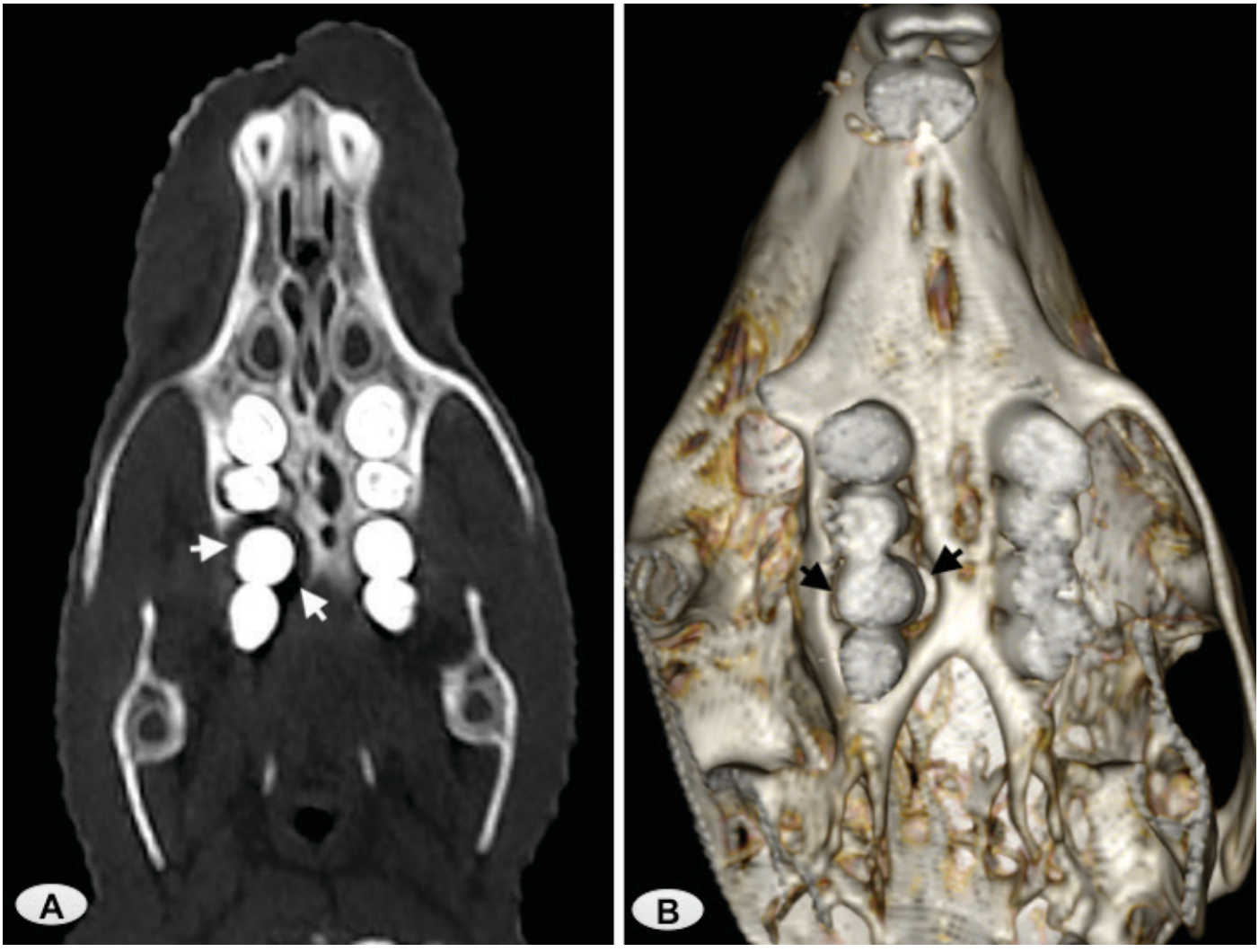

Figure 1: A) Dorsal MPR image of the porcupine head, bone window. The image displays an increase of the alveolar space corresponding to the third right maxillary molar tooth (white arrows). B) Volume-rendered reconstruction image of the porcupine head, displaying the dentary abscess (black arrows)

pulmonary algorithm), and two windows were applied by adjusting the window widths (WW) and window levels (WL): a bone window setting (WW=1500; WL=300) and a soft-tissue window setting (WW=350; WL=40). Multiplanar reconstruction (MPR) of CT images was performed to improve the visualization of the normal anatomy of the affected area as well as greater diagnostic accuracy of the extension of the disease. In addition, the original data were used to generate volume-rendered reconstructed images after manual editing of the transverse CT images to remove soft tissues using a standard Dicom 3D format (OsiriX MD, Geneva, Switzerland).

