

**Slovenian
Veterinary
Research**



**Slovenski
veterinarski
zbornik**

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The Impact of Academic Publishing on the Quality of Education, Research Work, and the Development of the Profession

Vpliv izdajanja znanstvene revije na kakovost izobraževanja, raziskovalnega dela in razvoja stroke

Key words

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This year, the 8th Slovenian Veterinary Congress, the largest professional and scientific meeting of veterinarians in Slovenia, is taking place in Portorož. The main theme of the congress, *Challenges of Modern Veterinary Medicine*, addressed key challenges such as staff shortages, the management of new generations of young professionals, the impact of environmental changes and the need for an interdisciplinary approach and the integration of new technologies. Veterinary medicine is at an important turning point that requires reflection on its role in the socio-political context and careful consideration of current challenges. One of the issues is the importance of developing scientific fields, disseminating new discoveries and scientific knowledge, promoting specialist discussions and linking science with the general public. Scientific journals in the field of veterinary medicine play an important role in this respect, and in Slovenia *Slovenian Veterinary Research (SVR)* fulfils this role. The journal, which is published by the Faculty of Veterinary Medicine of the University of Ljubljana as part of the University of Ljubljana Press, is characterised by the publication of articles in English with summaries in Slovenian, which increases its accessibility to the national and international professional audience. The SVR editorial team organised a workshop entitled The impact of publishing scientific journals on the quality of education,

Letos v Portorožu poteka Slovenski veterinarski kongres, največje strokovno in znanstveno srečanje veterinarjev v Sloveniji. Osrednja tema kongresa, *Izzivi sodobne veterinarske medicine*, naslavlja ključne izzive, kot so pomanjkanje kadra, upravljanje novih generacij mladih strokovnjakov, vplivi okoljskih sprememb ter potreba po interdisciplinarnem pristopu in vključevanju novih tehnologij. Veterinarska medicina se nahaja na pomembni prelomnici, ki zahteva refleksijo o njeni vlogi v družbenopolitičnem kontekstu in preišljeno obravnavo aktualnih izzivov. Med ključnimi vprašanji je bil poudarjen pomen razvoja znanstvenih področij, širjenja novih odkritij in znanstvenih spoznanj, spodbujanja strokovne razprave in povezovanja znanosti s širšo javnostjo. Pri tem imajo znanstvene revije na področju veterinarske medicine pomembno vlogo, ki jo v Sloveniji izpolnjuje Slovenski veterinarski zbornik (SVZ), ki jo izdaja Veterinarska fakulteta Univerze v Ljubljani v okviru Založbe Univerze v Ljubljani. Odlikuje jo objava prispevkov v angleškem jeziku z izvlečki v slovenščini, kar povečuje njeno dostopnost domači in mednarodni strokovni javnosti. Ožji uredniški odbor SVZ je v okviru veterinarskega kongresa organiziral delavnico z naslovom Vpliv izdajanja znanstvenih revij na kakovost izobraževanja, raziskovalno delo in razvoj stroke, ki je potekala v Zbornični dvorani Univerze

research and development of the profession, which took place in the Chamber Hall of the University of Ljubljana. The aim of the workshop was to increase the visibility of the journal, encourage authors to prepare manuscripts, improve the responsiveness of reviewers, shed light on the challenges of local publication of international scientific journals and strengthen cooperation between journals in related fields. We also discussed the importance of promoting evidence-based veterinary medicine as a central principle of professional practise and development.

After the opening speech by the Vice-Rector for Research and Development of the University of Ljubljana, Prof. Dr. Anton Ramšak, the editor of *Slovenian Veterinary Research (SVR)*, Dr. Klementina Fon Tacer, introduced the journal and its mission. *SVR* is an international, peer-reviewed, multidisciplinary journal that publishes research findings in the key areas of veterinary medicine, biomedicine, comparative medicine and the concept of one health. The journal focuses on the development of basic and clinical knowledge that contributes to improving the health and welfare of animals and humans. As a platform for scientific communication and knowledge exchange, it brings together scientists, clinical experts, students and educators. The editor took the opportunity to highlight the many challenges facing academic publishing. These were an important trigger for the organisation of a workshop with the aim of finding solutions and promoting dialogue between all those involved in the process of publishing scientific journals.

The first speaker, Dr. Vojteh Cestnik, professor emeritus of the Faculty of Veterinary Medicine at the University of Ljubljana, editor of the *Veterinary Journal* and recipient of the Dr. Janez Bleiweis Award, presented a brief history of the *SVR* (1). The journal, which has been published continuously since 1961, began its career as "Proceedings" of the Department of Veterinary Medicine of the Biotechnical Faculty of the University of Ljubljana. During its existence, it changed its name several times and in 2010 it was included in the list of journals with an impact factor, which consolidated its importance on the international stage. Throughout its development, the journal has maintained its core mission: to disseminate scientific knowledge and new discoveries among researchers, veterinarians, students and educators and to promote interdisciplinary cooperation for the welfare of animals, humans and their shared environment.

This was followed by a presentation by Dr. John Kastelic, Professor at the University of Calgary, Canada, recipient of numerous awards in the field of veterinary medicine and Editor-in-Chief of the *Canadian Veterinary Journal* (2). He has given lectures and workshops on scientific writing in more than 20 countries worldwide. In his lecture, he explained the key steps for successful publication of a scientific article, which include a clearly defined research question and objective hypotheses supported by an appropriate research design and adequate statistical power. In addition, he emphasised the importance of thorough data analysis and a structured presentation of the content. He emphasised that the article should be easy

v Ljubljani. Cilji delavnice so bili povečati prepoznavnost revije, spodbuditi avtorje k pripravi prispevkov, izboljšati odzivnost recenzentov, osvetliti izzive lokalnega izdajanja mednarodnih znanstvenih revij in okrepiti sodelovanje med revijami sorodnih področij. Razpravljali smo tudi o pomenu spodbujanja na dokazih temelječe veterinarske medicine kot osrednjega načela strokovnega delovanja in razvoja.

Po uvodnem nagovoru prorektorja za raziskovanje in razvoj Univerze v Ljubljani, prof. dr. Antona Ramšaka, je urednica *Slovenian Veterinary Research (SVZ)*, dr. Klementina Fon Tacer, predstavila revijo in njeno poslanstvo. *SVZ* je mednarodna, recenzirana, multidisciplinarna revija, ki objavlja rezultate raziskav na ključnih področjih veterinarske medicine, biomedicine, primerjalne medicine in ved o konceptu enega zdravja. Revija je osredotočena na razvoj temeljnega in kliničnega znanja, ki prispeva k izboljšanju zdravja in dobrega počutja živali ter ljudi. Kot platforma za znanstveno komunikacijo in izmenjavo znanja povezuje znanstvenike, klinične strokovnjake, študente in pedagoge. Urednica je ob tej priložnosti poudarila številne izzive, s katerimi se sooča akademsko založništvo, ki so bili ključni povod za organizacijo delavnice, namenjene iskanju rešitev in spodbujanju dialoga med vsemi deležniki, vključenimi v proces izdajanja znanstvenih revij.

Prvi predavatelj, dr. Vojteh Cestnik, zaslužni profesor Veterinarske fakultete Univerze v Ljubljani, urednik *Veterinarskega vestnika* in prejemnik priznanja dr. Janeza Bleiweisa, je predstavil kratko zgodovino revije *SVR* (1). Revija, ki neprekinjeno izhaja že od leta 1961, je svojo pot začela kot »Zbornik« Oddelka za veterinarsko medicino Biotehniške fakultete Univerze v Ljubljani. Med svojim obstojem je večkrat spremenila ime, leta 2010 pa je bila uvrščena na seznam revij s faktorjem vpliva, s čimer je utrdila svoj pomen v mednarodnem prostoru. Kljub spremembam je revija ves čas razvoja ohranjala svoje temeljno poslanstvo: širjenje znanstvenih spoznanj in novih odkritij med raziskovalci, veterinarji, študenti in pedagogi ter spodbujanje interdisciplinarnega sodelovanja za dobrobit živali, ljudi in njihovega skupnega okolja.

Predavanje je nadaljeval častni gost delavnice, dr. John Kastelic, redni profesor Univerze v Calgaryju, Kanada, prejemnik številnih nagrad s področja veterinarske medicine in glavni urednik revije *Canadian Veterinary Journal* (2). O znanstvenem pisanju izvaja predstavitve in delavnice v več kot 20 državah po vsem svetu. V predstavitvi je izpostavil ključne korake za uspešno objavo znanstvenega članka, ki vključujejo jasno definirano raziskovalno vprašanje in objektivne hipoteze, podprte z ustreznim raziskovalnim načrtom ter ustrezno statistično močjo. Poleg tega je poudaril pomen temeljite analize podatkov in strukturirane predstavitve vsebine. Opozoril je, da mora biti članek jasno berljiv, brez nejasnih izrazov ali nepotrebnih informacij, pri čemer naj besedilo dosledno sledi omejitvam obsega in zahtevam revije. Dobro zasnovani članki omogočajo ponovljivost raziskav, prispevajo k širjenju znanja, povečujejo možnosti za

to read, without unclear terms or unnecessary information, and that the text should strictly adhere to the scope limitations and requirements of the journal. Well-designed articles enable the replication of research results, contribute to the dissemination of knowledge, increase the chances of publication, strengthen scientific discourse and increase the visibility of researchers. You can also find content on scientific writing on our website *Slovenian Veterinary Research*.

Scientific writing includes not only text, but also images, diagrams and artistic representations. These serve as a visual supplement to interpret the results, illustrate key concepts and make the article more attractive and understandable so that it is accessible to a wider audience. For this reason, we invited our illustrator Pšenica Kovačič to the workshop. Pšenica Kovačič is a graduated painter and studied designer who has acquired additional certifications in the field of feline welfare and behaviour. She has focused on researching and understanding cat behaviour and is certified by international organisations such as the International Society of Feline Medicine (ISFM) and Compass Education and Training. She also devotes some of her time to illustrating the covers of SVR. The main purpose is visual communication, connecting scientific knowledge to the general public (3, 4). The aesthetic and informative qualities of illustrations are complemented by conceptual and technical precision, making scientific content more understandable, accessible and appealing. Scientists provide the expertise, and artists can help translate this information into compelling visual narratives. Moreover, the creative interaction between science and art can lead to unexpected and surprising results that enrich the work of both sides (5). The cover of this magazine features an owl designed by Pšenica Kovačič especially for our workshop. In the Western world, the owl symbolises wisdom and knowledge (6). The illustrations were also part of the exhibition space at the veterinary congress in Portorož.

Just as scientific writing is closely linked to the fine arts, the publication of an article is closely linked to a positive evaluation by peer reviewers. They are responsible for critically reading and evaluating the manuscript, providing respectful, constructive and honest feedback on the submitted work and discussing the strengths and weaknesses of the article, ways to improve the strengths and quality of the work, and evaluating the relevance and originality of the manuscript. Therefore, one of the topics of the workshop was how to write a good review of a scientific article. The topic was presented by our SVR editor and initiator of the workshop, Dr. Klementina Fon Tacer, Assistant Professor at Texas Tech University and Director of the Texas Centre for Comparative Cancer Research (TC3R). Dr. Tacer integrates veterinary and human medicine in the fields of reproduction and oncology through an interdisciplinary programme and a comparative approach. In addition to the tasks of the reviewers, she also presented the structure of the peer review procedure and the peer review process as an important part of scientific methodology, which ensures the quality, credibility and verification of research data before publication. The structure of the peer review should include a

objavo, krepijo znanstveni diskurz in povečujejo vidnost raziskovalcev. Vsebine, povezane z znanstvenim pisanjem, so na voljo tudi na naši spletni strani *Slovenian Veterinary Research*.

Znanstveno pisanje poleg besedila vključuje tudi slike, grafe in umetniške prikaze. Ti služijo kot vizualna dopolnitev za lažjo interpretacijo rezultatov, ponazoritev ključnih konceptov in večjo privlačnost ter razumljivost članka in tako izboljšujejo dostopnost članka širši javnosti. Zato smo na delavnico povabili tudi našo ilustratorko Pšenico Kovačič. Pšenica je diplomirana slikarka in univerzitetna diplomirana oblikovalka, ki je pridobila dodatne certifikate na področju dobrobiti, blagostanja in vedenja mačk. Usmerila se je v raziskovanje in razumevanje mačjega vedenja ter pridobila certifikate od mednarodnih organizacij, kot sta International Society of Feline Medicine (ISFM) in Compass Education and Training. Del svojega časa namenja tudi ilustriranju naslovnice SVZ, katerih ključni namen je vizualna komunikacija, ki povezuje znanstvene ugotovitve s širšo javnostjo (3, 4).

Estetsko in informativno noto ilustracij dopolnjuje konceptualna in tehnična natančnost, s čimer ilustracija prispeva k večjemu razumevanju, dostopnosti in privlačnosti znanstvenih vsebin. Znanstveniki zagotavljajo strokovno znanje, umetniki pa lahko pomagajo prevesti te informacije v prepričljive vizualne pripovedi. Še več, ustvarjalna povezanost med znanostjo in umetnostjo lahko vodi v nepričakovane in presenetljive rezultate ter obogati delo obeh (5). Naslovnico te revije krasi sova, ki jo je posebej za našo delavnico oblikovala Pšenica Kovačič. Sova namreč v zahodnem svetu simbolizira modrost in znanje (6). Ilustracije so bile tudi del razstavnega prostora Veterinarskega kongresa v Portorožu.

Kot je znanstveno pisanje tesno povezano z vizualno umetnostjo, je objava članka tesno povezana s pozitivno oceno ocenjevalcev. Ti so odgovorni za kritično branje in ocenjevanje rokopisa, zagotavljanje spoštljivih, konstruktivnih in iskrenih povratnih informacij o predloženem delu ter razpravo o prednostih in slabostih članka, načinih za izboljšanje moči in kakovosti dela ter oceno ustreznosti in izvirnosti rokopisa. Zato je bila ena izmed tem delavnice tudi vprašanje, kako napisati dobro oceno znanstvenega članka. Temo je predstavila urednica našega SVR in pobudnica izvedbe delavnice, dr. Klementina Fon Tacer, docentka na Texas Tech University in direktorica tekšaškega centra za primerjalne raziskave raka (Texas Center for Comparative Cancer Research, TC3R). Dr. Tacer z interdisciplinarnim programom in primerjalnim pristopom povezuje veterinarsko in humano medicino na področju reprodukcije in onkologije. Poleg dolžnosti ocenjevalcev je predstavila tudi sestavo ocene in proces recenziranja kot ključnega dela znanstvene metodologije, ki zagotavlja kakovost, verodostojnost in preverjanje raziskovalnih podatkov pred objavo. Struktura recenzije naj vključuje povzetek glavnega namena članka, konceptualni doprinos znanosti, primernost izbranih metod dela in oceno, ali so sklepi dobro podprti s podatki. Kljub nekaterim slabostim, kot so dolgotrajen postopek za avtorje in

summary of the main aim of the article, the conceptual contribution of the science, the appropriateness of the working methods chosen and an assessment of whether the conclusions are well supported by the data. Despite some disadvantages, such as a lengthy process for authors and tedious tasks for reviewers, the peer review process allows for a constructive contribution to science and provides reviewers with an insight into the latest research and a better understanding of the editorial and review process. Reviews become effective when authors and reviewers conscientiously participate in the process, because timely feedback encourages others to do the same (7).

High-quality and constructive peer review plays a key role in ensuring a high standard of published work, which has an impact on the reputation and future of scientific journals, especially in view of the rapidly evolving publication activities of large publishing houses, which are directly related to the purely bibliometric assessment of the quality of published research. To enrich the scientific debate in this field, we hosted Dr. Svjetlana Kalanj Bogнар, Professor at the Faculty of Chemistry and Biochemistry, Head of the Laboratory of Molecular Neurobiology and Neurochemistry at the Croatian Institute for Brain Research, Faculty of Medicine, University of Zagreb. As editor-in-chief of the *Croatian Medical Journal (CMJ)*, she has lectured on the key role of scientific journals in disseminating knowledge and discoveries, stimulating debate and facilitating communication within the scientific community. She emphasised the importance of vision and the need for qualitative, non-metric assessment of research supported by the responsible use of quantitative indicators. She shared her experience with the *CMJ* (8) and her reflections on the daily challenges faced by editors of a small journal, such as maintaining the journal's high quality, international visibility and impact factor, attracting top authors and articles, improving the efficiency of editorial and review work, and keeping pace with current trends in publishing while striving to maintain the journal's originality and authenticity.

The link between high quality peer review and the wider publication environment plays a key role in ensuring scientific excellence and the global visibility of research. In Slovenia, this role is played with great success by the University of Ljubljana Press, which with its long tradition is one of the leading scientific publishers in the region. As Dr. Matevž Rudolf, head of the UL publishing house, emphasised, the institution bundles the publishing activities of 26 faculties and academies and covers all scientific disciplines with a diverse programme for the publication of scientific works. With more than 1,700 titles in the online bookshop, 12,500 scientific articles on the web portal and more than 500 open access monographs on the e-book portal, UL Publishing makes an important contribution to the visibility of Slovenian science in the international environment. The publication of 56 international scientific open access journals is also of particular value, as it promotes global cooperation between researchers and strengthens the reputation of the University of Ljubljana as a central scientific institution in the region. The publisher's modern

obremenjujoče naloge za ocenjevalce, proces recenziranja omogoča konstruktiven prispevek k znanosti, ocenjevalcem pa ponuja vpogled v najnovejše raziskave ter omogoča boljše razumevanje uredniškega in ocenjevalnega dela. Recenzije postanejo učinkovite, ko se avtorji in ocenjevalci vestno vključijo v postopek, saj pravočasno zagotovljena povratna informacija spodbuja tudi druge k enakemu ravnanju (7).

Kakovostne in konstruktivne recenzije igrajo ključno vlogo pri zagotavljanju visokih standardov objavljenih del, kar vpliva na ugled in prihodnost znanstvenih revij, predvsem v luči hitro razvijajoče se založniške dejavnosti velikih založb, neposredno povezane z izključno bibliometričnim ocenjevanjem kakovosti objavljenih raziskav. Z namenom obogatitve znanstvene razprave na tem področju smo na delavnici gostili dr. Svjetlano Kalanj Bogнар, profesorico na Oddelku za kemijo in biokemijo, vodjo laboratorija za molekularno nevrobiologijo in nevrokemijo na Hrvaškem inštitutu za raziskovanje možganov Medicinske fakultete Univerze v Zagrebu. Kot glavna urednica revije *Croatian Medical Journal (CMJ)* je predavala o ključnih vlogah znanstvenih revij, ki vključujejo širjenje znanja in odkritij, spodbujanje razprave ter omogočanje komunikacije znotraj znanstvene skupnosti. Poudarila je pomen vizije in potrebe po kvalitativnem, nemetričnem ocenjevanju raziskav, ki je podprto z odgovorno uporabo kvantitativnih kazalnikov. Delila je izkušnje *CMJ* (8) in razmišljanja o vsakodnevih izzivih, s katerimi se srečujejo uredniki manjše revije, kot so ohranjanje visoke kakovosti in mednarodne prepoznavnosti revije in faktorja vpliva, privabljanje vrhunskih avtorjev in člankov, izboljšanje učinkovitosti uredniškega in recenzentskega dela ter sledenje sodobnim trendom v založništvu ob hkratnem prizadevanju za ohranjanje izvirnosti in pristnosti revije.

Povezava med kakovostnimi recenzijami in širšim založniškim okoljem ima ključno vlogo pri zagotavljanju znanstvene odličnosti in globalne prepoznavnosti raziskav. V slovenskem prostoru to vlogo izjemno uspešno opravlja Založba Univerze v Ljubljani, ki se s svojo dolgoletno tradicijo uvršča med osrednje znanstvene založnike v regiji. Kot je poudaril dr. Matevž Rudolf, predstojnik Založbe UL, institucija združuje založniške dejavnosti 26 fakultet in akademij ter pokriva vse znanstvene vede z raznovrstnim programom izdajanja znanstvenih del. Z več kot 1700 naslovi v spletni knjigarni, 12.500 znanstvenimi članki na spletnem portalu in več kot 500 odprto dostopnimi monografijami na portalu E-knjige Založba UL pomembno prispeva k vidnosti slovenske znanosti v širšem mednarodnem prostoru. Posebno vrednost dodaja tudi izdaja 56 mednarodnih znanstvenih revij v odprtem dostopu, kar spodbuja globalno sodelovanje med raziskovalci in krepi ugled Univerze v Ljubljani kot osrednje znanstvene ustanove v regiji. Sodobni pristopi k odprtemu dostopu in mednarodni distribuciji, ki jih uveljavlja založba, omogočajo večjo dostopnost, vplivnost in trajnost raziskovalnih dosežkov.

approaches to open access and international dissemination make research more accessible, impactful and sustainable.

Publication activity is directly linked to challenges and opportunities. This was the main topic of the round table discussion, which I moderated together with Dr. Uroš Rajčević, assistant professor at the University of Ljubljana and co-editor of SVZ. The discussion emphasised the importance of international scientific journals at the University of Ljubljana and for the development of scientific research in Slovenia and the region. The key role of publishing for the international visibility of the university, the development of research fields and the quality of study programmes was emphasised. Important challenges for academic publishing were mentioned, such as ensuring the quality of contributions, recruiting suitable reviewers and speeding up the editorial process of articles. Particular attention was paid to the professionalism of the peer review process, which is crucial for maintaining high publication standards. The editing of scientific journals is based on the voluntary nature of the work, which enables the journal to operate with minimal financial costs while ensuring a high level of professionalism and commitment. This way of working reflects and strengthens international solidarity and ensures access to scientific publications. However, the voluntary nature of the work can lead to overload and time delays. It is therefore essential to set up appropriate support systems to ensure high-quality publications. When discussing the future of local scholarly publishing, participants emphasised the need for systematic support from the university, including financial resources and measures to improve support for publishers. The discussion also addressed issues related to connecting science with the general public, which is crucial for trust in science and its impact on society. Therefore, collaboration between journals and editorial teams is of paramount importance as it can lead to improved editorial practise and help raise the profile of the different fields of science in Slovenia and beyond. Successful examples of collaboration between related journals were presented and the possibilities of transferring these models to other fields were discussed. Collaboration between the fields of veterinary medicine, neuroscience and other biological and medical sciences is particularly important for the development and promotion of younger generations of scientists (9). We have also paid particular attention to the potential impact of artificial intelligence on editorial work. Finally, we are committed to organising regular meetings of scientific journal editors to promote collaboration, mutual support and the involvement of young people in editorial work and to raise awareness of the importance of local publication of scientific journals for the scientific and wider societal community.

The panellists were John Kastelic, MD, PhD, professor in the Faculty of Veterinary Medicine at the University of Calgary (Canada) and editor-in-chief of the *Canadian Veterinary Journal (CVJ)* (10), who shared his experience of publishing the CVJ. The CVJ is a monthly peer-reviewed scientific journal that reports on scientific developments in veterinary medicine. It was founded in 1960 and is the official journal

Založniška dejavnost se neposredno prepleta z izzivi in priložnostmi, ki so bili osrednja tema razprave na okrogli mizi, ki sva jo vodila z dr. Urošem Rajčevićem, docentom na Univerzi v Ljubljani in sourednikom SVZ. Razprava je poudarila pomen mednarodnih znanstvenih revij za Univerzo v Ljubljani in za znanstvenoraziskovalni razvoj v Sloveniji in na širšem področju. Izpostavljena je bila ključna vloga založništva za mednarodno prepoznavnost univerze, razvoj raziskovalnih področij in kakovost študijskih programov. Prepoznali smo ključne izzive akademskega založništva, kot so zagotavljanje kakovosti prispevkov, pridobivanje ustreznih ocenjevalcev in pospeševanje procesa obdelave člankov. Poseben poudarek je bil namenjen profesionalnosti recenzijskega procesa, ki je ključen za ohranjanje visokih standardov objav. Uredništvo znanstvenih revij namreč temelji na prostovoljni naravi dela, ki omogoča delovanje revije z minimalnimi finančnimi stroški, hkrati pa zagotavlja visoko raven strokovnosti in predanosti. Takšen način dela odraža in krepi mednarodno solidarnost in zagotavlja dostop do znanstvenih objav. Kljub temu prostovoljna narava dela lahko vodi do preobremenjenosti in časovnih zamud, zato je nujno vzpostaviti ustrezne podporne sisteme za zagotavljanje visoke kakovosti publikacij. Pri razpravi o prihodnosti lokalnega znanstvenega založništva so udeleženci poudarili potrebo po sistemski podpori Univerze, vključno s financiranjem in ukrepi za izboljšanje podpore založnikom. Razprava je vključila tudi vprašanja, povezana s povezovanjem znanosti s širšo javnostjo, kar je ključno za krepitev zaupanja v znanost in njenega vpliva na družbo. Tako je sodelovanje med revijami in uredniškimi timi izjemno pomembno, saj lahko vodi v izboljšanje uredniških praks in pomaga povečati prepoznavnost različnih znanstvenih področij v Sloveniji in izven nje. Predstavljeni so bili uspešni primeri sodelovanja med sorodnimi revijami, razpravljali pa smo tudi o možnostih prenosa teh modelov na druga področja. Sodelovanje med področji veterinarske znanosti, nevroznanosti ter drugih bioloških in medicinskih ved je še posebej ključno za razvoj in napredovanje mlajših generacij znanstvenikov in znanstvenic (9). Posebno pozornost smo namenili tudi potencialnemu vplivu umetne inteligence na uredniškega dela. Na koncu smo se zavezali k periodični organizaciji srečanj urednikov znanstvenih revij s ciljem spodbujanja sodelovanja, medsebojne podpore, vključevanja mladih v uredniško delo ter krepitve zavesti o pomenu lokalnega izdajanja znanstvenih revij za znanstveno in širšo družbeno skupnost.

V razpravi so sodelovali: John Kastelic, dr. vet. med., doktor znanosti, profesor na Veterinarski fakulteti Univerze v Calgaryju (Kanada) in glavni urednik revije *Canadian Veterinary Journal (CVJ)* (10), ki je predstavil izkušnje izdajanja revije CVJ. CVJ je mesečna strokovno recenzirana znanstvena revija, ki pokriva znanstveni razvoj v veterinarski medicini. Ustanovljena je bila leta 1960 in je uradna revija Kanadskega veterinarskega združenja. CVJ je »glasnik veterinarske medicine v Kanadi«. V tej mesečni publikaciji so objavljeni različni strokovno pregledani članki, redne kolumne, novice in poslovne informacije, namenjene kanadskim

of the Canadian Veterinary Association. The CVJ is the “voice of veterinary medicine in Canada”. This monthly publication contains a variety of peer-reviewed articles, regular columns, news and business information aimed at Canadian veterinarians in private or public practise, industry or academia. Svjetlana Kalanj Bogнар, MD, PhD, professor at the Faculty of Medicine of the University of Zagreb and editor-in-chief of the *Croatian Medical Journal*, shared her experience of publishing the CMJ (8), which is published by the three main universities in Croatia, the University of Zagreb, the University of Rijeka and the University of Split. The journal has an impact factor of 1.5 and, like the SVR, combines science and art through its innovative covers, both of which aim to harness the power of collaboration between scientists and artists in presenting and communicating new discoveries. Gregor Serša, PhD, PhD, professor at the Ljubljana Oncology Institute, member of the Academy of Sciences and Arts and editor-in-chief of *Radiology and Oncology*. *Radiology and Oncology* is a journal published by the Association of *Radiology and Oncology* and has an impact factor of 2.1. Dr. Serša emphasised the historical importance of the journal, which was founded in 1964 as *Radiologia Jugoslavica* and at that time covered the field of radiology for the whole of Yugoslavia. In 1992, the focus of the journal shifted to radiologic imaging and its application in oncology. He emphasised the multidisciplinary nature of the journal and its aim to publish original and high quality scientific articles and reviews in the fields of oncological imaging, interventional radiology, nuclear medicine, radiotherapy, clinical and experimental oncology, radiobiology, medical physics and radiation protection, as well as articles on more general aspects of interest to radiologists and oncologists. Maša Čater, PhD in biotechnology, researcher at the Faculty of Biotechnology at the University of Ljubljana, editor-in-chief of the e-publication *eSinapsa*, which aims to disseminate knowledge and findings in the field of neuroscience. It is aimed at Slovenian neuroscientists and other Slovenian scientists and professionals with an interest in neuroscience. Publications include short scientific and technical papers, book presentations, conference reports and historical records. Dr. Čater is also the social media editor of *Slovenian Veterinary Research*. Publishing on social networks enables the effective dissemination of scientific information to a wider audience and thus contributes to increased visibility of the journal, strengthens its role in the scientific community and at the same time increases interest in scientific fields outside scientific circles.

The workshop on the importance of academic journals was organised because we felt the need for an in-depth discussion on the future of academic publishing and its independence in the age of large publishers. Academic journals are not only a means of publishing research results, but also play a key role in maintaining the high quality of scientific contributions and promoting the research work of academic institutions and scientific societies, fostering collaboration between researchers and increasing the international visibility of scientific fields and institutions. Finally, scientific journals play a key role in training new generations of scientists, promoting transparent and high-quality publication of scientific research

veterinarjem v zasebni ali javni praksi, industriji ali akademski sferi. Svjetlana Kalanj Bogнар, dr. med., doktorica znanosti, profesorica na Medicinski fakulteti Univerze v Zagrebu in glavna urednica revije *Croatian Medical Journal*, ki je predstavila svojo izkušnjo z izdajanjem CMJ (8), pri katerem sodelujejo tri glavne univerze na Hrvaškem – Univerza v Zagrebu, na Reki in v Splitu. Revija ima faktor vpliva 1,5 in podobno kot SVR združuje znanost z umetnostjo s svojimi inovativnimi naslovnici, s čimer oboji želimo izkoristiti moč sodelovanja znanstvenikov in umetnikov pri posredovanju in komuniciranju novih odkritij. Gregor Serša, univ. dipl. biol., doktor znanosti, profesor na Onkološkem inštitutu Ljubljana, član Akademije znanosti in umetnosti ter glavni urednik revije *Radiology and Oncology*, ki jo objavlja Združenje za radiologijo in onkologijo in ima faktor vpliva 2,1. Dr. Serša je osvetlil njen zgodovinski pomen, saj je revija začela izhajati leta 1964 kot *Radiologia Jugoslavica* in je pokrivala področje radiologije za tedanje celotno Jugoslavijo. Leta 1992 se je fokus revije usmeril v radiološko slikanje in njeno uporabnost v onkologiji. Izpostavil je multidisciplinarnost revije ter njen namen objavljanja izvirnih in visokokakovostnih znanstvenih člankov ter preglednih člankov s področja onkološkega slikanja, intervencijske radiologije, nuklearne medicine, radioterapije, klinične in eksperimentalne onkologije, radiobiologije, medicinske fizike in varstva pred sevanjem ter tudi prispevkov o splošnejših vidikih, ki zanimajo radiologe in onkologe.

Maša Čater, univ. dipl. bioteh., doktorica znanosti, raziskovalka na Biotehniški fakulteti Univerze v Ljubljani, glavna urednica e-publikacije *eSinapsa*, katere namen je širjenje znanja in spoznanj s področja nevroznanosti slovenskim nevroznanstvenikom in drugi širši slovenski znanstveni in strokovni javnosti, ki jo nevroznanost zanima. Objave zajemajo krajše strokovne in znanstvene prispevke, predstavitve knjig, poročila s konferenc in zgodovinske zapise. Dr. Čater je hkrati tudi urednica družbenih omrežij revije *Slovenski veterinarski zbornik*. Objavljanje na socialnih omrežjih namreč omogoča učinkovito širjenje znanstvenih informacij širši javnosti in s tem prispeva k večji prepoznavnosti revije, krepi njeno vlogo v znanstveni skupnosti in hkrati povečuje zanimanje za znanstvena področja tudi izven znanstvenih krogov.

Delavnico o pomenu znanstvenih revij smo organizirali, ker smo zaznali potrebo po poglobljeni razpravi o prihodnosti akademskega založništva in njegove neodvisnosti v času velikih založniških hiš. Znanstvene revije namreč niso le sredstvo za objavo raziskav, temveč imajo ključno vlogo pri ohranjanju visoke kakovosti znanstvenih prispevkov in promociji raziskovalnega dela akademskih institucij in znanstvenih društev, spodbujanju sodelovanja med raziskovalci ter povečanju mednarodne prepoznavnosti znanstvenih področij in institucij. Ne nazadnje so znanstvene revije pomembne za izobraževanje novih generacij znanstvenikov, promocijo transparentnega in kakovostnega objavljanja znanstvenih raziskav, komunikacijo znanosti s stroko in širšo javnostjo. Hkrati sta zelo relevantna tudi izobraževanje

and communicating science to the scientific community and the general public. At the same time, it is also very important to train new generations of peer reviewers and raise awareness of the importance of participating in peer review. We need to recognise that science is a team effort and that it is everyone's responsibility to create new knowledge for the benefit of animals and humans. Co-creation of scientific journals is therefore important to ensure quality and innovation in all these areas.

We would like to express our sincere gratitude to all participants who, with their knowledge, experience and commitment, greatly enriched the discussions and helped to raise awareness of the importance of harmonious collaboration between authors, reviewers and editors. At the JRC, we strive to continuously improve the editorial process and maintain the long tradition of local publication of a scientific journal. This tradition enables the networking of researchers, clinical professionals and educators, which contributes to the development of global knowledge, the promotion of scientific dialogue and the advancement of veterinary medicine.

Finally, we invite all readers to visit the website of our journal *Slovenian Veterinary Research*. There you will find the latest scientific articles, research results and current information from the field of veterinary medicine. I also invite you to follow our social networks (accessible via the website) to keep up to date with news, interesting events and opportunities for collaboration. We are also pleased to announce a special issue entitled "Comparative Cancer Biology and Oncology" (Call for Papers: Comparative Cancer Biology and Oncology | *Slovenian Veterinary Research*), which emphasises the importance of understanding the similarities and differences in cancer susceptibility, disease aggressiveness and response to therapy between different animal species and humans. Knowledge in this area will contribute significantly to the development of treatment options for all.

The editors of the special edition are:

- Klementina Fon Tacer, DVM, PhD, Texas Tech University School of Veterinary Medicine, Amarillo, Texas, USA
- Nataša Debeljak, Univ. Dipl. Biol., PhD, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
- Simona Kranjc Brezar, Univ. Dipl. Biol., PhD, Oncology Institute Ljubljana, Slovenia

If you have interesting research papers, clinical cases or review articles on this topic, we cordially invite you to submit your contributions! Your participation will make an important contribution to the understanding and development of oncology.

novih generacij ocenjevalcev in ozaveščanje o pomenu sodelovanja pri ocenjevanju člankov strokovnih kolegov. Zavedati se moramo, da je znanost skupinsko delo in odgovornost vseh, ki pri tem sodelujemo, da ustvarjamo novo znanje za dobrobit živali in ljudi. Soustvarjanje akademskih revij je zato ključno za zagotavljanje kakovosti in inovativnosti na vseh teh področjih.

Iskreno se zahvaljujemo vsem udeležencem, ki so z znanjem, izkušnjami in predanostjo pomembno obogatili razprave ter prispevali k ozaveščanju o pomenu usklajenega sodelovanja avtorjev, ocenjevalcev in urednikov. V SVZ si prizadevamo za stalno izboljševanje uredniškega procesa in ohranjanje dolgoletne tradicije lokalnega izdajanja znanstvene revije. Ta tradicija omogoča povezovanje raziskovalcev, kliničnih strokovnjakov in pedagogov, kar prispeva k razvoju globalnega znanja, spodbujanju znanstvenega dialoga ter napredku veterinarske medicine.

Vse bralce vabim k obisku spletne strani (*Slovenian Veterinary Research*) naše revije *Slovenski veterinarski zbornik*, kjer lahko dostopate do najnovejših znanstvenih člankov, raziskav in aktualnih informacij s področja veterinarske znanosti. Prav tako vas vabim k spremljanju naših socialnih omrežij (dostopnih preko spletne strani), kjer boste obveščeni o novostih, zanimivostih in priložnostih za sodelovanje. Obenem z veseljem napovedujemo posebno številko z naslovom: »Comparative Cancer Biology and Oncology« (Call for Papers: Comparative Cancer Biology and Oncology | *Slovenian Veterinary Research*). Ta zbirka člankov bo poudarila pomen razumevanja podobnosti in razlik v dovzetnosti za raka, agresivnosti bolezni ter odziva na terapijo med različnimi živalskimi vrstami in ljudmi. Znanje na tem področju pomembno prispeva k razvoju terapevtskih možnosti za vse.

Urednice posebne izdaje so:

- Klementina Fon Tacer, dr. vet. med., doktorica znanosti, Šola veterinarske medicine Univerze Texas Tech, Amarillo, Teksas, ZDA
- Nataša Debeljak, univ. dipl. biol., doktorica znanosti, Medicinska fakulteta Univerze v Ljubljani, Ljubljana, Slovenija
- Simona Kranjc Brezar, univ. dipl. biol., doktorica znanosti, Onkološki inštitut Ljubljana, Slovenija

Če imate zanimive raziskave, klinične primere ali pregledne članke na to temo, vas toplo vabimo k oddaji prispevkov! Vaše sodelovanje bo prispevalo k poglobljenemu razumevanju in razvoju onkologije.

References

1. Cestnik V. The cover and logo of Slovenian Veterinary Research contains the rod of asclepius. *Slov Vet Res* 2023; 60(2): 49–53. doi:10.26873/SVR-1750-2023
2. Kastelic J, Ogilvie T. Contributing to The Canadian Veterinary Journal; we're all in this together! *Can Vet J* 2021; 62 : 321–2.
3. Kubale V. Veterinary illustration: science and art telling a story together. *Slov Vet Res* 2023; 60(1): 5–7. doi: 10.26873/SVR-1723-2023.
4. Erjavec V, Kovačič P. Can illustration help us understand our cats better? *Slov Vet Res* 2023; 60(4): 181–3. doi: 10.26873/SVR-1917-2023.
5. Goldstein JL. The surprise element: a hallmark of creativity in scientists, artists, and comedians. *Cell* 2021;184(21): 5261–5. doi: 10.1016/j.cell.2021.08.007.
6. Bontzorlos VA, Johnson DH, Poirazidis K, Roulin A. Owl symbolism in Greek civilization over the last 5000 years: social perceptions and implications for conservation. *Eur Zool J* 2023; 90(2): 691–707. doi: 10.1080/24750263.2023.2254823.
7. Fon Tacer K. Reflecting and looking forward - the role of veterinary scientific journals in veterinary research and medicine. *Slov Vet Res* 2023; 60(4):177–9. doi: 10.26873/SVR-1922-2023.
8. Barić H, Petrak J, Kovačič N, Kalanj Bognar S. The Croatian Medical Journal over three decades: the impact beyond the impact factor. *Croat Med J* 2022;63(5):405–6. doi: 10.3325/cmj.2022.63.405.
9. Čater M. Pomen sodelovanja med uredništvi znanstvenih revij za rast znanosti in javno osveščenost. *Sinapsa* 2024; 27. https://www.sinapsa.org/eSinapsa/stevilke/2024-27/360/Pomen_sodelovanja_med_uredni%C5%A1tvi_znanstvenih_revij_za_rast_znanosti_in_javno_osve%C5%A1C4%8Denost. (4. 12. 2024)
10. Gyles C. The Canadian Veterinary Journal. *Can Vet J* 2017; 58(2): 113–4.

Ključne besede: akademsko založništvo; kakovost izobraževanja; raziskovalno delo; razvoj stroke; Slovenski veterinarski zbornik

Circulation and Presentation of Canine Distemper Virus Among Various fox Species

Key words

canine distemper virus;
fox;
Vulpes vulpes;
Canidae;
virus;
morbidity

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Abstract: Canine distemper virus (CDV) is a highly contagious and often fatal virus that affects members of the Canidae family. Foxes are susceptible to CDV, and outbreaks among fox populations have been reported in several species. The symptoms of distemper in foxes are similar to those in domestic dogs. Foxes may contract the virus directly from infected animals or via exposure to a contaminated environment. Among domestic animals' dogs are often a source of infection for domestic and wildlife populations, while among wildlife primary sources are other wildlife through direct contact or vectors such as insects. This review comprehensively discusses the virus itself, its pathology, clinical signs, diagnostic methods, treatment options, preventive measures, and its impact on fox populations. Wildlife veterinarians and researchers monitor and study the impact of diseases like distemper on fox populations. Understanding the spread and prevalence of such diseases is crucial for wildlife conservation efforts.

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Introduction

Canine Distemper Virus (CDV) is a pathogen causing significant morbidity and mortality in both domestic and wild carnivores (1). This virus is predominantly associated with dogs, which are largely responsible for its spread into wildlife populations that had not been previously exposed (2).

CDV's impact is a major concern for the conservation and health of wild species (3). In recent decades, this highly infectious disease has contributed to the decline of some wild carnivore populations due to its susceptibility to the virus (4). Factors facilitating the spread of CDV include a wide host spectrum, genetic variability, uncontrolled animal movement, environmental changes, and habitat reduction (5, 6).

Treatment options for CDV are limited, with vaccination being the primary preventative measure (6). Diagnosing CDV based solely on clinical signs can be challenging, as it shares respiratory, neurological, and gastrointestinal symptoms with other diseases like rabies, parvovirus, toxoplasmosis, and feline panleukopenia (4).

The prevalence of Canine Distemper Virus (CDV) in wildlife has been explored in a limited number of studies, most of which focus on symptomatic or dead animals (7). Among all wild carnivores, foxes are notably affected by CDV.

The Canidae family is divided into two tribes: the Canini tribe, which includes dogs, wolves, jackals, and coyotes, and the Vulpini tribe, which consists of foxes (8). Foxes, small to medium-sized omnivorous mammals (9, 10), are characterized by their upright triangular ears, pointed snouts, and long bushy tails (11, 12). They inhabit every continent except Antarctica and comprise 12 species (13, 14). Some fox species, such as the *Vulpes vulpes nescator*, *Vulpes bengalensis*, and *Vulpes chama* are classified as vulnerable or critically endangered (15). Foxes are highly adaptable predators, with species like *Vulpes vulpes* often found in urban areas that benefit from readily available food and shelter (16).

This review comprehensively discusses the virus itself, its pathology, clinical signs, diagnostic methods, treatment options, preventive measures, and its impact on different foxes species populations.

Canine Distemper Virus Structure

Negative-sense single-stranded RNA virus having a diameter of about 100-250nm (4, 17). It belongs to the genus Morbillivirus of the family Paramyxoviridae (4, 17). It possesses a helical nucleocapsid (N) surrounded by a lipoprotein envelop structure (17). The RNA genome consists of six genes that code for a single envelope-associated protein – matrix (M), two glycoproteins (hemagglutinin(H), fusion protein (F)), and two transcriptase-associated proteins (phosphoprotein (P), large protein (L) (4, 5) (Figure 1).

The haemagglutinin (H) protein is responsible for the first virus-host cell interaction and initial virus entry into a host (4). Due to its higher genetic and antigenic variation than other viral genes, the H gene is a pivotal target for investigating the genetic and antigenic diversity of the Canine Distemper Virus. Studies have revealed significant genetic diversity in the H gene, identifying various geographically distinct genotypes such as Asia-1, Asia-2, Europe-1, Arctic, America-1, America-2, Wildlife, Arctic-Like, and others. This diversity has made the H gene the primary focus of molecular epidemiological studies on CDV. Researchers have identified seven major lineages based on the genetic relationships among complete H gene sequences of different CDV strains. These lineages include America-1, America-2-5, Arctic-like, Asia-1-4, Asia-2, Europe-1, Europe-Wildlife, Caspian, Asia 4–6, Rockborn-like, India-1/Asia-5, Asia-6, South Africa, East Africa, and South America-2-3 (4, 17-19).

Host Range

In recent years, CDV has been detected in various hosts beyond canids (17). It is regarded as a multi-host and globally distributed pathogen (20). Among the vertebrate groups where CDV has been reported are included: Canidae (dogs, wolves, fox, dingo, African dogs), Ailuridae (red panda), Mephitidae (skunks), Mustelidae (ferrets, minks, otters, badgers), Procyonidae (raccoons), Ursidae (giant pandas), Felidae (lions, tigers, and leopards), Hyaenidae (hyenas), Viverridae (binturongs and civet), non-human primats (*Macaca mulatta*, *Macaca fascicularis*, *Macaca fuscata*), Tayassuidae (*Pecari tajacu*), and marine mammals (seals, dolphins, porpoises) (8, 20, 21).

Table 1 and Figure 2 describe the geographical distribution of CDV reports available to the moment of the writing of this manuscript, across 15 different fox species all around the world.