The CT and volume rendering reconstruction images displayed an increase in the alveolar space corresponding to the third right maxillary molar tooth (Figure 1). This alveolar space was hypoattenuating concerning the subjacent soft tissue. The rest of the oral cavity appeared unremarkable without any sign of disease. The nasal cavity and paranasal sinuses showed a large amount of fluid collection affecting both sides (more marked on the right side) that were hyperattenuated in the transverse and MPR images (Figure 2, 4a). No other remarkable findings were observed.

The MRI study was conducted with a 1.5-Tesla magnet (Toshiba, Vantage Elan, Japan) with the animal placed in ventral recumbence. A standard MRI protocol was used to generate spin-echo (SE) T1-weighted, and T2-weighted images in sagittal, transverse, and dorsal planes. SE T1-weighted transverse images were acquired with the following settings: Echo time (TE), 10 ms, repetition time (TR), 800 ms, acquisition matrix of 536 x 384, and 4,5 mm slice thickness with 4 mm spacing between slices. For SE T2-weighted transverse images, the TE 120 ms, TR 10541 ms, acquisition matrix 624 x 448, and 3 mm slice thickness with 3 mm interslice spacing. For SE T2-weighted sagittal images, the TE 120 ms, TR 7529 ms, acquisition matrix 512 x 804, and 2,8 mm slice thickness with 2 mm interslice spacing. For SE T2-weighted dorsal images, the TE 120 ms, TR 8282 ms, acquisition matrix 468 x 512, and 3,4 mm slice thickness with 3 mm interslice spacing. We used a medical imaging viewer (OsiriX MD, Geneva, Switzerland) to evaluate the images of the study.

Dorsal, transverse, and sagittal MR images of the porcupine head are presented (Figures 3, 4B). These images showed an increase of the alveolar space corresponding