Pathogenesis and transmission routes

CDV is known for its high contagiousness and rapid transmission among susceptible hosts (4). The virus causes

Table 1: Fox species and countries where the CDV has been reported

Specie	Country	Reference
Red foxes (<i>Vulpes vulpes</i>)	USA	(11, 22–27)
	Norway	(20)
	Italy	(6, 7, 18, 21, 28, 29)
	Spain	(5, 30, 31)
	Portugal	(2)
	Croatia	(7)
	Germany	(7, 9, 9, 32, 32–35)
	Denmark	(7, 16, 36)
	Switzerland	(7)
	Greece	(37)
	Austria	(38)
	Slovenia	(38)
	Czech Republic	(19)
	Belgium	(39)
	Luxembourg	(10)
Desert foxes (<i>Vulpes macrotis arsipus</i>)	USA	(40)
Grey foxes (<i>Urocyon cinereoargenteus</i>)	USA	(11, 22–24, 26, 41–45)
South American gray fox (<i>Lycalopex griseus</i>)	Brazil	(46, 47)
	Argentina	(48)
Culpeo (<i>Dusicyon culpaeus</i>)	Argentina	(48)
Darwin's foxes (<i>Lycalopex fulvipes</i>)	Chile	(49)
Fennec foxes (<i>Vulpes zerda</i>)	Sudan	(8)
Island fox (<i>Urocyon littoralis catalinae</i>)	California USA	(50, 51)
Artic fox (<i>Vulpes lagopus</i>)	Norway	(20, 25)
	USA	(52)
	Brazil	(53–55)
Crab-eating fox (<i>Cerdocyon thous</i>)	Colombia	(56)
	Argentina	(57)
	Brazil	(53, 58)
hoary fox (<i>Lycalopex vetulus</i>)	Brazil	(3, 54)
swift foxes (<i>Vulpes velox</i>)	USA	(59)
Indian foxes (<i>Vulpes bengalensis</i>)	India	(60)
Bat-eared foxes (<i>Otocyon megalotis</i>)	Africa	(61)

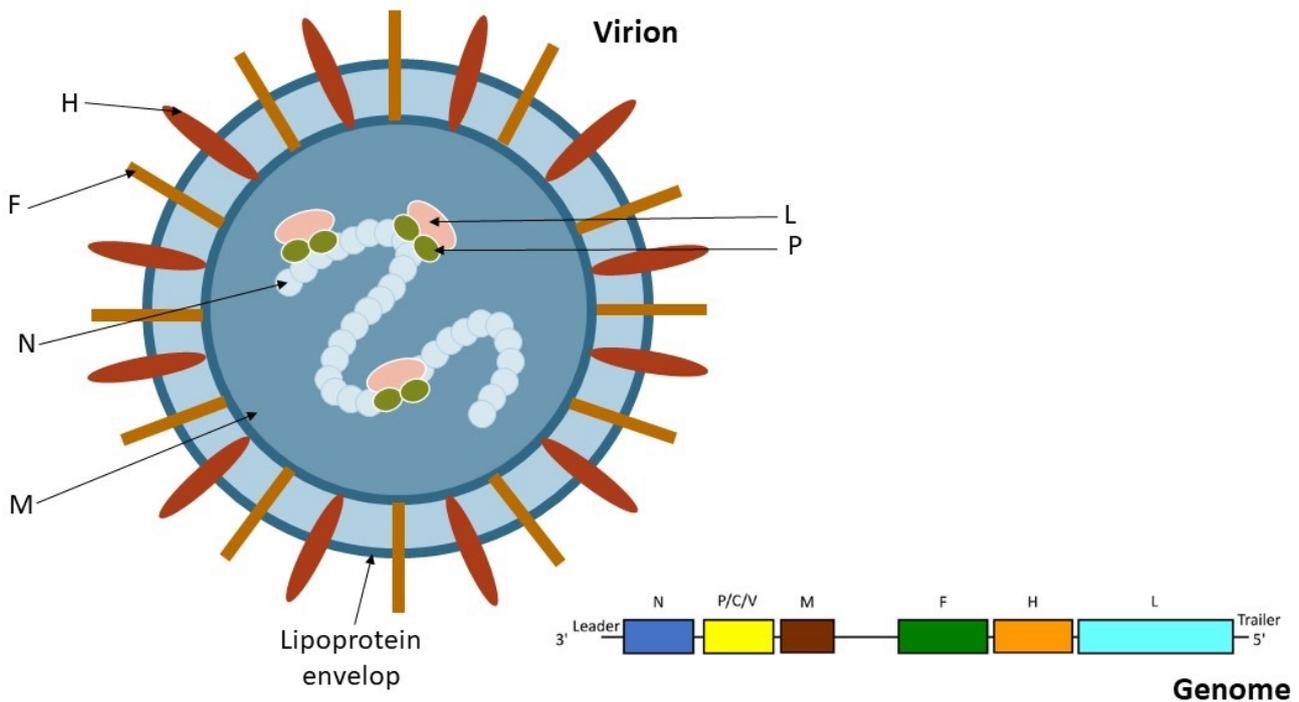


Figure 1: Structure of the Virion and Genome of Canine Distemper Virus (F- fusion protein, M-Matrix protein, P- phosphoprotein, L – large protein, H – hemagglutinin protein, N – nucleocapsid). (Adapted from 4).



Figure 2: Distribution worldwide of fox species where CDV was reported. (Author: Andreia Garcês)

an acute, highly immunizing infection, which requires dense host populations for its long-term persistence (18, 26, 62). CDV is resistant to cold temperatures but is rapidly inactivated by factors such as ultraviolet light, oxidizing agents, detergents, heat, lipid solvents, or dissection (4, 30). At room temperature (around 25°C), its viability is limited,

lasting only between 20 minutes to 3 hours when in contact with tissue or exudates (4).

The transmission of CDV occurs through direct contact or aerosolized fluids such as oral and respiratory secretions (including coughing, sneezing, licking, and biting), ocular

fluids, urine, faeces, and blood. It can also spread through shared contaminated food and water during the acute phase of infection (4, 21, 26). The host can shed the virus for 60–90 days post-infection (26). In domestic dogs, transplacental infection has been observed (21). CDV can also be transmitted via fomites at room temperature or lower for several hours (63). In wildlife, infections can occur throughout the year, but there is often a peak in cases during spring and summer, correlating with higher susceptibility in juveniles (63).

The virus often enters the host via the respiratory tract, primarily affecting the epithelial cells of both the respiratory and gastrointestinal tracts. Its initial replication takes place within these mucosal epithelial cells (20, 26) (Figure 3a).

The virus then spreads within the respiratory and gastrointestinal tissues and continues replicating. This leads to the development of respiratory and gastrointestinal clinical signs, as illustrated in Figure 3b. Rapidly, the virus spreads to regional lymph nodes (lymphotropism) (17), multiplies within macrophages and then enters the bloodstream, disseminating throughout the various organs and tissues of the body (25, 26) (Figure 3c). The virus can potentially affect immune cells during viremia, resulting in immunosuppression. It can also cross the blood-brain barrier, subsequently infecting neurons and glial cells. This invasion into the central nervous system leads to the development of neurological signs (3, 4, 28) (Figure 3d). Then, the virus is released by the apical surface of epithelial cells through aerosol and contaminates other animals

(Figure 2e). CDV demonstrates extended persistence in tissues such as neurons, urothelium, foot pads, and uvea. In healthy animals that develop antibodies, the virus is typically cleared approximately 14 days following infection. However, even if the animal recovers, it can continue to shed the virus in its urine for 60–90 days (42).

Clinical signs

The severity of the infection is influenced by various factors, including the host's immune status, age, and the virulence of the strain (21). Approximately 50-70% of dog infections are subclinical, presenting non-specific symptoms or mild, self-limiting respiratory signs (21). Similarly, such subclinical infections are likely to occur in wild hosts, such as foxes. The clinical signs in foxes are very similar to other members of the Canidae family (31).

The clinical manifestations of CDV infection include neurological, respiratory, and gastrointestinal signs (4, 5, 17). There are two main clinical forms: acute systemic and chronic nervous (4, 21). Early signs, such as listlessness, loss of appetite and fever, are often overlooked (4). The acute systemic phase, occurring 2-3 weeks post-infection, presents clinical signs such as fever, anorexia, rash, coughing, dyspnoea, dehydration, mucopurulent oculonasal discharge, optic neuritis, dermal manifestations including pustular dermatitis (distemper exanthema), chorioretinitis, rhinitis, uveitis, hyperkeratosis of the nose, foot pads and eyelids, vomiting, conjunctivitis, depression, jaundice, and

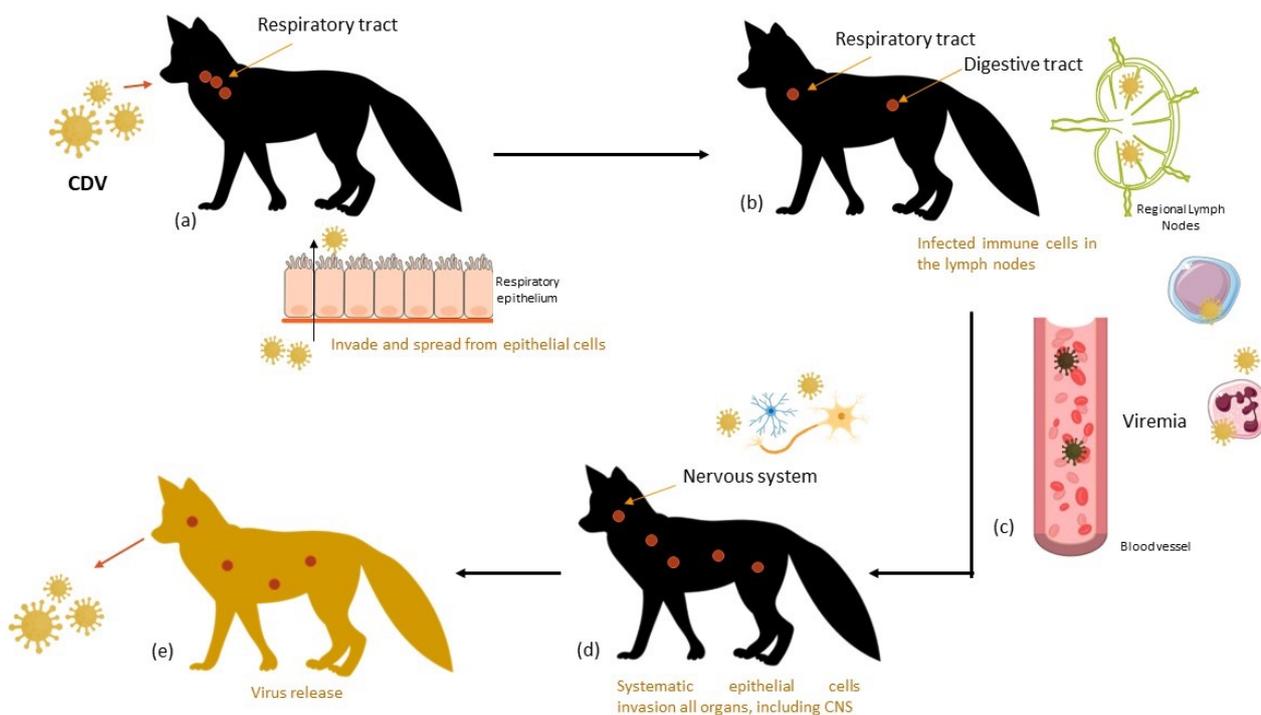


Figure 3a-3d: Pathogenesis of Canine Distemper Virus. (Author: Andreia Garcês)



Figure 4: *Vulpes vulpes* emaciated (A), ulceration of the cornea (B), diarrhoea (C), enamel hypoplasia (D). (Author: Andreia Garcês/Isabel Pires)



Figure 5: *Vulpes vulpes* pneumonia (A) where the lungs were incompletely deflated, diffusely and mildly oedematous, and the right cranial and middle lobes had multifocal to coalescing, dark red, consolidated area. Enteritis (B). (Author: Andreia Garcês/Isabel Pires)

diarrhoea (4). During this phase, the virus can be found in all body secretions (4, 42) (Figure 4).

The neurological phase typically arises 2-3 weeks after the initial infection, with acute and chronic presentations. The progression and variety of signs depend on the affected

brain region. These include abnormal behavior, diminished fear of humans, seizures, hyperesthesia, cervical rigidity, paresis or paralysis, tetraparesis with sensory ataxia, chewing gum-like mouth movements, incoordination, as well as cerebellar and vestibular signs, cycling motions, and blindness (4). Approximately 20 days post-infection,

inflammation, reduced myelin synthesis (demyelination), and metabolic dysfunction often result in the death of the animal within 2 to 4 weeks (42). Additionally, the compromised immune state post-CDV infection frequently exacerbates clinical signs due to secondary bacterial infections of the skin and respiratory tract (4).

CDV can affect tooth buds and ameloblast in very young animals, leading to enamel hypoplasia, juvenile cellulitis and bone lesions (4, 64).

In terms of clinical pathology, CDV infection may result in various hematological and biochemical changes, including lymphopenia, thrombocytopenia, regenerative anemia, reduced albumin levels, and increased concentrations of alpha- and gamma-globulins (64).

Post-mortem findings

During a necropsy, samples collected for examination should include the spleen, tonsil, lymph node, stomach, kidney, lung, duodenum, urinary bladder, and brain (63). The most significant gross lesions observed in cases of Canine Distemper Virus infection are pneumonia, depletion of lymphopoietic organs, hyperkeratosis of the nose, foot pads, and eyelids, emaciation and dehydration (Figure 5) (64).

Microscopic examination often reveals the presence of eosinophilic intracytoplasmic inclusion bodies, which are more common, and intranuclear inclusion bodies, particularly in the central nervous system, urinary bladder, and bronchial epithelium (4, 5). It is also possible to observe perivascular lymphoplasmacytic infiltration in areas of demyelination and neuronal degeneration of the CNS, lymphohistiocytic polyencephalitis, diffuse interstitial

pneumonia, broncho interstitial pneumonia, pustular dermatitis. Syncytial giant cells in the lungs and CNS white matter, anterior uvea, and lymph nodes may also be present (65) (Figure 6).

Canine Distemper Virus and Fox Populations

CDV poses significant risks to fox populations, with outbreaks leading to illness and high mortality rates, especially among young or immunocompromised individuals. The severity of the disease can vary among individuals and populations (4, 66). For instance, the Island fox population (*Urocyon littoralis*) on Santa Catalina Island, California, USA, has been experiencing a decline since 1999. Research indicates that CDV is partly to blame for this decline, likely due to the introduction of raccoons (50, 51).

Outbreaks of CDV can significantly influence the dynamics of fox populations. In certain instances, the virus may lead to fluctuations in population size, characterized by increased mortality rates during outbreaks. The degree to which CDV impacts these populations can vary, depending on factors like the density of the fox population, the presence of additional stressors, and the availability of susceptible individuals (17, 32, 34).

Fox populations may have developed some level of coexistence with CDV over time (67). Fox populations may have developed immune responses that provide some level of resistance to CDV (68). Through natural selection, individuals with genetic variations that confer greater immunity to the virus may be more likely to survive and reproduce, leading to the prevalence of these traits within the population over time (35, 69). Also, through passive

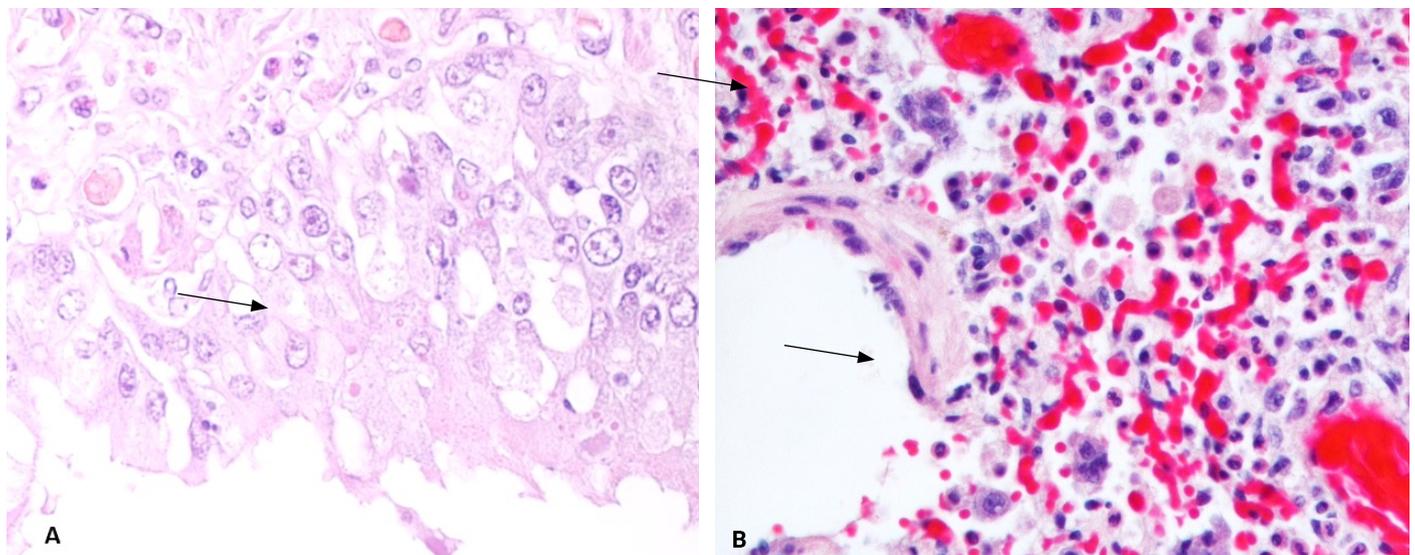


Figure 6: CDV infected *Vulpes vulpes* (A) eosinophilic inclusions in bronchial epithelium, H&E, 400x, (B) Lung syncytial cells, H&E, 200x. (Author: Isabel Pires)

immunity maternal antibodies could provide protection to fox pups during the critical early stages of life when they are most vulnerable to infections like CDV (70). Foxes might exhibit behaviors that reduce their risk of contracting CDV or limit the spread of the virus within their populations. For example, foxes could alter their social behavior to minimize direct contact with infected individuals, reducing the likelihood of transmission (71).

Diagnosis, treatment, and prevention

The diagnosis of CDV infection based only on clinical signs can lead to a false diagnosis since the clinical signs are similar to other diseases (64). *Ante-mortem* diagnosis can be achieved using methods like immunofluorescence assay (IFA), serological tests, cell culture, or reverse transcription–polymerase chain reaction (RT-PCR) (63). *Post-mortem* diagnosis can be done by observing CDV inclusion bodies in various organs (4).

To enhance the accuracy of serological results for canine distemper virus (CDV) in foxes using ELISA tests, it's imperative to consider potential adaptations of cut-off values tailored specifically for this species (72). This entails analyzing a robust dataset of serum samples collected from healthy fox populations to establish species-specific cut-off values. Additionally, accounting for geographic variation in CDV prevalence and antibody levels among fox populations is crucial, necessitating adjustments based on regional disease dynamics (5). Age-related differences in immune response should also be taken into account, potentially warranting age-specific cut-off values to accurately assess serological status across different age groups (73). Longitudinal monitoring of antibody levels in fox populations can provide insights into temporal trends, facilitating refinement of cut-off values over time. Furthermore, validation studies comparing the performance of alternative cut-off values in terms of sensitivity and specificity are essential to identify the optimal threshold for accurate detection of CDV antibodies in foxes (74). By integrating these potential adaptations and employing rigorous quality control measures, such as standardization of laboratory protocols and proficiency testing, we can improve the reliability of serological testing and enhance our understanding of CDV prevalence and dynamics in wild fox populations (51, 75).

Unfortunately, there is no specific antiviral treatment for CDV infection in foxes or any other species (4, 66). Treatment for CDV in foxes primarily focuses on supportive care to manage symptoms and complications associated with the disease. All infected animals should be isolated from other susceptible hosts. Symptomatic treatment, that includes fluid therapy, respiratory control, nutritional support (vitamin A supplementation can be added), temperature control, and control of secondary infections can be applied. Early intervention can improve the chances

of a more favourable outcome (42, 76). In cases where the CDV infection is severe, causing irreversible damage or suffering that cannot be alleviated by treatment, euthanasia may be considered to prevent further distress to the animal (50, 64).

Preventing CDV infection in foxes primarily involves implementing measures to reduce exposure to the virus and promoting immunity through vaccination (58, 62). Vaccination is the most effective strategy against CDV infection. Various vaccines have been developed, eliciting differing responses across species. Vaccines are available for domestic dogs and can indirectly benefit fox populations by reducing the circulation of the virus in the environment (70). Vaccination programs for domestic dogs should be comprehensive and include regular boosters to maintain immunity. Additionally, efforts should be made to vaccinate captive fox populations, such as those in rehabilitation centers or wildlife parks, where feasible (12, 32, 76). Avian cell-adapted CDV vaccines have proven highly effective in fennec and red and grey foxes. The canarypox-vectored vaccine has also been effective in fennec foxes (4). These vaccines can be administered via parenteral, intranasal, and intraduodenal routes. It is necessary to monitor continuously and study genetic and antigenic drift in circulating CDV strains to ensure the effectiveness of the vaccine in preventing infection (17).

Minimizing contact between foxes and other animals, particularly domestic dogs, reduces the risk of transmission (4, 58, 62). This may involve implementing measures to deter foxes from entering areas frequented by dogs or managing waste and food sources to reduce attraction (4, 38). Minimizing environmental contamination through proper disposal of carcasses and contaminated materials, as well as regular cleaning and disinfection of areas frequented by foxes, can help reduce the risk of transmission (38).

Regular surveillance and monitoring of foxes and other wildlife populations can provide valuable data on disease prevalence, including CDV, and help identify outbreaks or emerging threats (42, 76). This may involve collecting biological samples (e.g., blood, tissue, faeces) for laboratory testing, conducting necropsies on deceased animals to determine the cause of death, and implementing disease surveillance programs in collaboration with wildlife management agencies, research institutions, and conservation organizations (33, 47).

Conclusions

CDV infection poses a significant concern for wildlife conservation, particularly for endangered or threatened canid species. Outbreaks of the disease can exacerbate the threats to already vulnerable populations. It is crucial to understand the dynamics of CDV within fox populations, as this knowledge is vital for both wildlife management

and the health of domestic animals. This understanding underscores the interconnectedness of ecosystems and the potential impact of infectious diseases on wildlife populations. Efforts towards wildlife conservation may involve monitoring for disease outbreaks, implementing vaccination programs, studying population dynamics, and exploring interactions between domestic and wild canid populations.

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References

- Martinez-Gutierrez M, Ruiz-Saenz J. Diversity of susceptible hosts in canine distemper virus infection: a systematic review and data synthesis. *BMC Vet Res* 2016, 12(1): 78. doi:10.1186/s12917-016-0702-z
- Rosa GM, Santos N, Grøndahl-Rosado R, et al. Unveiling patterns of viral pathogen infection in free-ranging carnivores of northern Portugal using a complementary methodological approach. *Com Immunol Microbiol Infect Dis* 2020; 69: 101432. doi: 10.1016/j.cimid.2020.101432
- Silva ML, Caiaffa MG, da Costa ALM, et al. Canine distemper virus and canine adenovirus type 1 co-infection in a free-living hoary fox (*Lycalopex vetulus*) from Brazil. *Braz J Microbiol* 2023, 54(1): 587–95. doi: 10.1007/s42770-023-00921-7
- Loots A, Mitchell E, Dalton D, Kotze A, Venter E. Advances in canine distemper virus (CDV) pathogenesis research: a wildlife perspective. *J Gen Virol* 2017; 98(3): 311–21. doi: 10.1099/jgv.0.000666
- Oleaga Á, Vázquez CB, Royo LJ, et al. Canine distemper virus in wildlife in south-western Europe. *Transbound Emerg Dis* 2022; 69(4): e473–e85. doi: 10.1111/tbed.14323
- Ricci I, Cersini A, Manna G, et al. a canine distemper virus retrospective study conducted from 2011 to 2019 in central Italy (Latium and Tuscany Regions). *Viruses* 2021; 13(2): 272. doi: 10.3390/v13020272
- Prpić J, Lojkić I, Keros T, Krešić N, Jemeršić L. Canine distemper virus infection in the free-living wild canines, the red fox (*Vulpes vulpes*) and jackal (*Canis aureus moreoticus*), in Croatia. *Pathogens* 2023; 12(6): 833. doi: 10.3390/pathogens12060833
- Woo G-H, Jho Y-S, Bak E-J. Canine Distemper Virus Infection in Fennec Fox (*Vulpes zerda*). *J Vet Med Sci* 2010; 72(8): 1075–9. doi: 10.1292/jvms.09-0510
- Bourg M, Nobach D, Herzog S, et al. Screening red foxes (*Vulpes vulpes*) for possible viral causes of encephalitis. *Virol J* 2016; 13(1): 151. doi: 10.1186/s12985-016-0608-1
- Damien BC, Martina BEE, Losch S, Mossong J, Osterhaus ADME, Muller CP. Prevalence of antibodies against canine distemper virus among red foxes in Luxembourg. *J Wildl Dis* 2002; 38(4): 856–9. doi: 10.7589/0090-3558-38.4.856
- Kelly TR, Sleeman JM. Morbidity and mortality of red foxes (*Vulpes vulpes*) and gray foxes (*Urocyon cinereoargenteus*) admitted to the wildlife center of Virginia, 1993–2001. *J Wildl Dis* 2003; 39(2): 467–9. doi: 10.7589/0090-3558-39.2.467
- López-Peña M, Quiroga M, Vázquez S, Nieto J. Detection of canine distemper viral antigen in foxes (*Vulpes vulpes*) in northwestern Spain. *J Wildl Dis* 1994; 30: 95–8. doi: 10.7589/0090-3558-30.1.95
- Larivière S. Fox. In: Abella J, eds. *Encyclopaedia Britannica*. Chicago: Encyclopædia Britannica. <https://www.britannica.com/animal/fox-mammal> (26. 3. 2023)
- Larivière S, Stains HJ. Carnivore - Mammal Classification In: Abella J, eds. *Encyclopaedia Britannica*. Chicago: Encyclopædia Britannica. <https://www.britannica.com/animal/carnivore-mammal> (31. 10. 2023)
- The IUCN Red List of Threatened Species. IUCN Red List of Threatened Species. Cambridge: IUCN. <https://www.iucnredlist.org/es> (4. 2. 2022)
- Lempp C, Jungwirth N, Grilo ML, et al. Pathological findings in the red fox (*Vulpes vulpes*), stone marten (*Martes foina*) and raccoon dog (*Nyctereutes procyonoides*), with special emphasis on infectious and zoonotic agents in Northern Germany. Markotter W, editor. *PLoS One* 2017; 12(4): e0175469. doi: 10.1371/journal.pone.0175469
- Zhao J-J, Yan X-J, Chai X-L, et al. Phylogenetic analysis of the haemagglutinin gene of canine distemper virus strains detected from breeding foxes, raccoon dogs and minks in China. *Vet Microbiol* 2010; 140(1/2): 34–42. doi: 10.1016/j.vetmic.2009.07.010
- Monne I, Fusaro A, Valastro V, et al. A distinct CDV genotype causing a major epidemic in Alpine wildlife. *Vet Microbiol* 2011; 150(1): 63–9. doi: 10.1016/j.vetmic.2011.01.009
- Kličková E, Černíková L, Dumondin A, Bártová E, Budíková M, Sedlák K. Canine distemper virus in wild carnivore populations from the Czech republic (2012–2020): occurrence, geographical distribution, and phylogenetic analysis. *Life(Basel)* 2022; 12(2): 289. doi: 10.3390/life12020289
- Tryland M, Balboni A, Killengreen ST, et al. A screening for canine distemper virus, canine adenovirus and carnivore protoparvoviruses in Arctic foxes (*Vulpes lagopus*) and red foxes (*Vulpes vulpes*) from Arctic and sub-Arctic regions of Norway. *Polar Res* 2018; 37(1): 1498678. doi: 10.1080/17518369.2018.1498678
- Trogu T, Canziani S, Salvato S, et al. Canine distemper outbreaks in wild carnivores in northern Italy. *Viruses* 2021; 13(1): 99. doi: 10.3390/v13010099

22. Kimpston CN, Hatke AL, Castelli B, et al. High prevalence of antibodies against canine parvovirus and canine distemper virus among coyotes and foxes from Pennsylvania: implications for the intersection of companion animals and wildlife. *Microbiol Spectr* 2022; 26; 10(1): e02532. doi: 10.1128/spectrum.02532-21.
23. Davidson C. Declining downwind: amphibian population declines in California and historical pesticide use. *Ecol Appl* 2004; 14(6): 1892–902. doi: 10.1890/03-5224
24. Kimpston CN, Hatke AL, Castelli B, et al. High prevalence of antibodies against canine parvovirus and canine distemper virus among coyotes and foxes from Pennsylvania: implications for the intersection of companion animals and wildlife. *Microbiol Spectr* 2022; 10(1): e0253221. doi: 10.1128/spectrum.02532-21
25. Åkerstedt J, Lillehaug A, Larsen I-L, Eide NE, Arnemo JM, Handeland K. Serosurvey for canine distemper virus, canine adenovirus, leptospira interrogans, and toxoplasma gondii in free-ranging canids in Scandinavia and Svalbard. *J Wildl Dis* 2010; 46(2): 474–80. doi: 10.7589/0090-3558-46.2.474
26. Taylor K, Wilson JJ, Park AW, et al. Temporal and spatial patterns in canine distemper virus cases in wildlife diagnosed at the southeastern cooperative wildlife disease study, 1975–2019. *J Wildl Dis* 2021; 57(4): 820–30. doi: 10.7589/JWD-D-20-00212
27. Pluemer M, Dubay S, Drake D, et al. Red foxes (*Vulpes vulpes*) and coyotes (*Canis latrans*) in an urban landscape: prevalence and risk factors for disease. *J Urban Ecol* 2019; 5(1): 1–9. doi: 10.1093/jue/juz022
28. Martella V, Pratelli A, Cirone F, et al. Detection and genetic characterization of canine distemper virus (CDV) from free-ranging red foxes in Italy. *Mol Cell Probes* 2002; 16(1):77–83. doi: 10.1006/mcpr.2001.0387
29. Ferrara G, Brocherel G, Falorni B, Gori R, Pagnini U, Montagnaro S. A retrospective serosurvey of selected pathogens in red foxes (*Vulpes vulpes*) in the Tuscany region, Italy. *Acta Vet Scand* 2023; 65(1): 35. doi: 10.1186/s13028-023-00699-6
30. Sobrino R, Arnal MC, Luco DF, Gortázar C. Prevalence of antibodies against canine distemper virus and canine parvovirus among foxes and wolves from Spain. *Vet Microbiol* 2008; 126(1/3): 251–6. doi: 10.1016/j.vetmic.2007.06.014
31. López-Peña M, Quiroga MI, Vázquez S, Nieto JM. Detection of canine distemper viral antigen in foxes (*Vulpes vulpes*) in northwestern Spain. *J Wildl Dis* 1994; 30(1): 95–8. doi: 10.7589/0090-3558-30.1.95
32. Gras P, Knuth S, Börner K, et al. Landscape structures affect risk of canine distemper in urban wildlife. *Front Ecol Evol* 2018; 6: 136. doi: 10.3389/fevo.2018.00136
33. Frölich K, Czupalla O, Haas L, Hentschke J, Dedek J, Fickel J. Epizootiological investigations of canine distemper virus in free-ranging carnivores from Germany. *Vet Microbiol* 2000; 74(4): 283–92. doi: 10.1016/s0378-1135(00)00192-9
34. Denzin N, Herwig V, van der Grinten E. Occurrence and geographical distribution of canine distemper virus infection in red foxes (*Vulpes vulpes*) of Saxony-Anhalt, Germany. *Vet Microbiol* 2013; 162(1): 214–8. doi: 10.1016/j.vetmic.2012.08.031
35. Geiselhardt F, Peters M, Kleinschmidt S, et al. Neuropathologic and molecular aspects of a canine distemper epizootic in red foxes in Germany. *SciRep* 2022; 12(1): 14691. doi: 10.1038/s41598-022-19023-9
36. Pagh S, Chriél M, Madsen AB, et al. Increased reproductive output of Danish red fox females following an outbreak of canine distemper. *Canid Biol & Conserv* 2018; 21(3): 12–20.
37. Billinis C, Athanasiou L, Valiakos G, Mamuris Z, Birtsas P, Spyrou V. Phylogenetic analysis of canine distemper viruses from red foxes, Greece. *Vet Rec* 2013; 173(8): 194. doi: 10.1136/vr.101618
38. Nouvellet P, Donnelly CA, Nardi MD, et al. Rabies and canine distemper virus epidemics in the red fox population of northern Italy (2006–2010). *PLoS One* 2013; 8(4): e61588. doi: 10.1371/journal.pone.0061588
39. Garigliany M, Sarlet M, Franssen M, et al. Re-emergence of canine distemper in wildlife in Belgium. *Vet Rec* 2018; 182(15): 439. doi: 10.1136/vr.k1610
40. Randel CJ, Vanherweg WJ. Survey for select pathogens in the desert kit fox (*Vulpes macrotis arsipus*) in California, USA. *J Wildl Dis* 2022; 58(3): 631–5. doi: 10.7589/JWD-D-21-00102
41. Jacobson S, Ferro A, Navarro A, et al. Clostridium piliforme and canine distemper virus coinfection in 2 domestic dog littermates and a gray fox kit. *J Vet Diagn Invest* 2022; 34(5): 894–97. doi: 10.1177/10406387221109899
42. Pope JP, Miller DL, Riley MC, et al. Characterization of a novel canine distemper virus causing disease in wildlife. *J Vet Diagn Invest* 2016; 28(5): 506–13. doi: 10.1177/1040638716656025
43. Needle DB, Marr JL, Park CJ, et al. Concurrent infection of skunk adenovirus-1, *Listeria monocytogenes*, and a regionally specific clade of canine distemper virus in one gray fox (*Urocyon cinereoargenteus*) and concurrent listeriosis and canine distemper in a second gray fox. *Pathogens* 2020; 9(7): 591. doi: 10.3390/pathogens9070591
44. Hoff GL, Biler WJ, Proctor SJ, Stallings LP. Epizootic of canine distemper virus infection among urban raccoons and gray foxes. *J Wildl Dis* 1974; 10(4): 423–8. doi: 10.7589/0090-3558-10.4.423
45. Black JJ, Baumann PC. Carcinogens and Cancers in Freshwater Fishes. *Environ Health Perspect* 1991; 90: 27–33. doi: 10.1289/ehp.90-1519473
46. Acosta-Jamett G, Cunningham AA, Bronsvoort BM deC., Cleaveland S. Serosurvey of canine distemper virus and canine parvovirus in wild canids and domestic dogs at the rural interface in the Coquimbo Region, Chile. *Eur J Wildl Res* 2015; 61: 329–32. doi: 10.1007/s10344-014-0886-0
47. Acosta-Jamett G, Chalmers WSK, Cunningham AA, Cleaveland S, Handel IG, Bronsvoort BM deC. Urban domestic dog populations as a source of canine distemper virus for wild carnivores in the Coquimbo region of Chile. *Vet Microbiol* 2011; 152(3/4): 247–57. doi: 10.1016/j.vetmic.2011.05.008
48. Martino PE, Montenegro JL, Preziosi JA, et al. Serological survey of selected pathogens of free-ranging foxes in Southern Argentina, 1998–2001. *Rev Vet Sci* 2004; 23(3): 801–6. doi: 10.20506/rst.23.3.1521
49. Hidalgo-Hermoso E, Cabello J, Vega C, et al. An eight-year survey for canine distemper virus indicates lack of exposure in the endangered Darwin's fox (*Lycalopex fulvipes*). *J Wildl Dis* 2020; 56(2): 482–5. doi: 10.7589/2019-08-195
50. Timm SF, Munson L, Summers BA, et al. A Suspected canine distemper epidemic as the cause of a catastrophic decline in Santa Catalina Island foxes (*Urocyon littoralis catalinae*). *J Wildl Dis* 2009; 45(2): 333–43. doi: 10.7589/0090-3558-45.2.333
51. Clifford DL, Mazet JAK, Dubovi EJ, et al. Pathogen exposure in endangered island fox (*Urocyon littoralis*) populations: Implications for conservation management. *Biol Conserv* 2006; 131(2): 230–43. doi: 10.1016/j.biocon.2006.04.029
52. Stimmelmayer R, Rotstein DS, Maboni G, Person BT, Sanchez S. Morbillivirus-associated lipid pneumonia in Arctic foxes. *J Vet Diagn Invest* 2018; 30(6):933–6. doi: 10.1177/1040638718797382

53. Slaviero M, Ehlers LP, Lorenzo CD, et al. Anatomopathological and immunohistochemical aspects of distemper virus in Crab-eating-foxes and Pampa-foxes. *Acta Sci Vet* 2019; 47: 1632. doi: 10.22456/1679-9216.90120
54. Furtado MM, Hayashi EMK, Allendorf SD, et al. Exposure of free-ranging wild carnivores and domestic dogs to canine distemper virus and parvovirus in the Cerrado of Central Brazil. *EcoHealth* 2016; 13(3): 549–57. doi: 10.1007/s10393-016-1146-4
55. Courtenay O, Quinnell RJ, Chalmers WS. Contact rates between wild and domestic canids: no evidence of parvovirus or canine distemper virus in crab-eating foxes. *Vet Microbiol* 2001; 81(1): 9–19. doi: 10.1016/s0378-1135(01)00326-1
56. Echeverry-Bonilla DF, Buriticá-Gaviria EF, Orjuela-Acosta D, Chinchilla-Cardenas DJ, Ruiz-Saenz J. The first report and phylogenetic analysis of canine distemper virus in *Cercopithecus* from Colombia. *Viruses* 2022; 14(9):1947. doi: 10.3390/v14091947
57. Ferreyra H, Calderón MG, Marticorena D n, Marull C, Leonardo BC. Canine distemper infection in crab-eating fox (*Cercopithecus*) from Argentina. *J Wildl Dis* 2009; 45(4): 1158–62. doi: 10.7589/0090-3558-45.4.1158
58. Hübner S de O, Pappen FG, Ruas JL, Vargas GD, Fischer G, Vidor T. Exposure of pampas fox (*Pseudalopex gymnocercus*) and crab-eating fox (*Cercopithecus*) from the Southern region of Brazil to Canine distemper virus (CDV), Canine parvovirus (CPV) and Canine coronavirus (CCoV). *Braz Arch Biol Technol* 2010; 53(3): 593–7. doi: 10.1590/S1516-89132010000300012
59. Miller DS, Covell DF, McLean RG, et al. Serologic survey for selected infectious disease agents in swift and Kit foxes from the western United States. *J Wildl Dis* 2000; 36(4): 798–805. doi: 10.7589/0090-3558-36.4.798
60. Belsare AV, Vanak AT, Gompper ME. Epidemiology of viral pathogens of free-ranging dogs and Indian foxes in a human-dominated landscape in central India. *Transbound Emerg Dis* 2014; 61(suppl. 1): 78–86. doi: 10.1111/tbed.12265
61. Roelke-Parker ME, Munson L, Parker C, et al. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* 1996; 379(6564): 441–5. doi: 10.1038/379441a0
62. Meli ML, Simmler P, Cattori V, et al. Importance of canine distemper virus (CDV) infection in free-ranging Iberian lynxes (*Lynx pardinus*). *Vet Microbiol* 2010; 146(1/2): 132–7. doi: 10.1016/j.vetmic.2010.04.024
63. Kapil S, Yeary TJ. Canine distemper spillover in domestic dogs from urban wildlife. *Vet Clin Small Anim Pract* 2011; 41(6): 1069–86. doi: 10.1016/j.cvsm.2011.08.005
64. Deem SL, Spelman LH, Yates RA, Montali RJ. Canine distemper in terrestrial carnivores: a review. *J Zoo Wildl Med* 2000; 31(4): 441–51. doi: 10.1638/1042-7260(2000)031[0441:CDITCA]2.0.CO;2
65. Deem SL. Fungal diseases of birds of prey. *Vet Clin North Am Exot Anim Pract* 2003; 6(2): 363–76. doi: 10.1016/s1094-9194(03)00004-5
66. Karki M, Rajak KK, Singh RP. Canine morbillivirus (CDV): a review on current status, emergence and the diagnostics. *Virusdisease* 2022; 33(3): 309–21. doi: 10.1007/s13337-022-00779-7
67. Huang J, Cortey M, Darwich L, et al. Study of canine distemper virus presence in Catalonia's wild carnivores through H gene amplification and sequencing. *Animals (Basel)* 2024; 14(3): 436. doi: 10.3390/ani14030436
68. Rendon-Marin S, da Fontoura Budaszewski R, Canal CW, Ruiz-Saenz J. Tropism and molecular pathogenesis of canine distemper virus. *Virology* 2019; 16(1): 30. doi: 10.1186/s12985-019-1136-6
69. Duque-Valencia J, Sarute N, Olarte-Castillo XA, Ruiz-Sáenz J. Evolution and interspecies transmission of canine distemper virus—an outlook of the diverse evolutionary landscapes of a multi-host virus. *Viruses* 2019; 11(7): 582. doi: 10.3390/v11070582
70. Beineke A, Baumgärtner W, Wohlsein P. Cross-species transmission of canine distemper virus—an update. *One Health* 2015; 1: 49–59. doi: 10.1016/j.onehlt.2015.09.002
71. Wang H, Guo H, Hein VG, Xu Y, Yu S, Wang X. The evolutionary dynamics history of canine distemper virus through analysis of the hemagglutinin gene during 1930–2020. *Eur J Wildl Res* 2023; 69(3): 56. doi: 10.1007/s10344-023-01685-z
72. Muñoz-Hernández C, Wipf A, Ortega N, et al. Serological and molecular survey of canine distemper virus in red foxes (*Vulpes vulpes*): exploring cut-off values and the use of protein A in ELISA tests. *Prev Vet Med* 2023; 221: 106075. doi: 10.1016/j.prevetmed.2023.106075
73. Oluka GK, Namubiru P, Kato L, et al. Optimisation and validation of a conventional ELISA and cut-offs for detecting and quantifying anti-SARS-CoV-2 Spike, RBD, and Nucleoprotein IgG, IgM, and IgA antibodies in Uganda. *Front Immunol* 2023; 14: 1113194. doi: 10.3389/fimmu.2023.1113194
74. Aničić M, Vučičević I, Vasković N, et al. Histopathological characteristics and expression of CDV-NP antigen in the brain of serologically positive spontaneously infected red foxes (*Vulpes vulpes*) in Western Serbia. *Acta Vet (Beogr)* 2018; 68(4): 434–44. doi: 10.2478/acve-2018-0035
75. Zhang Y, Xu G, Zhang L, et al. Development of a double monoclonal antibody-based sandwich enzyme-linked immunosorbent assay for detecting canine distemper virus. *Appl Microbiol Biotechnol* 2020; 104(24): 10725–35. doi: 10.1007/s00253-020-10997-y
76. Sykes JE, Vandeveld M. Canine distemper virus infection. In: Sykes JE, ed. *Greene's infectious diseases of the dog and cat*. 5th ed. St. Louis: Elsevier, 2023: 271–88.

Kroženje in predstavitev virusa pasje kuge med različnimi vrstami lisic

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Izveček: Virus pasje kuge (CDV) je zelo nalezljiv in pogosto smrtonosen virus, ki prizadene pripadnike družine Canidae. Lisice so dovzetne za CDV, o izbruhih v populacijah lisic pa so poročali pri več vrstah. Simptomi kuge pri lisicah so podobni tistim pri domačih psih. Lisice se lahko z virusom okužijo neposredno od okuženih živali ali z izpostavljenostjo okuženemu okolju. Med domačimi živalmi so psi pogosto vir okužbe za domače in divje živalske populacije, med divjimi živalmi pa so glavni vir okužbe druge divje živali prek neposrednega stika ali prenašalcev, kot so žuželke. Ta pregled izčrpno obravnava virus, njegovo patologijo, klinične znake, diagnostične metode, možnosti zdravljenja, preventivne ukrepe in njegov vpliv na populacije lisic. Veterinarji in raziskovalci prostoživečih živali spremljajo in preučujejo vpliv različnih bolezni, kot je kuga, na populacije lisic. Razumevanje prenosa in razširjenosti teh bolezni je ključno pri prizadevanjih za ohranjanje prostoživečih živali.

Ključne besede: virus pasje kuge; lisica; *Vulpes vulpes*; Canidae; virus; obolevnost

Comparison of hCG and GnRH for Synchronization of the Follicular Wave in Saanen Goats During the Breeding Season

Key words

estrus synchronization;
follicle;
hCG;
GnRH;
goat

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Abstract: This study aimed to compare the effects of a single dose of GnRH or hCG administered at the beginning of the short-term oestrus synchronization protocol on ≥ 4 mm diameter follicles in Saanen goats during the breeding season. The goats estrus cycles were synchronized with intravaginal sponges containing 60 mg of medroxyprogesterone acetate for five days. Additionally, 1 ml of physiological saline solution (control-group; n=30), 0.004 mg of buserelin acetate (GnRH-group; n=31) or 150 IU of hCG (hCG-group; n=31) were injected intramuscularly to the goats during the sponges insertion. Transrectal ultrasonographic examination was performed immediately before and 24 h after intravaginal sponge application into all goats and follicles with a diameter of ≥ 4 mm in the ovaries were counted. Blood samples were collected on the same days to determine serum progesterone (P4) and estradiol (E2) concentrations. At the first ultrasonographic examination, the percentages of ≥ 4 mm diameter follicles were 56.66% (17/30), 54.83% (17/31) and 70.96% (22/31) in the control, GnRH and hCG groups, respectively. The percentage of goats with reduced follicle diameters 24 h later was 29.41% (5/17), 52.94% (9/17) and 59.09% (13/22) in the same groups, respectively. The mean regression rates of follicle diameters between days 0 and 1 in each group were significantly different ($P < 0.05$). Serum E2 concentrations were significantly different ($P < 0.05$) between days 0 and 1 in hCG group. There were no differences in serum E2, P4 concentrations and mean regression rates in follicle diameters between days 0 and 1 for all groups. As a result, a significant relationship between the administration of hCG or GnRH and the reduction of large follicle diameters could not be established.