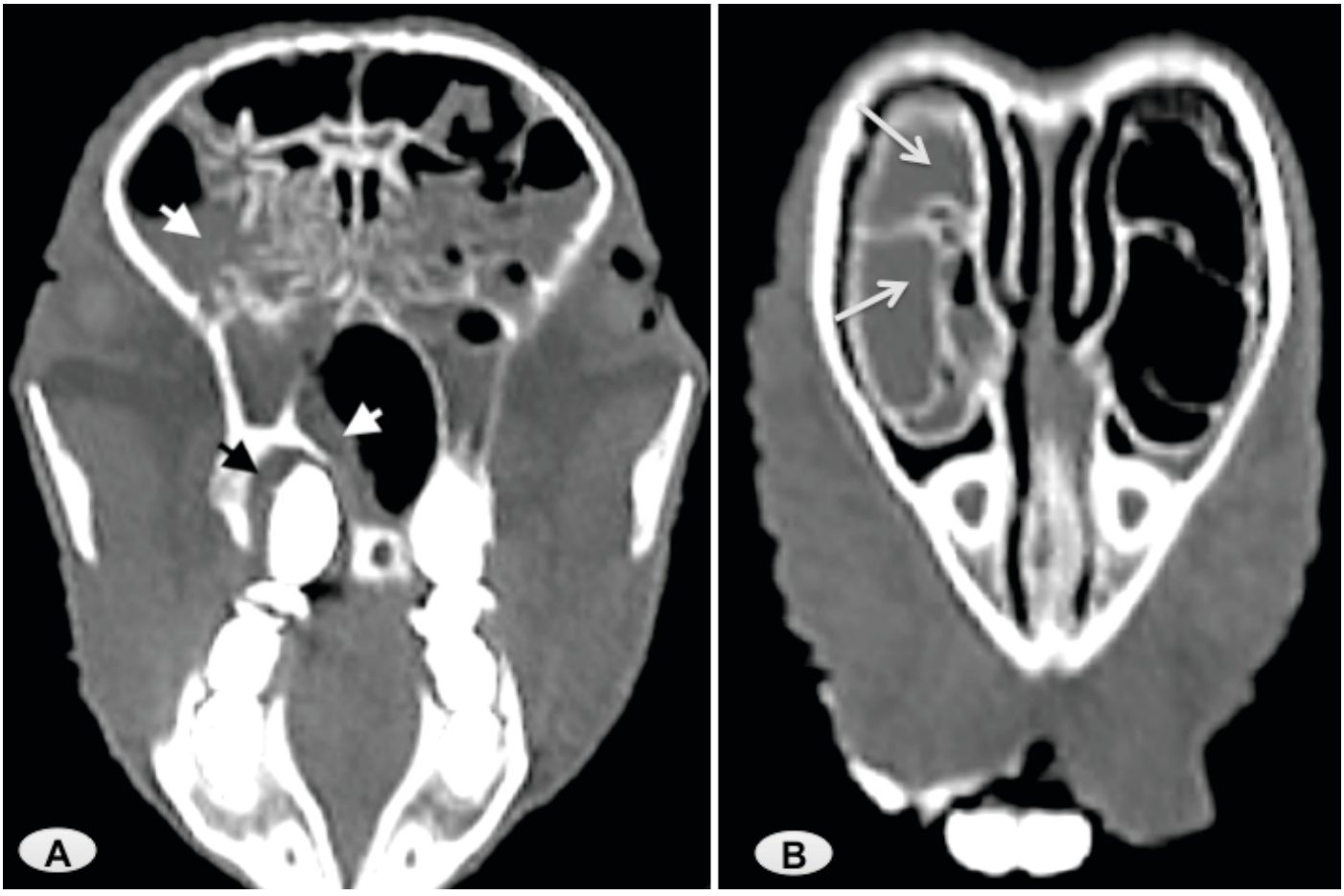

Figure 2: A) Transverse CT image of the porcupine head, bone window. The image shows an increase of the alveolar space corresponding to the third right maxillary molar tooth (black arrow). This increased space produced deviation to the right midline of the right wall of the nasopharynx (white arrow). In addition, there was a fluid collection in the paranasal sinuses (white arrow). B) Transverse CT image of the porcupine head, bone window. The image shows fluid collection in the dorsal and ventral nasal conchae (white arrows)

to the third right maxillary molar tooth. The increase of the alveolar space was hyperattenuated in T2W images when compared with the subjacent soft tissue. The dorsal and sagittal T2W images depicted abundant fluid collection in the nasal cavity and paranasal sinuses (Figures ·3A, 4B). In addition, the tympanic bullas showed a slight amount of fluid that was hyperintense in the T2W transverse images (Figure 3B). No other Imaging findings were identified.

We submitted samples of the fluid collection for aerobic and anaerobic bacterial culture. These were performed using sterile cotton swabs in a transport medium (Eurotubo®, Rubi, Barcelona, Spain). The swab was introduced 2-4 cm into the medial aspect of each nare and was stored and kept at 4 ºC until further processing in the laboratory. Later, samples were inoculated on Blood agar, MacConkey agar, Baird parker agar, and sabouraud agar. Plates were incubated at 37 ºC for 24 hours. There was bacterial growth on blood agar and Baird parker agar. We also performed a Gram stain, resulting in gram-positive cocci in pure culture. Subsequently, the API 20 Staph gallery confirmed the presence of *Staphylococcus aureus*. The antimicrobial resistance was tested to select effective drugs for treatment with those antimicrobials used in people against staphylococcal infection (12). The antimicrobial sensitivity discs (Oxoid, England) were cephalexin, enrofloxacin, ciprofloxacin, and amoxicillin-clavulanic acid. With this result, we recommend enrofloxacin (15 mg/kg s.c. q24h) for 14 days. Unfortunately, medical treatment was ineffective so surgical therapy was performed to extract the affected tooth and instilment the surgical site with antibiotic preparations (doxycycline-containing polymer gel). Later, the porcupine was maintained with amoxicillin–clavulanate 7,5 mg/kg q48h s.c. for six weeks. Further evaluation of the animal revealed no further complications.