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Introduction

Goat breeds located in temperate and subtropical latitudes generally show seasonal breeding behavior (1), resulting in seasonal fluctuations in the availability of goat products such as milk and meat. Controlling the seasonality of goat reproduction is a way to overcome this problem. Nowadays, assisted reproductive technologies such as artificial insemination (AI) and multiple ovulation and embryo transfer (MOET) allow faster genetic progress on farms by increasing the number of offspring from animals with high genetic merit, also creating a great opportunity for farmers and industry (2, 3). The induction and synchronization of estrus using exogenous hormones in goats is the most

important reproductive management tool for the application of these techniques (1-4). The success of these technologies depends on the developmental stage of the follicles in the ovaries at the time of application of exogenous hormones (3, 5). Hence, the synchronization of the follicular wave is important to achieve high fertility rates in both AI and MOET. Because of their ease of use, and availability, progestogen sponges such as medroxyprogesterone acetate (MAP) or fluorogestone acetate (FGA) have been the most commonly used exogenous hormones in goats (4-7). The target of traditional long-term protocols developed with a focus on the lifespan of the corpus luteum is to

reduce the secretion of luteinizing hormone (LH) that prevents the occurrence of estrus, the preovulatory LH surge, and ovulation, and to allow them to occur after progestagen removal (6, 7). Nevertheless, in long-term protocols, serum progesterone concentrations decrease to subluteal levels 6 days after treatment and remain at subluteal level until days 10-14 when the sponge is withdrawn (8). Subluteal progesterone concentrations cannot adequately suppress LH, leading to the development of abnormal and permanent follicles and adversely affect the fertility of oocytes, and the function of the corpus luteum (6, 8-10). Currently, data based on the dynamics and regulation of follicular development have led to the design of new follicle-focused protocols (8). New short-term protocols based on 5-7 days of progesterone administration have provided better control of preovulatory events and ovulation, resulting in similar or higher pregnancy rates (3, 11). In short-term protocols subluteal progesterone levels do not occur and the quality of preovulatory follicles, appearing 5 to 6 days after the emergence of a new follicle wave, is not affected (4, 8, 11-13). Our hypothesis was to examine whether hCG or GnRH administered at the beginning of the short-term estrus synchronization protocol will cause regression of all large follicles present on the ovaries at administration. Therefore, the objective of the present study was to compare the effect of a single dose of GnRH or hCG administered at the start of the short-term estrus synchronization protocol on the diameter of all ≥ 4 mm follicles in Saanen goats, during the breeding season.

Materials and methods

Animal management

This study was conducted at the Research and Application Farm of the Veterinary Faculty, University of Bursa Uludag, Bursa (latitude 40° 11' N, longitude 29° 04' E, altitude 155 m), Turkey. A total of 92 clinically healthy, free of reproductive disorders, and non-lactating, multiparous Saanen does were used during the breeding season in the region. The does were kept outdoors in a sheltered paddock under natural photoperiod and temperature conditions and were fed dry grain wheat hay (1500 g/doe/day) supplemented with commercial pellets (18% crude protein; 800 g/doe/day, 2800 Kcal). No additional feed supplement was given to the goats during the study. The goats were provided ad libitum with clean drinking water and mineralized salt.

Estrus synchronization and hCG or GnRH treatment

Estrus was synchronized in all goats by the insertion of an intravaginal sponge impregnated with 60 mg medroxyprogesterone acetate (MAP, Esponjavet, Hipra, Spain) for 5 days. The insertion day of intravaginal sponges was considered as day 0 of the study period. Subsequently all goats were divided into three groups according to their age, body weight, and body condition score (scale 0 to 5, according to

the model proposed by Morand-Fehr et al (14). In groups 1 to 3, the age averaged 37.00 ± 5.36 , 37.90 ± 4.90 , and 36.70 ± 3.24 months, body weights averaged 50.25 ± 3.04 , 50.58 ± 2.92 , and 50.94 ± 2.17 kg, and the body condition score averaged 3.09 ± 0.18 , 3.10 ± 0.19 , and 3.05 ± 0.14 , respectively; these parameters were not statistically different between the groups. Does in the control group ($n=30$), hCG group ($n=31$) and GnRH group ($n=31$) received i.m. 1 ml of physiological saline solution (0.9% NaCl), 150 IU of hCG (hCG, Chorulon, MSD, Netherlands), or 0.004 mg of buserelin acetate (GnRH, Buserin, Alke, Turkey), respectively, as soon as intravaginal sponges were placed.

Ovarian ultrasonography examination

All examinations to determine ovarian structures in goats were performed on a real-time transrectal ultrasonographic scanner (RTU, Prosound 2, Hitachi Aloka Medical, Ltd., Tokyo, Japan) with a 7.5 MHz linear transducer (model UST-660) and by the same operator. Before ultrasonic examination, the goat was placed in a standing position in a raised narrow wooden box, after which the hydro-soluble contact gel was applied, and the transducer was gently guided into the rectum. Briefly, after imaging the urinary bladder and the uterus horns on the monitor, the transducer was rotated 45°-90° clockwise or counterclockwise to observe both ovaries (15). Ultrasound examination of all goats was performed just prior to sponge insertion on day 0 and repeated on the following day. The number and size of all follicles equal or greater than 4 mm in diameter in both ovaries were recorded. After freezing the image of each ovary on the screen, the follicle diameter was measured using the built-in electronic caliper system and each ovary schematic map was drawn on a sheet of paper. The diameters of the follicles were characterized as large (i.e. ≥ 4 mm) on day 0 and day 1. Ovarian data were then combined for both ovaries of each doe.

Hormonal analysis

Blood samples (8 ml) were collected from all goats by jugular venipuncture into vacuum blood tubes (Ref. Hp. 0013, Hema & Lab. Ankara, Turkey) just before sponge insertion and 24 h later. The tubes were immediately placed on an ice pack, transported to the laboratory, and then centrifuged at 4 °C for 10 min at 1500 x g. After centrifugation, serum was transferred to 1.5 ml micro-tubes and stored at -20 °C until assayed for progesterone (P4) and estradiol (E2). The concentrations of P4 (SRB-T-86624) and E2 (SRB-T-87401) in the blood serum were determined with commercial ELISA kits and the results were read by the ELISA reader (ELX-808IU Ultra Microplate Reader, BioTek, USA) according to the manufacturer's instructions. The sensitivity of the P4 and E2 assay were 0.048 ng/ml and 0.925 pg/ml, respectively. The mean intra- and inter-assay coefficients of variation were <10% and <12% for P4 and E2, respectively.

Table 1: Percentage of Saanen goats with large (≥ 4 mm diameter) follicles before (Day 0) and 24 h after (Day 1) at the intravaginal sponge insertion in the Control, GnRH and hCG groups. All goats were synchronized using intravaginal sponges, and divided into three groups according to the treatment with either GnRH, or hCG (Day 0) or left untreated (control) group

	Control Group	GnRH Group	hCG Group	Total
Percentage of goats with large follicles at on Day 0	56.67% (17/30)	54.84% (17/31)	70.97% (22/31)	60.87% (56/92)
Mean number of large follicles in goats	1.18 \pm 0.10	1.18 \pm 0.10	1.18 \pm 0.11	1.17 \pm 0.06
Percentage of goats with regression of large follicles on Day 1	29.41% (5/17)	52.94% (9/17)	59.09% (13/22)	48.21% (27/56)

Table 2: Regression rate of large (≥ 4 mm diameter) follicles and serum E2 and P4 concentrations before (Day 0) and after 24 h (Day 1) after intravaginal sponge insertion in Saanen goats in the Control, GnRH and hCG groups. All goats were synchronized using intravaginal sponges, and divided into three groups according to the treatment with either GnRH, or hCG (Day 0) or left untreated (control) group

	Large follicles (≥ 4 mm)			E2 concentrations (pg/ml)		P4 concentrations (ng/ml)	
	Day 0 (mm)	Day 1 (mm)	Regression rate (mm)	Day 0	Day 1	Day 0	Day 1
Control Group(n=5)	4.73 \pm 0.34 ^a	3.50 \pm 0.33 ^b	1.37 \pm 0.37	29.57 \pm 4.11	21.49 \pm 2.99	2.29 \pm 0.15	2.85 \pm 0.40
GnRH Group(n=9)	4.52 \pm 0.19 ^a	3.26 \pm 0.24 ^b	1.10 \pm 0.33	38.29 \pm 4.57	27.59 \pm 4.59	1.59 \pm 0.23	1.66 \pm 0.43
hCG Group(n=13)	4.41 \pm 0.09 ^a	3.44 \pm 0.23 ^b	0.87 \pm 0.30	37.95 \pm 5.45 ^x	19.19 \pm 4.71 ^y	1.67 \pm 0.18	1.53 \pm 0.28
Total(n=27)	4.51 \pm 0.98	3.38 \pm 0.14	1.04 \pm 0.20	36.51 \pm 3.11	22.42 \pm 2.81	1.76 \pm 0.12	1.82 \pm 0.22

^{a,b,x,y} Values for each parameter in the same row with different superscripts differ significantly ($p < 0.05$)

Statistical analysis

SPSS for Windows, Version 20 was used to analyze the study data. Results were presented as mean (\pm SEM) and differences were considered significant when the p value was below 0.05. The normality of the distribution data was tested by Shapiro-Wilk test. Kruskal-Wallis test was chosen to analyze the findings. After that, Mann-Whitney U test was used to discover the statistical differences between the groups.

Results

Ovarian structures

The first ultrasonographic examination (day 0) performed in all goats just before the insertion of intravaginal sponges showed that 60.87 % (56/92) of the goats had follicles ≥ 4 mm in diameter (Table 1). In detail, these ratios were 56.67% (17/30) in the control, 54.84% (17/31) in GnRH and 70.97% (22/31) in hCG groups, respectively. In the ultrasonographic examination performed 24 h after the intravaginal sponge application, it was observed that ≥ 4 mm follicle diameters were decreased in 29.41% (5/17) of goats in the control, 52.94% (9/17) in GnRH and 59.09% (13/22) in hCG groups, respectively. The mean rates of ≥ 4 mm follicles diameter reduction were significantly different for each group between days 0 and 1 ($P < 0.05$), but not between the groups.

Serum P4 and E2 profile

Serum progesterone concentrations at the time of sponge insertion (day 0) revealed that 83 of 92 (90.21%) goats had a functional corpus luteum (> 1 ng/ml). Serum E2 concentrations were significantly different between days 0 and 1 in hCG group ($P < 0.05$), but not in control and GnRH groups (Table 2). There was no difference in serum E2 and P4 concentrations between days 0 and 1 for all groups.

Discussion

This study showed that the insertion of sponges containing medroxyprogesterone acetate caused the regression of ≥ 4 mm follicles in 29.41% (5/17) of the goats in the control group. According to the results of ultrasonographic measurement, an average reduction of the diameters by 1.37 mm in of the initially ≥ 4 mm follicles was observed in approximately a third of these goats after 24 h ($P < 0.05$), while the diameters of similar follicles did not change in the remaining goats in the group. In addition, there was no difference between days 0 and 1 in serum P4 and E2 concentrations in the control group. In the present trial, the mean diameter reduction of the large follicles in the control group was 1.37 mm (mm/day), which is higher compared with those previously reported in Shiba goats (0.8-0.9 mm/day) (16) and in Serrana goats (0.79 mm/day) (17). In addition, in these studies, no significant differences were observed in the regression rates of dominant follicles between follicular

waves in natural estrous cycles in goats, except for the ovulatory wave. The higher regression rate observed in our study, suggests that this difference might be probably due to fluctuation in naturally occurring plasma P4 concentrations on different days of the estrous cycle. Unlike other studies, this difference may also be due to the exogenous administration of a progesterone analogue (medroxyprogesterone acetate). In goats synchronized with different methods, plasma P4 concentration peaked on day 10 of the estrous cycle and remained high until day 15, and then declined rapidly (15, 16, 18).

In addition, high plasma P4 concentrations in goats caused that the third and fourth wave emerge earlier compared to the first and second wave of the estrous cycle (16-18), what supports our study. Furthermore, the high regression rate found in our study also supports the negative effect of high plasma/serum P4 concentrations on follicular growth, as observed in Saanen (18) and Shiba goats (16), and as claimed by other researchers (16, 18, 19), due to the variation in plasma P4 concentrations between the middle and the onset or end of the estrous cycle in cycling goats, depending on the developmental stage of the corpus luteum. A positive correlation was observed between high plasma P4 concentrations and regression rates of large follicles in P4-treated goats, and high plasma P4 concentrations were considered as an accelerating factor in follicular turnover (6, 8, 12). As a result, it can be claimed that in 29.41% of the goats in the control group a new follicle wave emerged. Daily transrectal ultrasonographic examination results in goats showed that follicles on the ovaries developed in wave-like patterns throughout an interovulatory interval (15) and the first wave emerged just after ovulation (Day 0) (16-19). This could also be a reason why most of the studies have focused on the manipulation of the first wave, which emerges on the ovulation day of the previous cycle, as it is very difficult to predict when other waves would emerge (6, 8). Therefore, our study, as in researchers' studies, has been designed to regress large follicles on the ovaries with the insertion of intravaginal devices containing progesterone or its analogues, thus creating a new follicle wave after the withdrawal of intravaginal devices (Day 0 Protocol) (8, 20). Also, it has been reported that a new wave of follicles does not emerge unless the large follicles on the ovaries regress (5, 8, 21). In a previous study, Año-Perello et al (20) reported that 81.1% (30/37) of large follicles regressed 24 h after insertion of a controlled internal drug releasing device (CIDR) in Segureña meat ewes, and plasma E2 concentrations decreased significantly between days 0 and 1. Despite using the same synchronization protocol in our study, no change in serum E2 concentrations was observed between days 0 and 1 in the control group. This difference may be due to the negative relationship between high serum/plasma P4 concentrations and the development of large follicles and E2 synthesis. As previously reported, plasma progesterone concentrations increase rapidly within the first 48 h after insertion of a progesterone or progestogen-containing intravaginal device, reaching a peak after 3 days and then

gradually decrease (22). Similarly, Rubianes and Manchaca (6) reported that large follicles that grow in the mid-late luteal phase do not synthesize high levels of estradiol-17 β due to the high P4 concentrations produced by the corpus luteum. This variation may be due to natural progesterone, breed, age, nutrition and development stage of follicles (2, 6, 8). Our results support previous studies showing that exposure to high levels of exogenous progesterone or progestogens affects the development of large follicles and, as a result, increases follicular turnover (2, 7).

In the current study, administration of 0.004 mg buserelin acetate (GnRH analogue) at the time of sponge insertion resulted in regression of large follicles in 52.94% (9/17) goats in the GnRH group. An average reduction of 1.10 mm in the diameters of larger follicles was observed in these goats. No significant change in serum P4 and E2 concentrations was observed between days 0 and 1. Unlike our study results, Año-Perello et al (20) reported significant differences in the percentages of sheep with regression of large follicles 24 h after CIDR insertion after treatment with GnRH (50 μ g gonadorelin, 100%) or without GnRH (81.1%) during the breeding season. Furthermore, they also observed a significant decrease in E2 concentration between days 0 and 1 in GnRH-treated ewes. In the present trial, serum E2 concentrations did not decrease from day 0 to day 1 in the GnRH group, suggesting that possibly large follicles were not fully responsive to GnRH and yet not dependent on LH, possibly due to high serum P4 concentrations. Similarly, it has been reported that administration of GnRH 24 h after sponge withdrawal does not improve the time of ovulation or pregnancy rate in estrus synchronization in ewes (23) and also did not change plasma P4 and E2 concentrations in goats (4). On the other hand, Epplston et al (24) reported that administration of GnRH at the end of progestogen treatment induces preovulatory secretion of LH by the anterior pituitary gland within 1-4 hours which ovulation improve in ewes. In conclusion, the result of this experiment supported that regression rate of large follicles in goats in the GnRH group was probably associated with high serum P4 concentration, but not dependent on GnRH administration. Our observation was similar to data from other researchers (5, 18, 19).

In the current study, administration 150 IU of hCG i.m. at the time of the sponge insertion resulted in regression of large follicles in 59.09% (13/22) goats, with an average reduction of 0.87 mm in large follicle diameters between days 0 and 1 ($P < 0.05$). E2 concentrations were significantly different between both days ($P < 0.05$). hCG, a powerful luteotropic agent is a glycoprotein hormone that has a similar chemical structure to LH, binds to the same receptor, and has a longer half-life and rapid absorption than LH (25). hCG shows its effect on follicular growth by binding directly to LH receptors on granulosa cells in large follicles that emerge in the luteal phase of the estrous cycle (5). As observed in our study, hCG administration in the luteal phase may cause faster atresia or luteinization of the large follicles and so,

decrease estradiol-17 β production. Early findings in goats (5, 16, 18) showed that estradiol-17 β was mostly produced by the largest follicle of the wave and E2 produced by other follicles contributed less to the plasma E2 concentrations. Therefore, the absence of such an increase in E2 concentrations on day 1 in our study may reflect a lower production of E2 in all or some of the large follicles. In contrast, injection of hCG in the presence of preovulatory follicles caused a sharp increase in LH, which resulted in luteinization or ovulation but no effect on the fertility rate in goats (26).

Our results indicated that the insertion of a MAP-loaded intravaginal sponge, regardless of the GnRH and hCG treatment caused the regression of large follicles in 48.21% (27/56) of all the treated goats. The regression of all gonadotropin-dependent follicles was complete in about half of these goats, and as a result, a new follicular wave was initiated in these goats. Regardless of GnRH or hCG administration, our results support previous studies indicating that high serum P4 concentrations affect the development of large follicles and cause faster follicular turnover. Similarly, administration of a single dose of GnRH or hCG at sponge insertion did not affect the regression rate of large follicles and serum P4 and E2 concentrations.

Conclusions

In conclusion, a significant relationship between the administration of hCG or GnRH and the reduction of large follicles diameters or the serum concentrations of P4 and E2 could not be established.

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Ethical approval: All of the methods and management procedures in this study were evaluated and approved by the Animal Experiments Local Ethics Committee of the Uludag University (approval reference number: B.30.2.ULU.08Z.00.00).

Conflict of interest: The authors declare no conflict of interest.

References

1. Fatet A, Pellicer-Rubio MT, Leboeuf B. Reproductive cycle of goats. *Anim Reprod Sci* 2011; 24: 211–9. doi: 10.1016/j.anireprosci.2010.08.029
2. González-Bulnes A, Baird DT, Campbell BK, et al. Multiple factors affecting the efficiency of multiple ovulation and embryo transfer in sheep and goats. *Reprod Fertil Dev* 2004; 16: 421–35. doi: 10.1071/RD04033
3. Menchaca A, dos Santos-Neto PC, Cuadro F, Souza-Neves M, Crispo M. From reproductive technologies to genome editing in small ruminants: an embryo's journey. *Anim Reprod* 2018; 15(suppl.1): 984–95. doi: 10.21451/1984-3143-AR2018-0022
4. Dogan I, Toker MB, Alcay S, Udum Kucuksen D. Effect of GnRH treatment following a short-term estrous induction protocol on estrus and ovulation in Saanen goats, during the transitional period. *J Hellenic Vet Med Soc* 2020; 71: 2569–76. doi: 10.12681/jhvms.25943
5. Driancourt MA. Regulation of ovarian follicular dynamics in farm animals. Implication for manipulation of reproduction. *Theriogenology* 2001; 55: 1211–39. doi: 10.1016/S0093-691X(01)00479-4
6. Rubianes E, Menchaca A. The pattern and manipulation of ovarian follicular growth in goats. *Anim Reprod Sci* 2003; 78: 271–87. doi: 10.1016/S0378-4320(03)00095-2
7. Gonzalez-Bulnes A, Menchaca A, Martin GB, Martinez-Ros P. Seventy years of progestogen treatments for management of the sheep estrous cycle: where we are and where we should go. *Reprod Fertil Dev* 2020; 32: 441–52. doi: 10.1071/RD18477
8. Menchaca A, Rubianes E. New treatments associated with timed artificial insemination in small ruminants. *Reprod Fertil Dev* 2004; 16: 403–13. doi: 10.1071/RD04037
9. Viñoles C, Meikle A, Forsberg M, Rubianes E. The effect of subluteal levels of exogenous progesterone on follicular dynamics and endocrine patterns during the early luteal phase of the ewe. *Theriogenology* 1999; 51: 1351–61. doi: 10.1016/S0093-691X(99)00079-5
10. Viñoles C, Forsberg M, Banchemo G, Rubianes E. Effect of long-term and short-term progestogen treatment on follicular development and pregnancy rate in cyclic ewes. *Theriogenology* 2001; 55: 993–1004. doi: 10.1016/S0093-691X(01)00460-5
11. Menchaca A, Miller V, Salveraglio V, Rubianes E. Endocrine, luteal and follicular responses after the use of the Short-Term Protocol to synchronize ovulation in goats. *Anim Reprod Sci* 2007; 102: 76–87. doi: 10.1016/j.anireprosci.2006.10.001
12. Menchaca A, Rubianes E. Relation between progesterone concentrations during the early luteal phase and follicular dynamics in goats. *Theriogenology* 2002; 57: 1411–9. doi: 10.1016/S0093-691X(02)00638-6
13. Hameed N, Khan MIUR, Ahmad W, et al. Follicular dynamics, estrous response and pregnancy rate following GnRH and progesterone priming with or without eCG during non-breeding season in anestrus Beetal goats. *Small Rumin Res* 2020; 182: 73–7. doi: 10.1016/j.smallrumres.2019.106026
14. Morand-Fehr P, Hervieu J, Santucci P. Notation de l'état corporel: a vos stylos. *La Chèvre* 1989; 175: 39–42.
15. Dogan I, Toker MB, Alcay S, Udum Kucuksen D. Comparison and assessment of ovarian follicular dynamics during the breeding and non-breeding season in Saanen goats. *Wien Tierärztl Monat* 2020; 107: 63–71.
16. Medan MS, Watanabe G, Sasaki K, Sharawy S, Groome NP Taya K. Ovarian dynamics and their associations with peripheral concentrations of gonadotropins, ovarian steroids, and inhibin during the estrous cycle in goats. *Biol Reprod* 2003; 69: 57–63. doi: 10.1095/biolreprod.102.013334
17. Simões J, Almeida JC, Valentim R, et al. Follicular dynamics in Serrana goats. *Anim Reprod Sci* 2006; 95: 16–26. doi: 10.1016/j.anireprosci.2005.09.005

18. de Castro T, Rubianes E, Menchaca A, Rivero A. Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. *Theriogenology* 1999; 52: 399–411. doi: 10.1016/S0093-691X(99)00138-7
19. Ginther OJ, Kot K. Follicular dynamics during the ovulatory season in goats. *Theriogenology* 1994; 42: 987–1001. doi: 10.1016/0093-691X(94)90121-X
20. Año-Perello A, Santoz-Jimenez Z, Encinas T, Martinez-Ros P, Gonzalez-Bulnes A. Use of GnRH for synchronization of the follicular wave in assisted reproductive technologies in sheep: A preliminary study. *Animals* 2020; 10: 1208. doi: 10.3390/ani10071208
21. Hunter MG, Robinson RS, Mann GE, Webb R. Endocrine and paracrine control of follicular development and ovulation rate in farm species. *Anim Reprod Sci* 2004; 82-83: 461–77. doi: 10.1016/j.anireprosci.2004.05.013
22. Greyling JPC, Kotze WF, Taylor GJ, Hagendijk WJ, Coloete F. Synchronization of estrus in sheep: use of different doses of progestagen outside the normal breeding season. *S Afr J Anim Sci* 1994; 24: 33–7.
23. Cavalcanti ADS, Brandão FZ, Nogueira LAG, Da Fonseca JF. Effects of GnRH administration on ovulation and fertility in ewes subjected to estrous synchronization. *R Bras Zootec* 2012; 41: 1412–8. doi: 10.1590/S1516-35982012000600014
24. Eppleston J, Evans G, Roberts EM. Effect of time of PMSG and GnRH on the time of ovulation, LH secretion and reproductive performance after intrauterine insemination with frozen ram semen. *Anim Reprod Sci* 1991; 3-4: 227–37. doi: 10.1016/0378-4320(91)90049-6
25. Saleh M, Shahin M, Wuttke W, Gaulty M, Holtz W. Pharmacokinetics of human chorionic gonadotropin after i.m. administration in goats (*Capra hircus*). *Reproduction* 2012; 144:77–81. doi: 10.1530/REP-12-0093
26. Alvarado-Espino AS, Meza-Herrera CA, Carrillo E, et al. Reproductive outcomes of Alpine goats primed with progesterone and treated with human chorionic gonadotropin during the anestrus-to-estrus transition season. *Anim Reprod Sci* 2016; 167: 133–8. doi:10.1016/j.anireprosci.2016.02.019

Primerjava hCG in GnRH za sinhronizacijo folikularnega vala pri kozah Saanen med sezono parjenja

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Izvleček: Namen te študije je bil primerjati učinke enkratnega odmerka GnRH ali hCG danega na začetku kratkotrajnega protokola za sinhronizacijo estrusa na folikle s premerom ≥ 4 mm pri kozah pasme Saanen med sezono parjenja. Cikle estrusa pri kozah smo pet dni sinhronizirali z intravaginalnimi gobicami z vsebnostjo 60 mg medroksi progesteron acetata. Poleg tega smo jim v času vstavljanja gobice intramuskularno aplicirali 1 ml fiziološke fiziološke raztopine (kontrolna skupina; $n=30$), 0,004 mg buserelin acetata (skupina GnRH; $n=31$) ali 150 IU hCG (hCG-skupina; $n=31$). Neposredno pred in 24 ur po intravaginalni uporabi gobice smo pri vseh kozah opravili transrektalni ultrazvočni pregled in prešteli jajčne folikle s premerom ≥ 4 mm. Ob istih dnevih smo odvzeli tudi vzorce krvi za določitev serumskih koncentracij progesterona (P4) in estradiola (E2). Ob prvem ultrazvočnem pregledu so bili odstotki foliklov s premerom ≥ 4 mm 56,66 % (17/30) v kontrolni, 54,83 % (17/31) v GnRH in 70,96 % (22/31) v hCG skupini. Odstotek koz z zmanjšanim premerom foliklov 24 ur pozneje je bil 29,41 % (5/17) v kontrolni, 52,94 % (9/17) v GnRH in 59,09 % (13/22) v hCG skupini. Povprečna stopnja regresije premerov foliklov med dnem 0 in 1 se je pomembno razlikovala ($P<0,05$) v vseh skupinah. Koncentracija E2 v serumu se je med dnem 0 in 1 pomembno razlikovala ($P<0,05$) v skupini hCG. V serumskih koncentracijah E2 in P4 ter povprečni stopnji regresije v premeru foliklov med dnevi 0 in 1 ni bilo razlik v nobeni skupini. Posledično ni bilo mogoče potrditi povezave med dajanjem hCG ali GnRH ter zmanjšanjem premera velikih foliklov.

Ključne besede: inhronizacija estrusa; folikel; hCG; GnRH; koza

Chambers in Slovenia – so Similar and yet so Different; A Comparison of Structures and Working Methods Using the Example of the Veterinary Chamber and the Detective Chamber in Republic of Slovenia

Key words

chambers;
Veterinary chamber;
Detective chamber

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Abstract: Chambers are associations of legal and natural persons engaged in profitable activity. By definition, they are a community governed by public law that is pursuing a similar activity, founded primarily to protect the interests of its members. In Slovenia, chambers are established to represent persons engaged in various business activities from companies, sole traders, farmers, foresters, craftsmen, private detectives, veterinarians and similar. The chambers differ slightly from one another, and can essentially be divided into three groups, namely chambers of commerce, professional chambers and mixed-type chambers. The tasks of the chambers, their bodies and their duties, the criteria for membership as well as the termination, financing or acquisition of funds for operation and other important information are specified in their by-laws, the respective law or other legal act. In this article the entire system of chambers of the Republic of Slovenia is presented using the example of three randomly selected chambers that differ in terms of their functioning, the exercise of public powers or financing in general, with the Chamber of Veterinarians and the Detective Chamber being explained in more detail. We have selected these two chambers as we wanted to present one natural science and one social science chamber. In the comparison between the detective and veterinary chambers, we can therefore research and analyse many aspects that reflect the diversity and specificity of each professional field. One of the reasons, why we have chosen these two chambers is also the fact, that we as authors are or were active members of our two chosen chambers. The comparison makes it easier to understand how they work, as well as their similarities and differences. We found that not only the chambers we selected, but most chambers operate very similarly, perform similar tasks and have many of the same organs required for their functioning. This is probably due to the fact that all chambers in Slovenia have developed or started to develop according to the model of the first chamber – the Chamber of Commerce and Industry of Slovenia.

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Introduction

Various definitions of the term "chamber" can be found in professional literature. According to Virant (1), chambers are registered entities that unite companies or individuals practicing a particular profession on a non-profit basis. The

Dictionary of the Slovenian Literary Language (2) states that a chamber is a public-law community of institutions, organisations engaged in a similar, related activity, established with the aim of protecting the professional and business

interests of its members, or a public administration community of people of the same profession, established to protect the interests of its members. The Statute of the Chamber of Commerce and Industry of Slovenia defines a chamber as an independent, voluntary, interest-driven, and non-profit association of legal and natural persons engaged in profit-making activities on the market (3).

The chamber system in the Republic of Slovenia is extremely important, especially for the members of chambers who perform certain activities, whether they are companies, sole traders, farmers or foresters, craftsmen, doctors, private detectives, veterinarians, and many others. Chambers are classified as chambers of commerce, professional chambers and mixed-type chambers (1).

All chambers have been established on the basis of certain legal acts or agreements that define the functioning of the chamber, its bodies, the financial resources required for its operation and other information that is important for understanding the establishment of the chamber and its smooth functioning. In addition to the legal acts, chambers prepare annual reports, work programmes, presentations, rules, codes, standards, and others.

The main tasks of the chambers are to achieve the goals and realise the interests of their members. Each chamber also has its own vision, mission and certain fundamental tasks that it pursues. The chambers can also perform certain tasks within the scope of public powers. Public powers are carried out in accordance with the laws governing the chambers' remit, in accordance with the regulations, and on the basis of an appropriate concession or possibly an authorisation granted to the chamber by the respective competent ministry. One task is the issuing and revoking of licences to practise a particular profession, such as veterinarian and private detective.

The article presents the Slovenian chamber system, focusing on the definition of the importance of granting public powers and the role of chambers in Slovenia. It includes a comparison and analysis of three chambers of different types as well as a comparison and detailed presentation of the Veterinary Chamber and the Detective Chamber.

Overview of the system of chambers in the Republic of Slovenia and a comparison of the operation of selected chambers of commerce and professional chambers

The law according to which the entire system of chambers was developed in Slovenia is the Chambers of Commerce and Industry Act (4), which serves as the basis for the establishment of chambers of commerce as well as professional chambers and mixed-type chambers, which operate

according to their own laws. Each chamber a chamber of commerce, professional or mixed-type chamber, has its by-laws that define the general characteristics of the chamber, its goals, values, main tasks and the public powers it exercises, the way it is structured, its functioning bodies and their tasks, the financing of the chamber, relationship to legal entities, the rights and obligations of its members and others.

The first chamber established after the adoption of the Act was the Chamber of Commerce and Industry of Slovenia (Gospodarska zbornica Slovenije – GZS), which is also the largest chamber in the country with the most members. After its foundation, other chambers of commerce were established. Professional chambers and mixed-type chambers were established on the basis of an umbrella law, the by-laws, or the laws under which they operate (1).

Depending on their status, method of establishment, purpose and obligation of membership, chambers are divided into as public-law and private-law chambers. The former are characterised by the fact that, as legal entities under public law, by-law or any other national legal act, or by a decree, and have public powers and can perform tasks in the public interest (1). Within the scope of their powers, they can decide on the rights and obligations of their members. Article 121 of the Constitution of the Republic of Slovenia stipulates that by or on the basis of a law, self-governing associations, companies and other organisations as well as natural persons, may be granted with public authority to perform certain tasks of state administration (5).

Within the scope of their public powers, the chambers can keep records, issue certificates, conduct administrative proceedings and issue decisions and regulations. Public powers are awarded by law. One principle that is very important in the exercise of public powers is the principle of legality. This means that all decisions made by a chamber must be legally justified and comply with the content of the legislation. It must also be understood that the chambers do not set their own conditions for granting permits, certificates or licences, but carry out a specific activity prescribed by law and thus only implement the law (1).

Private-law chambers, on the other hand, operate according to the Anglo-American principle, which means that the membership fees are not compulsory and they are predominantly market oriented. They are founded by natural and legal persons on the basis of a private-law act, i.e., a contract, by-laws or some other founding instrument (1). They generally promote the interests of their members in relation to their activities (6).

Comparing public-law and private-law chambers, it can be summarised that the former are established by the state through a legal act, while the latter are established by natural and legal persons through a contract or other founding instrument. It is also worth mentioning the implementation

of tasks: the public-law chambers perform tasks for the entire country, whereas private-law chambers focus more on their members and their interests. There are notable differences in terms of membership – it is generally compulsory for public-law and voluntary in private-law chambers (7). In Slovenia we just have public-law chambers, but in some other countries, such as Belgium, Great Britain, Denmark, Ireland, Finland, Norway, Portugal, Sweden and Switzerland the chambers of commerce are a part of private-law chambers (5).

According to the type of entity, chambers are categorised as chambers of commerce, professional chambers and a special type of chambers, i.e., so-called mixed-type chambers. Chambers of commerce are associations of economic entities, mostly companies, professional chambers are associations of individuals engaged in a professional activity as an independent profession, while mixed-type chambers combine economic entities and natural persons who perform a professional activity (1).

In Slovenia, chambers of commerce include the Chamber of Commerce and Industry of Slovenia (GZS), under whose auspices there are numerous other chambers and associations as well as the Chamber of Craft and Small Business of Slovenia (OZS), a mixed-type chamber is the Chamber of Agriculture and Forestry of Slovenia (KGZS), whereas all the other chambers are professional chambers, such as the Chamber of Engineers, the Chamber of Pharmacy, the Occupational Therapists Chamber, the Chamber of Architecture and Spatial Planning of Slovenia, the Detective Chamber, the Bar Association, the Slovenian Advertising Chamber, the Social Chamber, the Chamber of Notaries, the Veterinary Chamber, the Medical Chamber and others.

Public power is an authorisation to perform certain tasks of the state or municipal administration (8), where the entity exercising public powers is determined by law (9). In the case of public powers held by the chambers, individual laws and by-laws of the chambers define and specify the tasks to be performed on the basis of the public powers. These tasks relate in particular to the granting and revoking of licences, keeping records and registers, the performance of professional supervision, the determination of the training programme, the conduct of professional examinations and similar (10).

A public power is non-transferable, i.e. the holder cannot transfer it to another person, nor can it be relinquished, as public powers are considered to be rights and duties involved in the performance of administrative tasks. The state may revoke the power if conditions are not fulfilled or violations occur in the course of exercising the power, whereby the conditions for revocation must also be specified in the law (11).

Article 121 of the Constitution of the Republic of Slovenia (5) stipulates that public authority to perform certain tasks

of the state administration may be vested by law or on the basis thereof. If public power is granted by law, that law must determine the holder and their tasks. There is usually a single holder of a public power and the public authority is vested for a longer term. If public power is granted pursuant to a law, there can be several holders and it is important that the law specify tasks as well as the granting authority (8). The administrative tasks of the state as defined in the State Administration Act comprise participation in policy-making, executive tasks, inspection, monitoring, developmental tasks and the provision of public services. These tasks are primarily performed by state administration bodies and by legal and natural persons under public authority (10).

Public powers are exercised in three ways, i.e., by issuing general acts, by issuing individual acts and by performing material actions. The mere exercise of a public power by issuing general acts constitutes the right of legal or natural persons to regulate issues or relationships with their general act in a compulsory manner. The exercise of a public power by issuing an individual legal act is the most common form of exercising it. The law stipulates that the holder decides on the rights, obligations and legal benefits of legal or natural persons with individual acts. Exercising public powers by performing material actions means an entitlement to perform authoritative material actions. Implementation of these actions requires the use public authority, at least indirectly, such as for instance means or powers of restraint, keeping records and others (12).

In order to better understand the functioning and inter-connectedness of the chambers, we randomly selected three different chambers, described the main characteristics of their operations, the public powers they exercise and the sources of funding, and compared them with each other. The Veterinary Chamber and the Detective Chamber were chosen for comparison, while the other randomly selected chambers are the Chamber of Commerce and Industry of Slovenia and the Chamber of Craft and Small Business of Slovenia, which are chambers of commerce, and the Medical Chamber of Slovenia, which is a professional chamber.

Furthermore, in the paper we compare the Veterinary Chamber of the Republic of Slovenia and the Detective Chamber of the Republic of Slovenia. The comparison can be very interesting if one wants to explore the similarities and differences between professional associations in various industries especially in terms of regulation and control, education and training, legislation, ethical issues and public interest.

Table 1: The operation, public powers and financing of the selected three Chambers in Slovenia

Name of the chamber	Operation	Public powers	Financing
Chamber of Commerce and Industry of Slovenia	<ul style="list-style-type: none"> - Represents the common and general interests of its members before government authorities, bodies of the European Community, the Economic and Social Council and in other forms of economic and social dialogue as well as in the conclusion of cross-industry collective agreements for the corporate sector; - participates in designing the economic system and preparing economic policies; - plans vocational education and training, participates in practical training and the conducting of exams in line with the law; - implements tasks involving the exercise of public powers vested in the Chamber of Commerce and Industry of Slovenia pursuant to the law; - fulfils the obligations arising from membership in Eurochambres, the International Chamber of Commerce and other forms of multilateral and bilateral association between chambers and cooperates with other domestic and foreign institutions; and - implements common tasks within the scope specified in the annual programme of the Chamber of Commerce and Industry of Slovenia (3). 	<ul style="list-style-type: none"> - Competent ministries supervise the exercise of public powers on the basis of annual reports. - In the field of transport, it holds the same powers as the Chamber of Craft and Small Business. 	<ul style="list-style-type: none"> - Membership fee; - special appropriated funds provided by members on an interest basis; - payments for services provided; - funds from the national budget for the exercise of delegated public powers; - donations and gifts; - other sources (3).
Chamber of Craft and Small Business of Slovenia	<ul style="list-style-type: none"> - Represents the interests of its members before state bodies, in shaping the economic system and economic policy by participating in the procedures for amending them and forming economic opinions, positions and proposals for changes to the economic system and economic policy; - acts as a partner in the conclusion of a social agreement between the social partners; - acts as a partner in the concluding of collective agreements; - provides professional assistance to members (economic, legal, tax, business consulting); - informs the members; - cooperates with the National Assembly, the National Council, the Government and individual ministries on all matters relevant to its membership; - analyses legal regulations and proposes new ones, as well as amendments and supplements thereto in order to create conditions that provide for the protection and development of the craft sector of the economy; - participates in coordinating the common and individual interests of the members in the framework of formulating the development policy of the Republic of Slovenia; - coordinates and represents the interests of its members in standardisation, metrology, and the adoption of technical regulations as well as in the field of regular specialist education in line with the requirements of a modern, market-oriented economy; - performs tasks with the aim of connecting the corporate sector and the academic-scientific community and transferring knowledge from the academic-scientific sphere to small and micro enterprises as well as promotes innovation and the development of small and micro enterprises; - cooperates with other chambers in the country on all matters relevant to its membership; - is involved in the educational process of short vocational education, vocational secondary education, upper secondary technical education, vocational-technical and short-cycle higher vocational education and professional higher education; - arranges and conducts various forms of education and training; - organises activities related to economic relations with foreign countries involving the promotion of goods and services as well as economic propaganda, organises trade shows, takes part in the development of the foreign trade network, facilitates foreign trade deals and fosters economic cooperation with the chambers of crafts and small business and relevant institutions in other countries; 	<ul style="list-style-type: none"> - Issues and revokes craft licences; - performs tasks in the field of vocational education; - controls the professional qualification of craft operators, the persons providing vocational education and the suitability of the facilities at which students undergo vocational training; - manages the register of trade licences, the register of facilities, the register of persons authorised to educate students, the register of facilities where vocational training is carried out, and the register of master craftsman certificates; - issues certificates to natural persons or legal persons as proof of legal establishment in the Republic of Slovenia for the purpose of performing craft activity and proving that they are not temporarily or permanently prohibited from performing such activity at the time of issuing the certificate; - carries out the procedure of recognising professional qualifications for citizens and member states of the European Union, the European Economic Area, the Swiss Confederation and the country with which a relevant international agreement has been concluded, third-country citizens who have obtained professional qualifications in a member state of the European Union, the European Economic Area or in the Swiss Confederation and third-country citizens, in accordance with the second paragraph of Article 2 of the Act Regulating the Procedure for Recognition of Professional Qualifications for Practising Regulated Professions or Professional Activities in the Republic of Slovenia for Nationals of the EU Member States, European Economic Area and Swiss Confederation, Official Gazette of the RS, nos. 21/02, 92/07, 85/09 and 55/12); - notifies the temporary cross-border performance of craft activities by natural and legal persons from a member state of the European Union, the European Economic Area and the Swiss Confederation; - issues Community Licences and copies thereof for international cargo and passenger transport services; - issues certificates to drivers who are not citizens of Community member states; - withdraws Community licences; - issues, refuses the issue and withdraws licences and copies of licences for the provision of transport; - distributes permits for international transport of goods to domestic carriers, including CEMT permits; - issues certificates for international passenger transport operations for own needs; and 	<ul style="list-style-type: none"> - Membership fee; - special appropriated funds allocated by members on an interest basis; - payments for services provided; - local government budget; and - other sources (13).

- develops an information system and disseminates up-to-date marketing and technical-technological information in Slovenia and abroad, integrating with other information systems for this purpose;
- is involved in the promotion of business relations in domestic and foreign markets;
- is a member of and joins international associations of craft and of SMEs;
- promotes the introduction of modern technologies and quality standards, modern methods of managing and organising companies and craft cooperatives;
- provides for the preservation and development of traditional and artistic crafts;
- performs other tasks in the interest of its members, as defined in its by-laws and the annual action programme (13).
- issues signs for taxi transport services indicating data concerning the transport operator and licence number (13).

Medical Chamber of Slovenia	<ul style="list-style-type: none"> - Adopts the Code of Medical Deontology, specifies actions that constitute a violation of it; - monitors compliance with the Code, verifies the conduct of doctors and takes action in the case of breaches of the Code; - plans, monitors and supervises specialisations and other forms of postgraduate professional training of its members through qualification testing, determining the conditions for the nomination of mentors and appointing them; - conducts expert supervision with counselling; - concludes agreements in its own name and on its own behalf and in its own name and on behalf of doctors regarding matters specified by the by-laws, other general acts of the Chamber or a special doctor's authorisation; - concludes agreements in the name and on behalf of doctors or based on individual or collective doctors' authorisation; - enters into collective agreements on behalf of private doctors in their capacity as employers; - gives opinions and consents in procedures, participates in procedures and issues general and individual acts in accordance with the law as well as the by-laws and internal acts of the Chamber; - participates in procedures prescribed by the law and the by-laws; - keeps records of companies and other legal entities under private law that perform medical services; - participates in the design of the undergraduate training programme for doctors; - organises and conducts professional seminars, professional meetings and other forms of professional training; - carries out scientific and research activities related to its tasks; - represents the professional and social interests of doctors; - provides advice and assistance and furthers the interests of its members in legal, economic, analytical, financial and other areas related to the performance of a medical service; - co-determines the starting points for concluding contracts with the Health Insurance Institute of Slovenia and other insurance companies and represents the interests of private doctors in the conclusion of contracts between them and other legal entities; - creates and manages the trust fund of its members and provides assistance to its members and their families; - prevents a medical service from being performed in an unauthorised manner; - monitors the need for doctors, offers help to unemployed doctors in search of employment, and assistance in providing replacements for periods of absence; - performs publishing and issuing activities: issuing and supplying forms and documents, - publishing and supplying its newsletters, books and other publications; - promotes cooperation between members, addresses and settles mutual disputes; 	<ul style="list-style-type: none"> - Keeps a register of its members and issues membership cards; - grants, extends and revokes independent practice permits of doctors; - plans, monitors and supervises the secondment, specialisations and other forms of postgraduate professional training of its members through qualification testing; and - conducts expert supervision with counselling (14). 	<ul style="list-style-type: none"> - Membership fee and other contributions by members; - the organisation and implementation of activities in accordance with the by-laws; - investing the Chamber's funds with the goal of preserving and increasing the value of the assets and property of the Chamber; - payments for services that are not financed from membership fees and funds for exercising public powers; and - donations and other sources (14).
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- fosters the cultural and social activities of its members, organises and conducts cultural, sports and other social events and activities;
- performs all other activities that support the performance of the core activities;
- designates the representatives of the Health Insurance Institute for negotiations and other agreements with healthcare institutions;
- carries out other tasks in line with legal regulations, the by-laws and its other general acts (14).

Veterinary Chamber of Slovenia

- Issues and revokes veterinary licences of members who have passed the state examination;
- keeps a register of issued and revoked licences of its members;
- plans, monitors and organises the professional training of doctors of veterinary medicine and the professional competency examination of members in veterinary organisations;
- conducts expert supervision in veterinary organizations;
- verifies veterinary organisations and keeps a register of them;
- lays down the basis and criteria for the pricing of the public veterinary service in cooperation with the council of users;
- keeps a register of members of the Chamber;
- formulates the standard operating procedures for veterinary services, evaluates them and sets veterinary tariffs;
- participates in the drafting of regulations in the field of veterinary medicine and marketing of medicinal products and their use in veterinary medicine;
- is involved in the planning and implementation of professional training;
- takes part in conducting veterinary specialisation, supervising it and awarding the title of veterinary specialist;
- provides legal, business and professional assistance to its members;
- seeks to ensure the employment of its members;
- promotes cooperation among members, addresses and settles mutual disputes and conflicts;
- publishes and issues professional and other publications and documents;
- selects candidates to the electoral body that elects the representative of professional occupations in the National Council;
- adopts and amends the Veterinary Code of Conduct;
- prevents and sanctions irregularities in the performance of veterinary activities;
- organises and performs other activities for the needs of its members; and
- carries out other tasks in accordance with the law and the by-laws (15).

- Issues and revokes veterinary licences of members who have passed the state examination;
- keeps a register of issued and revoked licences of its members;
- plans, monitors and organises the professional training of doctors of veterinary medicine and the professional competency examination of members in veterinary organisations;
- conducts expert supervision in veterinary organizations;
- verifies veterinary organisations and keeps a register of them;
- lays down the basis and criteria for the pricing of public veterinary services in cooperation with the council of users (15).