Discussion

To the authors' knowledge, the present study is the first to characterize CT and MRI findings of a dental abscess in a crested porcupine. Different reports have postulated that rodents can develop dental diseases in their lifetime and be

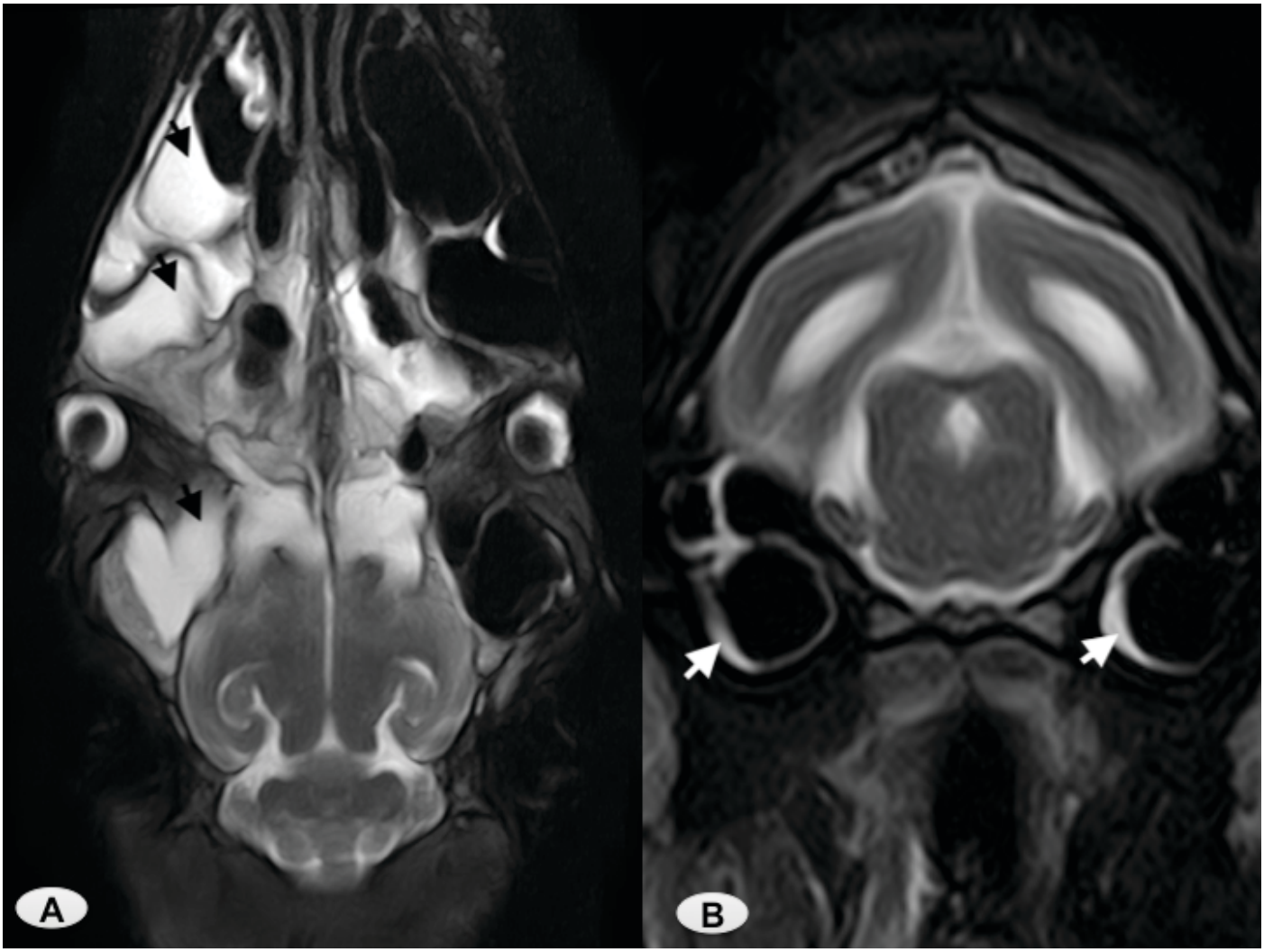

Figure 3: MRI of the porcupine head. A) T2W, dorsal image, displaying the fluid collection in the nasal cavity and paranasal sinuses (black arrows). B) T2W, transverse image. The image shows the tympanic bullas with a slight amount of fluid that is hyperintense (white arrows)

a cause of morbidity (13, 14) since these diseases may lead to infection of teeth and surrounding structures. Among these diseases, we highlighted dental abscesses, which are common in lagomorphs and rodents (10, 11, 15, 16). However, the singular aspects of its dental anatomy, physiologic characteristics, and host response make abscess diagnosis and treatment quite difficult (17). The aetiology of dental abscesses implies food and microorganisms being able to track up a loosened or broken tooth into the periodontal tissues and alveolar socket, resulting in the generation of an abscess associated with the maxilla or mandible, which can extend to the nasal cavity (17, 18). In this study, CT and MR images supported the diagnosis of rhinosinusitis secondary to dental associated infection. Information concerning rhinosinusitis in captive and free-ranging wildlife species is sparse. Thus, only a few reports have described this finding in rodents such as ground squirrels (19) or an orange-spined hairy dwarf porcupine (20), and as in our case, the nasal cavity inflammation was associated to *Staphylococcus* spp infection.