- The joining fee, membership fee and other contributions of its members;
- organising and performing professional tasks;
- preparing and conducting mandatory professional training for its members;
- specialisation fees;
- investing its funds in commercial transactions;
- publishing and issuing activities;
- budget funds for the exercise of public powers;
- donations; and
- in other permissible ways or from other sources (15).
- The financing of the tasks performed by the Chamber based on public powers and funded from the budget of the Republic of Slovenia, as well as the amount of budget funds allocated for this purpose, are regularly agreed between the Ministry in charge of veterinary medicine and the Chamber in accordance with the law governing veterinary medicine (15).

Detective Chamber of the Republic of Slovenia

- Performs the tasks defined by the law and the regulations issued on its basis as well as the tasks stipulated in the by-laws or defined in the Chamber's programme, development acts or other general acts;
- monitors and addresses private detective work and related issues, represents the professional and social interests of its members, defends the honour and good name of the private detective profession and the Chamber's members against unjustified and unfounded accusations, reprimands and interventions by state authorities, providing them with legal, material and moral assistance and, if necessary, professional and legal aid in proceedings before the state and other authorities;

- Issues and revokes private detectives' licences;
- in agreement with the ministry in charge of internal affairs, determines the private detective exam programme and the method of taking the private detective exam referred to in Article 8 of the Act (16).
- keeps the records prescribed by the Act; and
- conducts professional supervision of the work of private detectives and private investigation companies (17).

- Membership fee and other contributions by private detectives;
- registration fees to be entered on the register of private detectives;
- registration fees to be entered on the candidate register for taking the private detective exam;
- fees paid by private detective applicants for taking the private detective exam;
- funds from the sale of services covered by the Chamber's business activity;
- fines imposed on private detectives in disciplinary proceedings;
- gifts and legacies;
- interest and other income from the performance of the Chamber's tasks (16).

- keeps and manages records of private detectives who are citizens of the Republic of Slovenia; foreign private detectives entitled to perform private detective activity under the title of "private detective" in the Republic of Slovenia; foreign private detectives entitled to perform private detective activity in the Republic of Slovenia under the professional title bestowed by their home country; records of issued and revoked private detective licences; private detective companies that perform private detective services in Slovenia; imposed disciplinary measures; consents issued for the registration of a private investigation company in the court register; other records;
- regulates the disciplinary liability of private detectives, constituting a breach of professional duty as well as actions which amount to a breach of a private detective's due care and diligence, and regulates the procedure before the Tribunal of Honour (16);
- adopts the code of professional ethics;
- proposes to the ministry in charge of internal affairs (hereinafter: the Ministry) a professional training programme, professional standards and catalogues of professional knowledge and skills, and the method of taking the private detective exam;
- proposes to the Ministry a private detective identification card; and
- organises and conducts periodic trainings for private detectives (17).

The Veterinary Chamber of the Republic of Slovenia and its operations

The purpose of veterinary medicine in general is to maintain and improve animal health, protect animals from infectious and other diseases, diagnose diseases and treat sick animals, ensure animal welfare, provide measures to protect the public from various diseases and infections, and pursue other objectives for the benefit of animals (18).

The Veterinary Chamber is an autonomous and independent professional organisation. Its members are doctors of veterinary medicine from the entire territory of the Republic of Slovenia. It represents and promotes their professional, economic and social interests. Another purpose of the Chamber is to ensure the quality of its members' work and uphold its reputation (19). It supports all its members in their work, encouraging cooperation between them and with the Chamber. It is also committed to preventing and resolving any disagreements and disputes amicably and in a dignified manner (20).

The law that contains several articles that regulate the Veterinary Chamber is the Veterinary Practice Act (18). Based on Articles 58 and 60 of the Veterinary Practice Act, the Assembly of the Veterinary Chamber of Slovenia adopted the by-laws of the Veterinary Chamber of Slovenia in 2014. These by-laws regulate the status, organisation, powers and operation of the Chamber (15).

As defined, Chamber membership is on an individual basis and represents a professional honour common to all doctors of veterinary medicine. It can be mandatory, voluntary or honorary (15). Membership is mandatory for every doctor of veterinary medicine practising veterinary activity in

Slovenia as referred to in Article 51 of the Veterinary Practice Act (18), who must hold a veterinary licence. Voluntary members are doctors of veterinary medicine with permanent residence in Slovenia who are not in practise. Honorary membership is awarded by the Assembly of the Chamber on the proposal of the Board of Directors, which must be in accordance with the Rules (21). All rights, duties, and other information on membership are specified in the by-laws of the Veterinary Chamber. The membership fee is payable quarterly and is determined by the Annual General Assembly based on the financial statement for the previous year and other factors. An individual can become a member of the Chamber after submitting a complete written application showing that he or she meets all legal membership criteria. If a doctor of veterinary medicine has been subject to a precautionary measure prohibiting them from practising their profession as part of criminal proceedings, the application will be rejected or the member will be removed from the membership list if they are an existing member. After the measure has ended, they may reapply for membership in the Chamber. Membership is also terminated if a member resigns from the Chamber in accordance with the by-laws, if certain membership criteria are not met, and in cases referred to in Articles 13, 17 and 54 of the by-laws (15).

According to the Act (18), the licence, which is the main document for the practise of veterinary medicine (activity), is granted to a doctor of veterinary medicine who has completed a university degree in veterinary medicine in the Republic of Slovenia or another EU member state or a recognised diploma who has passed the state exam or a recognised professional examination and is a member of the Veterinary Chamber of Slovenia. The licence is valid for the period in which the prescribed conditions are met. The

decision on the licence is issued to the doctor of veterinary medicine based on a written application upon the meeting of the prescribed conditions. The licence is renewed every 10 years. If during a period of 10 consecutive years, the veterinarian does not meet the conditions regarding professional training or fails the professional competency examination, the Veterinary Chamber sends them a written notification warning them in advance of licence suspension and setting a 1-year period to fulfil the conditions. A veterinarian can also fulfil the professional training requirement by passing the prescribed knowledge examination in accordance with the law governing veterinary medicine and the Rules on further expert training and proficiency tests in private veterinary practice. If the veterinarian does not complete the prescribed training within the additional period referred to in the preceding paragraph, the Director of the Veterinary Chamber will issue a decision revoking the licence until the prescribed conditions are fulfilled (22).

At the request of the Veterinary Chamber's tribunal, the licence can also be revoked for a period of three months to three years on the basis of a decision by the Director if it is proven that the veterinarian has committed serious violations or has not completed his professional training. At the end of this period, the veterinarian can reapply for the licence provided that he has completed his training. If a veterinarian is banned from practicing their profession as part of criminal proceedings, their licence will also be revoked for the duration of the measure imposed (18).

The Chamber performs numerous tasks that are also specified in the by-laws. The most important task of the Chamber is the granting and revoking of veterinary licences. In addition, the Chamber keeps a register of issued and revoked licences, organises the professional training of veterinary doctors and conducts the professional qualification examination of its members. It carries out regular and extraordinary inspections of veterinary organisations, verifies them and keeps a register of them. Moreover, it establishes standard operating procedures for services. One of its tasks is to participate in the preparation of regulations in the field of veterinary medicine and the marketing of medicinal products and their daily use. The Chamber is an essential element in the planning and implementation of professional training, specialisation, and employment of its members, to whom it provides legal, business and professional support. It promotes and strengthens cooperation and settles disputes and disagreements between the members. The Chamber adopts and possibly amends the Veterinary Code of Conduct, prevents irregularities and sanctions them, and performs other tasks in the field of veterinary medicine in line with the laws and the by-laws (15).

Some of the mentioned tasks are performed by the Chamber on the basis of public powers according to the law governing veterinary medicine, namely issuing and revoking veterinary licences, keeping a register of licences, planning, monitoring and organising professional training

and professional competency examinations, carrying out professional supervision in organisations, verifying organisations and keeping a corresponding register and setting prices for veterinary services. The Ministry responsible for veterinary medicine regularly agrees with the Chamber on the financing of these tasks in accordance with the law (15). In order to carry out these tasks, the Chamber must fulfil certain personnel, spatial and other requirements (18). The exercising of public powers requires the Assembly of the Veterinary Chamber of Slovenia to adopt regulations governing the issuance, renewal and revocation of licences and the keeping of the register of issued licences (22), the verification and control of the conditions prescribed for veterinary activity and the keeping of the register of veterinary organisations (23); expert supervision with consultation (24), provision of professional training and professional competency examination.

The Chamber is headed by the Director who manages the Chamber's Board of Directors and performs operational and executive tasks on its behalf. The Director is appointed on the basis of a public call for applications. It should be noted that the Director must be a veterinarian (18). The bodies of the Chamber include the Assembly, the President, the Vice President, the Board of Directors, the sections, the Prosecutor, the Deputy Prosecutor and the tribunal, as well as the Expert Committee, the Supervisory Board and the Election Committee. The Chamber Assembly is the highest body of the Chamber and comprises all members of the Chamber. It has certain powers, while its organisation and working methods are defined and regulated by the Rules of Procedure of the Chamber's Assembly. A regular meeting is convened at least once a year at the request of the President of the Chamber, whereas an extraordinary meeting can also be held in certain cases to address various matters. The Chamber is represented by its President, who also directs the work and chairs the Board of Directors. The term of office of the President is four years, with the possibility of re-election. Any member of the Chamber can run for President, provided such member has collected 50 handwritten signatures of the other members in support of his candidacy. The President performs certain tasks specified in the by-laws and other acts. The President is assisted by the Vice President, who contributes to their work. The Vice President is also responsible for the Chamber's international operations and represents the President in his absence. The conditions for the post of Vice President are the same as for nominating and electing the President i.e. any member of the Chamber who has collected at least 50 handwritten signatures of the other members can apply for the position. A candidate may apply for the position of President and Vice President at the same time (15).

Another important body of the Chamber is the Board of Directors, which is a decision-making and consultation body. It consists of the President, the Vice President, the Chair of the Expert Committee, the presidents of sections, the Director and one representative for every 150 members.

The Board of Directors has certain powers and tasks as specified in Article 39 of the by-laws and is accountable for its decisions to the Assembly of the Chamber. The members of the Chamber form the sections for the purpose of ensuring specialised operations of the Chamber and its members. The Chamber has two permanent sections, both of which have their own bodies and perform specific tasks. Another body of the Chamber is the Prosecutor, who is an autonomous and independent body of the Chamber. The candidate who receives the most votes is elected as the Prosecutor. Each Prosecutor has a deputy. The tribunal is also an autonomous and independent body of the Chamber, consisting of first- and second-instance tribunals. It is tasked with initiating proceedings against an individual on the proposal of the Prosecutor. Other bodies include the Expert Commission, which consists of seven members and carries out specific tasks, the Supervisory Board, a three-member autonomous and independent body, whose task is to supervise the material and financial operations of the Chamber, and the Election Committee, which also consists of three members and certain powers and duties in accordance with the by-laws (15).

The Assembly of the Chamber adopts the annual work programme and the annual financial plan, based on which its operations are financed. Two types of financing are defined, namely regular financing for the period between two consecutive Annual General Assembly meetings, and temporary financing, which is initiated if the proposed financing plan for the following year is not adopted at the Annual General Assembly. However, temporary funding can only be provided for a maximum of 3 months (15).

The funds for the operation of the Veterinary Chamber are acquired through joining fees, membership fees and other contributions from members, by organising and performing various professional tasks, by preparing and implementing mandatory professional training, from specialisation fees, by investing funds in commercial transactions, publishing and issuing activities, budget funds for the exercise of public powers, through donations and in other ways or from other sources (15).

The Veterinary Chamber also collaborates with authorities on the international level and is thus a member of the Federation of Veterinarians of Europe, in which three sections are represented: the Practising Veterinarians, the Veterinarians working in Education, Veterinary Research and Industry, and the Hygienists and Public Health Veterinarians. In addition, the Veterinary Chamber of Slovenia is a member of the Višegrad Vet Plus Group, which includes the chambers and associations of Central and Eastern Europe. It comprises 13 members, who meet twice a year. The Chamber has signed a protocol on cooperation, and the exchange of practices and experiences with other chambers in both Europe and around the world (25).

The Detective Chamber of the Republic of Slovenia and its operations

The Detective Chamber of the Republic of Slovenia (DeZRS) is an organisation that protects the reputation, credibility and development of the activity of private detectives. In addition, it represents the interests of private detectives and performs other tasks stipulated by the law, the by-laws and other acts (16). It was founded in 1994 at the first Founding General Assembly of the Detective Chamber of the Republic of Slovenia, at which the first by-laws and the symbol of the Chamber were adopted, and its initial management team (26) was elected.

In the course of their work, private detectives are bound by the Code of Professional Ethics, whereby they undertake to carry out all private detective duties in accordance with the highest moral and ethical principles. "I recognise the badge of my profession as a symbol of social trust awarded time to guard by the Detective Chamber of the Republic of Slovenia. I shall protect it throughout, with loyalty to the ethics of private detective profession. I shall always strive to achieve this goal and the ideals of my chosen profession and be an honourable private detective" (27).

On the basis of the Private Detective Services Act (17), the Assembly of the Detective Chamber of the Republic of Slovenia adopted its by-laws at an Extraordinary General Assembly on December 23rd, 2011 and at the Annual General Assembly on April 18th, 2012 (16).

Membership in the chamber is mandatory for all domestic and foreign private detectives who perform private detective work as a freelance profession in the Republic of Slovenia or are employed by a legal or natural person registered as a business entity performing private detective activity in the Slovenian Business Register (16).

The tasks of the Chamber include monitoring and addressing private detective work and issues, representing the professional and social interests of its members, defending the honour and good reputation of the private detective profession and its members against unjustified and unfounded accusations, reprimands and interventions by state authorities, providing them with legal, material and moral assistance and, if necessary, providing professional and legal assistance in proceedings before the state and other authorities. In addition, the Chamber keeps and manages records of private detectives who are citizens of the Republic of Slovenia; foreign private detectives who are authorised to work in the Republic of Slovenia under the professional title "private detective"; foreign private detectives entitled to perform private detective activity in the Republic of Slovenia under the professional title granted by their home country; records of issued and revoked private detective licences; private detective companies providing private detective services in Slovenia; disciplinary measures imposed; permits issued for the registration of a private detective company

in the court register and other records. In addition,, the Chamber regulates the disciplinary liability of private detectives, constituting a breach of professional duties, as well as actions which amount to a breach of a private detective's duty of care, and regulates the procedure before the Tribunal of Honour (16).

In addition to the tasks described above, the Chamber performs tasks such as adopting the Code of Professional Ethics; proposing to the Ministry responsible for internal affairs a programme for professional training, professional standards and catalogues of professional knowledge and skills, and the method of taking the private detective exam; proposing to the Ministry a private detective identification card, and organising and conducting regular training for private detectives. The work and operation of the Detective Chamber are supervised by the Ministry of the Interior, which is primarily responsible for monitoring the performance of the Chamber's tasks and its exercise of public powers (28).

Within the scope of its public powers, the Chamber performs tasks such as granting and revoking private detectives' licences and, in agreement with the Ministry of internal affairs, determines the examination programme for private detectives and the manner in which the private detective examination is taken. It also conducts professional supervision over the work of private detectives and private investigation companies. The Board of the Chamber decides on the granting or revocation of a licence to perform private detective work as well as on the issuance of consent for the registration of foreigners or a foreign private investigation company on the register of private detectives operating in the territory of the Republic of Slovenia, and on other matters related to the performance of tasks within the scope of public authority, in accordance with the provisions of the Private Detective Services Act (17). It is also important to point out that in certain cases the Ministry can withdraw the public powers of the competent body. This is the case when the body, in this case the Chamber, grants or revokes a licence illegally, without grounds or in violation of the law; when records are not kept in accordance with the law; if the Chamber does not comply with the deadlines and procedures for the recognition of professional qualifications of foreign private detectives and if when does not have a qualified person to manage the administrative procedures and regarding the recognition of professional qualifications and to make decisions. If the body fails to eliminate the irregularities within a certain period, the Ministry will withdraw its public powers until the irregularities are eliminated (17).

The bodies of the Chamber include the Assembly, the Board of Directors, the President, the Supervisory Board and the Tribunal of Honour. The Assembly is the highest body of the Chamber and consists of all private detectives who are entered in the register of private detectives kept by the Chamber. Its powers comprise the adoption of the Chamber's by-laws and other general legal acts;

the adoption of the Code of Professional Ethics of Private Detectives of the Republic of Slovenia; the adoption of the Chamber's Rules of Procedure, the election and discharge of the President and Vice-President, the members of the Board of Directors, the Supervisory Board, the Chair and members of the Tribunal of Honour and others. The Assembly is convened as required, and at least once a year on the initiative of the Board of Directors or at the request of at least 30 private detectives. Decisions on amendments and supplements to the founding charter, adoption of and amendments and supplements to the by-laws, status changes of the Chamber, the cessation of operations of the Chamber and other matters are adopted with a majority of the Chamber's members present. Another of the Chamber's bodies is the Board of Directors, which manages the work and operations of the Chamber. It consists of nine members elected by the Assembly for a four year term of office. The work of the Board of Directors is managed by the President of the Chamber or, in their absence, by the Vice-President. The Board of Directors has certain powers, such as convening the Assembly of the Chamber and preparing the relevant materials, implementing resolutions adopted by the Assembly; formulating draft by-laws, the Code of Professional Ethics of Private Detectives and other general acts, organising and conducting regular professional training, appointing and dismissing the Chamber's Secretary, adopting general legal acts of the Chamber that have not been adopted by the Assembly, and more. Meetings are convened and chaired by the President of the Chamber, who also represents the Chamber and acts on its behalf in Slovenia and abroad, implements the resolutions of the Assembly and the Board of Directors, grants detectives' licences and cards, awards Chamber awards, issues resolutions and decisions, approves payments and performs other tasks. In addition to the above-mentioned bodies, the Chamber has a Supervisory Board, consisting of three members, one of whom is the Chair. The main task of the Supervisory Board is to monitor the Chamber's financial transactions and, in particular, to review the financial transactions and financial situation of the Chamber at least twice a year and report thereon to the Board of Directors and the Assembly. After reviewing the year-end accounts of the Chamber, the Supervisory Board draws up a report thereon. The final body of the Chamber is the Tribunal of Honour, which consists of seven members, who are elected by the Assembly for a term of four years. It decides in disciplinary proceedings against detectives due to a breach of professional duty or a breach of a private detective's due care and diligence, and also in the case of the violation of the Code of Professional Ethics of Private Detectives, the Chamber's by-laws and other acts. Moreover, it decides in case of disputes between members of the Chamber or between the members and third parties. The proceedings against a detective due to a violation of Articles 38 and 52 of the by-laws may be instigated at the proposal of the Supervisory Board, the national supervisory authority, a member of the Chamber or the party concerned (16).

In addition to the above-mentioned bodies, the Professional Department of the Chamber and the Secretary managing it play an important role. The Secretary is also responsible for preparing materials required for the work of the Board of Directors and the Assembly as well as other bodies of the Chamber, the working bodies of the Assembly and the Board of Directors, and coordinates the work of the Chamber's bodies. The Secretary is appointed, for a fixed or indefinite period, and is dismissed by the Board of Directors of the Chamber. This position, for which an employment contract or a work contract is concluded, requires at least higher education in the relevant field and five years of professional experience (16).

The work and financial operations of the Chamber are financed by membership fees and other contributions from detectives; the Chamber's income from the performance of public powers and other services, the sale of services within the scope of the Chamber's business activity, fines imposed against detectives in disciplinary proceedings, gifts and legacies as well as interest and other income from the performance of tasks (16).

Membership is terminated when the detective's licence expires, if one of the conditions laid down in Article 15 of the Private Detective Services Act (ZDD-1) is met or if the licence is definitively revoked in accordance with Article 9 of the by-laws, or if the Chamber ceases to operate (16).

In the event of a disciplinary breach by a detective, disciplinary measures may be imposed, such as a warning, a fine or a proposal to the Board of Directors to revoke the licence or prohibit the provision of cross-border services. The measure is imposed in the case of a minor breach that is particularly insignificant in view of the circumstances in which it was committed and does not have serious consequences. A fine up to EUR 1,000 is imposed if the detective has obtained an unjustified financial advantage in the performance of a service, with a payment period of no less than 30 days and no more than 6 months. The proposal to revoke a licence refers either to a permanent revocation, a temporary suspension or a conditional revocation or a ban on the provision of cross-border services (16).

Comparison of the operation of chambers in Slovenia

As can be seen from Table 1, it is not surprising that there are no specific differences between the chambers, given that all chambers are based on the same basic law. The main or basic task of all chambers is to represent the interests of their members and to assist them in their business. Each of the chambers focuses on its own field of activity, e.g. the Chamber of Commerce and Industry on the corporate sector and the economy, the Chamber of Craft and Small Business on crafts and companies, and the Medical Chamber on healthcare, medicine, and doctors.

Likewise, practically all chambers perform certain tasks within the scope of public authority. The performance of public tasks is carried out in accordance with the laws that regulate the scope of the chambers' tasks, in accordance with the regulations, on the basis of a corresponding concession or, if applicable, an authorization granted to the chamber by the relevant ministry. The chambers differ in terms of whether membership is mandatory or voluntary. In most professional chambers, membership is mandatory, in the vast majority of chambers of commerce membership is voluntary, and in mixed-type chambers membership is either mandatory or voluntary. Chambers' by-laws specify the conditions in which a person can become a member. As already mentioned, each chamber represents the interests of its members in the respective professional field for which it was founded. For example natural and legal persons who perform a craft or handicraft activity or any other for-profit or non-profit activity may join the Chamber of Craft and Small Business (13), companies and sole traders can join the Chamber of Commerce and Industry on a voluntary basis (3), everyone practising medicine in the capacity of physician in the territory of the Republic of Slovenia must join the Medical Chamber (14), doctors of veterinary medicine can join the Veterinary Chamber on mandatory or voluntary basis or as honorary members (15), and domestic and foreign private detectives who perform detective services in the Republic of Slovenia as a freelance profession or in a private investigation company are obliged to become members of the Detective Chamber (16).

A review of the chambers' by-laws shows that all chambers of commerce obtain funds for their operation from the monthly or annual membership fees. In addition, the vast majority of chambers of commerce receive designated funds provided by members on an interest basis, funds as payments for services rendered, funds acquired in the market, donations and gifts, and funds from the state budget. Some chambers receive funds from support contributions, funds based on European projects, publications issued, payments for granted public powers and from other sources. All professional chambers also finance their operations with membership fees or members' contributions, i.e., membership fees, joining fees and other payments, as well as from donations, gifts and other sources. Almost all professional chambers gain funds also from the activity for which they were founded. In addition, the vast majority of professional chambers secure financial resources through designated funds which their members provide on an interest basis.

The chambers differ in terms of the number of members. This number varies from chamber to chamber, which is mainly due to the different areas of competence and the scope of certain activities in Slovenia.

A closer comparison of the Veterinary and Detective Chambers – the central chambers in our research – immediately reveals that their fields of activity are completely

different. The Veterinary Chamber operates in the field of veterinary medicine, while the Detective Chamber operates in the field of detective activity. The Veterinary Chamber includes doctors of veterinary medicine practising throughout Slovenia (19), while the Detective Chamber includes domestic and foreign private detectives who perform detective services in various ways in Slovenia (16).