Diseases of incisors and cheek teeth result in clinical signs that could require appropriate imaging techniques to obtain a definitive diagnosis, formulate a prognosis, and develop a treatment plan. The use of radiographic imaging can be contemplated as a primary diagnostic tool to evaluate these processes. Unfortunately, the small size of rodents and the overlapping of the dental quadrants make radiographic evaluation quite arduous (18). Advanced diagnostic imaging such as CT and MRI have become popular in exotic mammal medicine since these techniques improve anatomic identification and lesion detection that allows accurate assessment, detailed prognosis, the diagnosis of underlying lesions and treatment choice (10, 18). CT has been widely used in rabbits to evaluate acquired dental disease and its associated problems, such as deformities and osteomyelitis, as well as the extension of the infection process to different bone cavities of the skull as the nasal or the paranasal cavities and the tympanic bullae (10, 11, 12). Therefore, the clinical signs are commonly related to the primary dental problem or complications connected with dental disease. The clinical signs observed in our animal such as anorexia, reduced food intake, excessive salivation, or nasal discharge were similar to those described in previous reports (10, 11, 12). Additionally, mild anemia, marked lymphopenia, and hypoglycaemia have also been reported in other animals with dental diseases (3, 14, 15).

In this study, a third-generation CT scan provided transverse and three-dimensional reconstructed images that gave an adequate overview of head morphology, displaying a good depiction of the affected areas. Thus, the transverse CT images were helpful to delineate the dental abscess, as well as the extension of the process to other locations, such as the nasal cavity and the tympanic bulla. Interestingly, other studies performed on rabbits showed similar Imaging findings (2, 3, 15, 16). Three-dimensional CT reconstruction is a helpful procedure to evaluate the extension of bony lesions with excellent detail by cropping part of the volume to evaluate deeper anatomic structures (5, 21). Hard and soft tissues can be added virtually or subtracted to different extents and degrees of density, providing a comprehensive relationship between soft and hard tissues. Shaded surface displays present a contoured surface map of the entire image volume, converting CT data into an image very similar to the depiction of an anatomic specimen (10). Despite these arguments, this technique has been infrequently used in exotic veterinary medicine. Three-dimensional CT reconstruction may be of critical importance for diagnostic accuracy and selecting the best

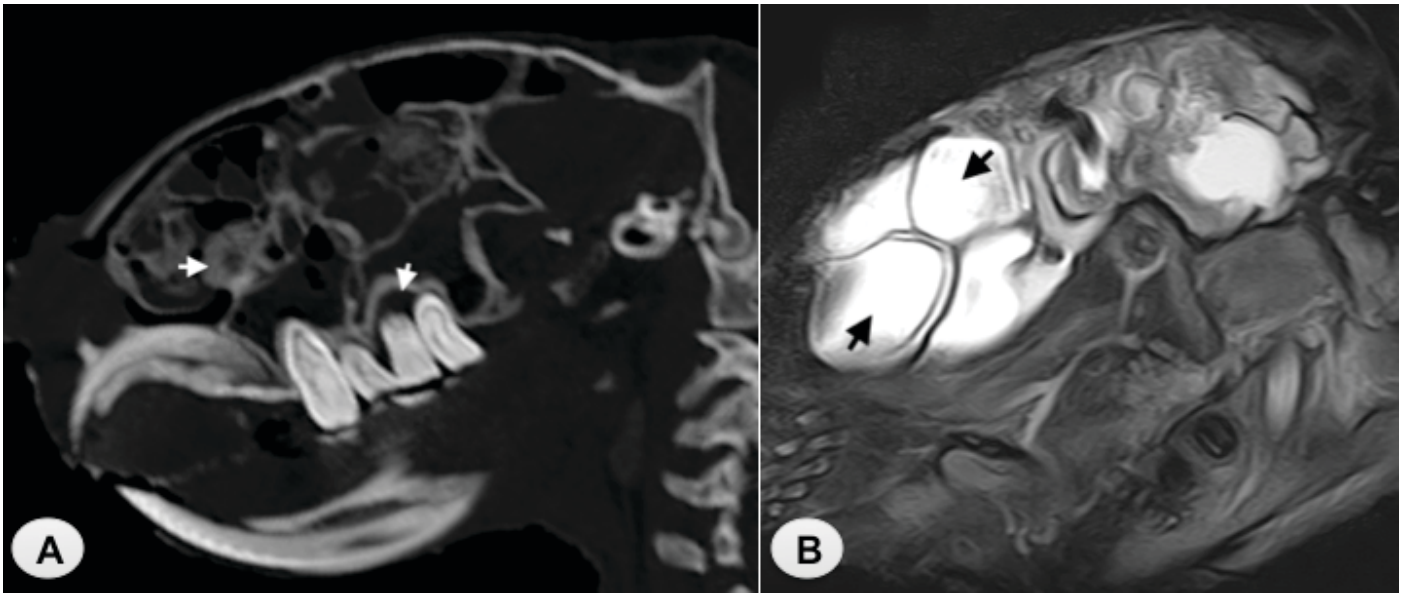

Figure 4: A) Sagittal MPR image of the porcupine head displaying the tooth abscess and the fluid collection in the paranasal sinuses (white arrows). B) T2W, sagittal image, displaying the abundant fluid collection in the nasal cavity and paranasal sinuses (black arrows)

surgical approach, depending on the nature and extension of the process. Thus, the use of this technique in rabbits has been quite helpful evaluating osteomyelitis, or dental and skull abnormalities (10). In our study, the images obtained by three-dimensional CT reconstruction displayed excellent detail of the dental abscess and the extension of the process to the nasal cavity, providing additional information to the transverse CT images.

In recent years, the contribution of MRI to the knowledge of exotic animals has increased (10, 11, 16). This Imaging technique displays soft tissues with excellent resolution. Therefore, an MRI of the head for reasons other than studies of the brain is a very helpful tool in pet rabbits and rodents to diagnose the presence and the extent of abscesses (10, 11, 16). In our study, we used a magnet of 1.5 T that provided T2W images with high resolution. These images displayed abundant fluid collection affecting the nasal cavity and the tympanic bulla, thus, diagnosing the presence and extension of the abscess.

Anaerobic and aerobic bacteria have been cultured from dental abscesses when pertinent techniques are used (17). Specific bacteria such as *Staphylococcus Aureus,* previously described to be important etiologic in rabbit dental infections (22), was isolated in our study. It is a versatile opportunistic pathogen that causes a wide spectrum of pathologies. It is also a mammalian commensal and opportunistic pathogen that colonizes niches such as skin, nares, and diverse mucosal membranes. The prevalence in animals varies from host species but colonization and infection have only been superficially investigated in small rodent wild animals (23) such as beavers, ground squirrels, red squirrels, or wood mice (24).

To summarize, advanced imaging diagnostic techniques such as computed tomography and magnetic resonance imaging were helpfully delineating the dental abscess and the extension of this process to other locations such as the nasal cavity and the tympanic bulla. It is the first time this pathology is described in crested porcupines by modern diagnostic techniques.

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Uporaba računalniške tomografije in magnetne resonance za slikanje rinosinuzitisa, nastalega zaradi zobnega abscesa pri afriškem ježevcu (*Hystrix cristata*)

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Izvleček: Odraslega samca afriškega ježevca (*Hystrix cristata*) v ujetništvu smo slikali zaradi zmanjšanega vnosa hrane, anoreksije, nosnega izcedka, sprememb v količini in velikosti iztrebkov ter težav z dihanjem. Uporabili smo napredne tehnike slikovne diagnostike, kot sta računalniška tomografija in magnetna resonanca. Te tehnike so bile zelo koristne pri opredelitvi zobnega abscesa in razširitve procesa na druga mesta, kot sta nosna votlina in timpanični del temporalne kosti. To je prvi opis rinosinuzitisa, ki je posledica zobnega abscesa pri afriškem ježevcu.

Ključne besede: ježevec; zobni absces; računalniška tomografija; magnetna resonanca

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