The by-laws of the Veterinary Chamber were adopted in 2014 (15) and those of the Detective Chamber at an extraordinary meeting in 2011 and at a regular meeting in 2012 (16). Both documents define membership in the respective chamber. Membership in the Veterinary Chamber is mandatory, voluntary or honorary, whereas in the Detective Chamber it is mandatory for all detectives working in the Republic of Slovenia. Members of both chambers have certain rights and duties, which include the payment of a predetermined membership fee.

All the tasks performed by the chambers are defined in their by-laws. Some are very similar to each other; namely, both chambers issue and revoke licences in their respective field, keep registers or records of licences and supervise the operations – of veterinary organisations and detectives, respectively. Other tasks differ, but what is important is that both chambers perform their tasks with the aim of representing the interests of their members, defending their honour and reputation or the chamber's good name. Some tasks specified in the by-laws or laws are performed by the chambers based on public powers in accordance with the laws governing either veterinary medicine or private detective work.

Both chambers have certain bodies that perform various tasks and are very important for the chamber's overall operation. It is evident from the chambers' by-laws and legal acts that the Veterinary Chamber has many more bodies than the Detective Chamber. Nevertheless, the two chambers have some identical bodies, which perform very similar and even identical tasks. In both chambers, the highest body is the Assembly, in which all members are represented. Another body that operates in both chambers is the Board of Directors, i.e., a decision-making and consultation body, which also manages the work and operations of the respective chamber. Both chambers have a President, who carries out certain tasks and in the Veterinary Chamber answers to the Assembly, and in the Detective Chamber to the Board of Directors. In the absence of the President, the Vice President acts in their place. The Supervisory Board in both chambers consists of three members and is an autonomous and independent body supervising the material and financial operations of the Veterinary Chamber (15), whereas in the Detective Chamber it conducts only financial supervision (16).

The chambers are financed in a very similar way, i.e. through membership fees, admission fees and other contributions from members, donations, gifts and legacies as

well as other sources. The chambers also obtain funds in other ways, namely the Veterinary Chamber through the organisation and performance of various professional tasks, through specialisation fees and in other ways (15). The performance of the public powers by the Veterinary Chamber is financed by the Ministry of Agriculture, Forestry and Food through the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection. The Detective Chamber also acquires funds through various membership fees and payments, fines, and funds from the sale of services, as well as in other ways (16). In reviewing the two statutes, we noted a difference in the funding of functions based on public powers. The Veterinary Chamber receives funds from the budget of the Republic of Slovenia for the tasks it performs on the basis of public powers. The Ministry in charge of the veterinary sector and the Chamber regularly agree on the amount in accordance with the law (15). The by-laws of the Detective Chamber do not contain an article on the financing of tasks based on public powers. It can therefore be concluded that the Chamber does not receive any funding for this purpose. The activities of the Veterinary Chamber and Detective Chamber can be described as to be very similar, despite of the fact that they cover very different professions. Although both chambers are specialised organisations, they have common goals namely ensuring high standards of practice, ethical conduct, and concern for the public good. In this way, they contribute to the development of the profession and to the lawful and professional performance of tasks assigned to them by the state within the scope of public powers and public tasks, in numerous fields and as part of various financial and other frameworks. In summary, both chambers operate similarly, in a similar way, naturally in a manner adapted to the profession in respect of which they were originally established.

Conclusions

There are many interpretations and definitions of what a chamber actually is, and each differs from the other in a few small details. Nevertheless, it can be stated that chambers are a type of community that brings together individuals or groups practicing the same profession. Chambers differ in terms of their status, their entities, their number of members, their powers, their tasks and other aspects. In terms of their status, they are divided into public-law and private-law, and in terms of entities, they are classified into chambers of commerce, professional chambers, and mixed-type chambers. What all chambers have in common is that they have developed or emerged from the same law, i.e., the Chambers of Commerce and Industry Act (4). Each chamber has its own by-laws, wherein it defines its tasks, public powers, bodies, financing, and all other relevant matters.

The main tasks of each chamber is to pursue the objectives and interests of its members and to represent them. Also very important are the tasks performed within the framework of public powers, which differ from chamber to

chamber depending on the activity or field for which the chamber was established.

The research examines in more detail the Veterinary Chamber and the Detective Chamber, and includes a comparison of the two.

The Veterinary Chamber (15) and the Detective Chamber and practically all other chambers in the chamber system of the Republic of Slovenia are financed by membership fees and from other contributions, income of the chamber from the performance of public powers and other services (16). In this respect, we would like to point out that not all chambers are financed for the performance of tasks within the scope of public authority, which leaves room for improvement in such a way that all chambers receive funds in the same way for the tasks they perform for the state on the basis of public powers. The legislation to be applied to the activities of the chambers must also be regulated and defined. In this context, the General Administrative Procedure Act (29) should be reasonably applied or its application defined. We would like to emphasise that in any amendment of laws and other regulations and acts relevant to the chambers they should be considered as partners participating in the procedures and processes, and only merely as enforcers of legislation enacted without their participation. In view of our focus and the detailed presentation of veterinary and detective activities, it is also important to point out some of the risks and obstacles faced by the two chambers. Particularly problematic is the fact that many people decide for important services, such as veterinary services, only because of the low prices and discounts they receive, which means they are more exposed to the risk of a lesser-quality service and the use of cheap materials and medicines. As far as the Detective Chamber is concerned, the biggest problem are the individuals who perform detective work without a licence, which the inspectors do not discover at all, as they are more focused on checking whether private investigation companies with valid licences are operating and carrying out their activities, and whether other companies that are not allowed to perform detective work or this work does not fall under their jurisdiction are doing it anyway. Another major problem is public's lack of awareness or ignorance about the detective work itself and what detective work actually is. Many people do not realise that they can turn to private investigation companies in many situations for help, because too little is said about it and the clarification is inadequate. It can be concluded that the chambers are very similar and operate in a similar way. Both the Veterinary Chamber and the Detective Chamber perform core tasks in their field of activity; nevertheless, like everyone else, they face various challenges in their operations and activities that need to be resolved immediately to ensure smooth operations, which is particularly important for all their members, i.e. veterinarians and detectives.

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References

1. Virant G. Vrsta in značilnosti pravnih oseb: gospodarske in poklicne zbornice. *Podjetje in delo* 1999; 25(6): 6–7.
2. Slovar slovenskega knjižnega jezika: SSKJ 2. 2. dopolnjena in deloma prenovljena izd. Ljubljana: Cankarjeva založba, 2014.
3. GZS EN. By-laws of the Chamber of Commerce and Industry of the Republic of Slovenia. Ljubljana: Chamber of Commerce and Industry of Slovenia, 2006. <https://eng.gzs.si/> (24. 1. 2024).
4. Zakon o gospodarskih zbornicah (ZGZ). *Ur List RS* 2006; 16(60): 6549–53.
5. Odlok o razglasitvi ustave Republike Slovenije. *Ur List RS* 1991; 1(33): 1373–86.
6. SDS. Obstoječi zbornični sistem potrebuje prenavo. Ljubljana: Slovenska demokratska stranka, 2012. <https://www.sds.si/novica/obstojecci-zbornicni-sistem-potrebuje-prenovo-1897> (25. 01. 2023).
7. Virant G. Gospodarske in poklicne zbornice. In: Juren P, ed. *Dnevi slovenskih pravnikov*, od 14. do 16. oktobra 1999 v Portorožu. Ljubljana: Gospodarski vestnik, 1999: 938–48.
8. Rakar I, Tičar B. *Pravo javne uprave*. Ljubljana: Univerza v Ljubljani, Fakulteta za javno upravo, 2019. <http://www.dlib.si/stream/URN:NBN:SI:doc-IDXIARZQ/f64b23a1-1a7d-4e64-9a5c-cef768a-6c0a0/PDF> (24. 1. 2024).
9. DTS. *Pravilnik o finančnem in materialnem poslovanju zbornice*. Ljubljana: Zbornica delovnih terapevtov Slovenije, 2018. <https://www.zdts.si/index.php/dokumenti/send/11-pravilniki/127-pravilnik-o-financnem-in-materialnem-poslovanju-zbornice> (25. 01. 2023).
10. Kovač P. *Javno pooblastilo v Republiki Sloveniji*. Ljubljana: Univerza v Ljubljani, Pravna Fakulteta, 2005. Doktorska disertacija.
11. DTS. *Poslanstvo*. Ljubljana: Zbornica delovnih terapevtov Slovenije, 2019. <https://www.zdts.si/index.php/home/poslanstvo> (24. 01. 2023).
12. Zakon o zasebnem varovanju (ZZasv-1). *Ur List RS* 2011; 21(17): 2055–72.
13. OZS. *Statut Obrtno-podjetniške zbornice Slovenije*. Ljubljana: Obrtno-podjetniška zbornica Slovenije, 2016. https://www.ozs.si/datoteke/ozs/staro/Media/Dokumenti/OSTALO%20NA%20OZS/Statut%20OZS_%C4%8Distopis22122016.pdf (24. 1. 2024).
14. *Statut Zdravniške zbornice Slovenije*. *Ur List RS* 1994; 4(65): 3651–4.
15. *Statut Veterinarske zbornice Slovenije*. *Ur List RS* 2014; 24(79): 8728–37.
16. *Statut Detektivske zbornice Republike Slovenije*. *Ur List RS* 2021; 31(64): 3857–62.
17. Zakon o detektivski dejavnosti (ZDD-1). *Ur List RS* 2011; 21(17): 2047–55.
18. Zakon o veterinarstvu (Zvet-1). *Ur List RS* 2001; 11(33): 3674–93.
19. VZbSi. *O zbornici*. Ljubljana: Veterinarska zbornica Slovenije, 2023. <https://www.vzb.si/o-zbornici> (25. 01. 2023).

20. VET-MAGAZIN. Veterinarska zbornica. Wien: Wet-magazin.com, 2022. <https://vet-magazin.si/Novice/veterinarska-zbornica.html?SID=ZU0NiahXkDveeP8N@s1kUAAA> (26. 01. 2023).
21. VZbSi. Članstvo VzbSi. Ljubljana: Veterinarska zbornica Slovenije, 2023. <https://www.vzb.si/o-zbornici/clanstvo-vzbsi> (26. 01. 2023).
22. Pravilnik o licenci za opravljanje veterinarskih dejavnosti. Ur List RS 2006; 16(115): 12117–8.
23. Pravilnik o pogojih, ki jih morajo izpolnjevati veterinarske organizacije, in o postopku njihove verifikacije. Ur List RS 2003; 13(35): 4079–83.
24. Pravilnik o pogojih, ki jih mora izpolnjevati veterinarska zbornica za opravljanje nalog, ki se opravljajo kot javna pooblastila po zakonu, ki ureja veterinarstvo. Ur List RS 2001; 11(86): 8637.
25. VZbSi. Mednarodno sodelovanje. Ljubljana: Veterinarska zbornica Slovenije, 2023. <https://www.vzb.si/o-zbornici/mednarodno-sodelovanje> (26. 01. 2023).
26. Dvojmoč M, ed. Detektivska dejavnost in upravičenost nadzorovanja zaposlenih: priročnik za naročnike in ponudnike detektivskih storitev v Republiki Sloveniji. Maribor: Univerzitetna založba Univerze, 2020. <http://www.dlib.si/stream/URN:NBN:SI:DOC-7G71NAXE/7c204339-2479-4960-8ceb-2b719f98df0e/PDF> (24. 1. 2024).
27. Detektivska zbornica Republike Slovenije. Kodeks poklicne etike. Ljubljana: Detektivska zbornica Republike Slovenije, 2017. <https://www.detektivska-zbornica-rs.si/kodeks-poklicne-etike/> (25. 01. 2023).
28. Dvojmoč M. Detektivska dejavnost v Sloveniji: (normativen) razvoj dejavnosti in pogled v prihodnost. Revija za kriminalistiko in kriminologijo 2017; 68(3): 280-297. https://www.policija.si/images/stories/Publikacije/RKK/PDF/2017/03/RKK2017-03_MihaDvojmoč_DetektivskaDejavnostVSloveniji.pdf (20. 1. 2023).
29. Zakon o splošnem upravnem postopku (ZUP-UPB2). Ur List RS 2006; 16(24): 2477–513.

Zbornice v Sloveniji – tako podobne in tako različne; primerjava struktur in načinov dela na primeru Veterinarske zbornice in Detektivske zbornice v Republiki Sloveniji

O. Podpečan, M. Dvojmoč

Izvleček: Zbornice so združenja pravnih in fizičnih oseb, ki opravljajo pridobitno dejavnost. Po definiciji so javnopravna skupnost, ki opravlja podobno dejavnost, ustanovljena pa je predvsem za zaščito interesov svojih članov. V Sloveniji so ustanovljene zbornice za zastopanje oseb, ki opravljajo različne dejavnosti - od gospodarskih družb, samostojnih podjetnikov, kmetov, gozdarjev, obrtnikov, detektivov, veterinarjev ipd. Zbornice se med seboj nekoliko razlikujejo in jih lahko razdelimo v tri skupine, in sicer na gospodarske zbornice, poklicne zbornice in mešane zbornice. Naloge zbornic, njihovi organi in zadolžitve, merila za članstvo ter prenehanje, financiranje ali pridobivanje sredstev za delovanje in drugi pomembni podatki so določeni v njihovih statutih, z ustreznim zakonom ali drugim pravnim aktom. V prispevku je na primeru treh naključno izbranih zbornic, ki se med seboj razlikujejo po delovanju, izvajanju javnih pooblastil ali financiranju nasploh, predstavljen celoten sistem zbornic Republike Slovenije, Veterinarska zbornica in Detektivska zbornica pa sta podrobneje pojasnjeni. Za ti dve zbornici smo se odločili, ker smo želeli predstaviti zbornico, ki deluje na področju naravoslovja in zbornico, ki deluje na področju družboslovja. S primerjavo veterinarske in detektivske zbornice lahko torej preučimo in analiziramo številne vidike, ki odražajo raznolikost in specifičnost posameznega poklicnega področja. Eden od razlogov, zakaj smo izbrali ti dve zbornici, je tudi dejstvo, da smo kot avtorji aktivni člani naših dveh izbranih zbornic. Primerjava omogoča lažje razumevanje njihovega delovanja ter njihovih podobnosti in razlik. Ugotovili smo, da ne samo zbornice, ki smo jih izbrali, ampak večina zbornic deluje zelo podobno, opravlja podobne naloge in ima veliko enakih organov, potrebnih za njihovo delovanje. Verjetno je to posledica dejstva, da so se vse zbornice v Sloveniji razvile ali začele razvijati po vzoru prve zbornice – Gospodarske zbornice Slovenije.

Ključne besede: zbornice; Veterinarska zbornica; Detektivska zbornica

Anatomical Structures in the Rabbit Carpal Tunnel: Comparison with Human

Key words

carpal canal;
morphometry;
histology;
median nerve;
ulnar nerve;
flexor retinaculum

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Abstract: In this study, it was aimed to reveal the morphological and morphometric changes of anatomical structures passing through the carpal tunnel, which is an entrapment neuropathy location for the median nerve. It was also aimed to evaluate the potential of rabbits as an animal model for carpal tunnel research. New Zealand rabbit cadavers were enrolled, and their wrists were investigated using the histological method in this study. ImageJ was used to measure histological sections stained with hemox-ylin-eosin. The carpal tunnel was examined at three levels: entrance, inside, and exit. Flexor retinaculum comprises two layers, encircling the carpal tunnel from the palmar side. The deep and superficial digital flexor tendons, and radial carpal flexor tendon were observed within the carpal tunnel but not the flexor pollicis longus tendon. The median and ulnar vascular nerve bundles reside between two layers of flexor retinaculum in the carpal tunnel. The areas of these anatomical structures were measured from images at three levels. Since the flexor retinaculum was located at the entrance and inside, the carpal tunnel area was measured at both levels.

The narrowing in the area from the carpal tunnel entrance to the inside of the carpal tunnel in rabbits and the decrease in connective tissue bring the anatomical structures here closer together. This clearly demonstrates that the carpal tunnel is an entrapment neuropathy area, particularly for the median nerve. Upon comparing rabbit and dog carpal tunnel anatomy, it has been shown that rabbits exhibit a greater resemblance to humans, particularly with regard to the flexor retinaculum. This study emphasizes the importance of using the rabbit model to gain insights into carpal tunnel syndrome. It demonstrates similarities between rabbit and human anatomy, underscoring the value of this animal model for future research.

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Introduction

The carpal tunnel is an osteofibrous canal located along the palmar aspect of the wrist, formed the carpal bones and the flexor retinaculum. This tunnel contains flexor tendons, the median nerve, median artery, and median vein, which are surrounded by the flexor retinaculum from the palmar side (1-3). The carpal tunnel syndrome (CTS), which is a common entrapment neuropathy in humans and is caused by compression of the median nerve in the wrist region (4, 5). This condition can lead to loss of work and time, and treatment can be economically costly (6-9). Enhancing the diagnostic precision of carpal tunnel syndrome (CTS) might

be achieved with a comprehensive analysis of the anatomical characteristics of the median nerve.

Apart from carpal tunnel syndrome in humans, entrapment neuropathy of the ulnar nerve is also mentioned in this region. The region where the ulnar nerve passes through the carpal canal is called the Guyon canal (10-14).

Understanding the normal morphology of the carpal region contributes to the diagnosis and treatment of carpal tunnel syndrome. The carpal tunnel investigations conducted

on animal models has also enhanced comprehension of the carpal tunnel and carpal tunnel syndrome (CTS) (1, 15, 16). Rabbits have been commonly utilised as a preferred animal model in carpal tunnel investigations due to their resemblance to humans (1, 5, 15-18). Moreover, rabbits are particularly preferred for CTS study due to their convenient accessibility and cost-effective feeding and care conditions (17-20). Nevertheless, these investigations often contain insufficient and inaccurate anatomical information, and there is no detailed morphometric study on the anatomical structures passing through the carpal tunnel in rabbits.

The objective of this study was to assess the morphological and morphometric characteristics of the anatomical structures passing through the carpal tunnel and obtain accurate reference values in rabbits using histological sections.

Materials and methods

Animal material

In this study, cadavers of thirteen adult New Zealand rabbits (7 male and 6 female) (*Oryctolagus cuniculus L.*) were used, which prepared for a previous anatomical study. The mean weight of rabbits was determined to be 2113 ± 299.70 g, with a range of 1774–2660 g. These rabbits were anesthetized with an intramuscular mixture of xylazine (5 mg/kg, Rompun; Bayer) and 35 mg/kg ketamine (Ketanes; Alke) and weighted. Then, they were exsanguinated by make an incision in their hearts. They were fixed by administering 10% formaldehyde through the aorta with a plastic catheter. For around nine months, the cadavers were kept in a 10% formaldehyde solution. After, performing histological sections were prepared from the cadavers. The study was approved by Animal Experiments Local Ethics Committee of Aydin Adnan Menderes University (No: 645583101/2017/141).

Histological section (HS)

Carpal tissue was preserved in 10% buffered formalin (NBF) and decalcified in 10% nitric acid. It was trimmed after sufficient decalcification for around 5 days. After multiple treatments with xylol and increasing alcohol solutions (70°, 80°, 90°, 96°, and 100°), the tissues were taken to the Leica TP1020 tissue tracking device and blocked in paraffin. These blocks were cut with a microtome (Leica RM 2135) and stained with hematoxylin and eosin (H&E) (21). At each three levels of carpal tunnel, serial sections of 5 μ m thickness were taken at 5 x 5 μ m intervals. The digital images taken using an Olympus SZX-LGR66 stereomicroscope were stored. Histological slices were photographed with a stereomicroscope and measurement paper. After transferring the photos to BSD-2-licensed ImageJ, measurements were taken.

Measurements

The carpal tunnel was examined at three levels: entrance (1. Level), inside (2. Level), and exit (3. Level). The first level was defined as the entrance of the carpal tunnel at the ends of the radius and ulna, where the palmar projection of the accessory carpal bone could be seen. The second level was defined as the inside of the carpal tunnel and included the proximal row of the carpal bones. The third level was defined as the exit of the carpal tunnel, where the proximal row of carpal bones ends and the distal row of carpal bones begins (Figure 1).

The area of the deep digital flexor tendon, superficial digital flexor tendon, and radial carpal flexor tendon that are passing through the carpal tunnel were measured. These measurements were taken by including synovial sheaths. The area of the median and ulnar nerve bundles were measured. These measurements were taken by including the fibrous band surrounding these structures. The inside area

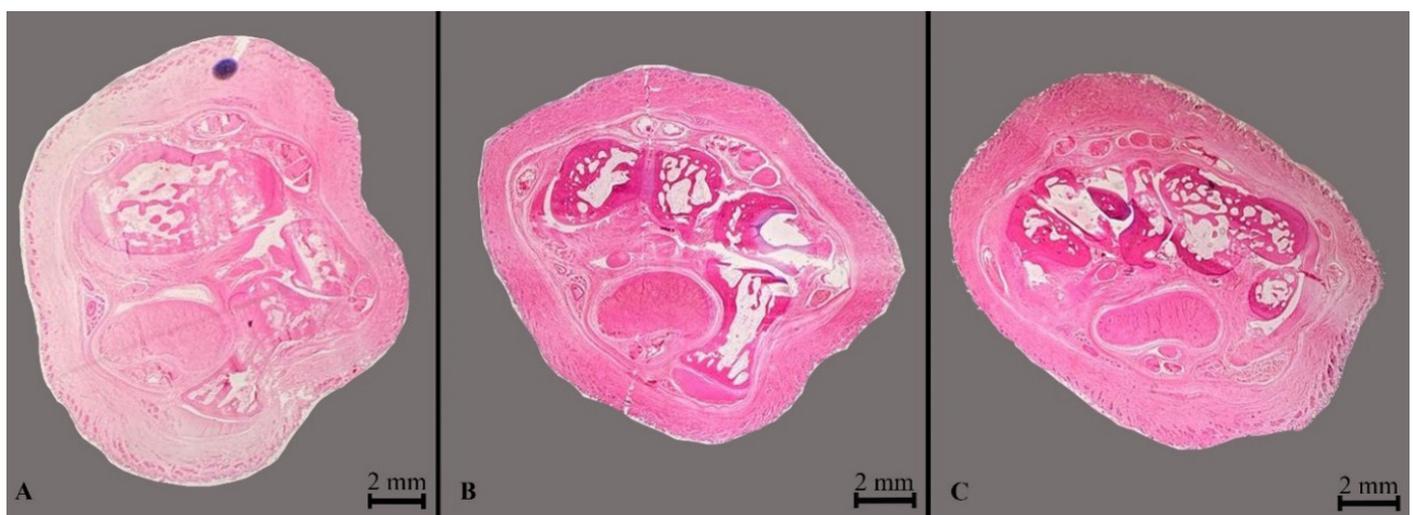


Figure 1: Images from histological section at three levels (H&E, x20 magnification). A: carpal tunnel entrance (1. Level), B: carpal tunnel inside (2. Level), C: carpal tunnel exit (3. Level)

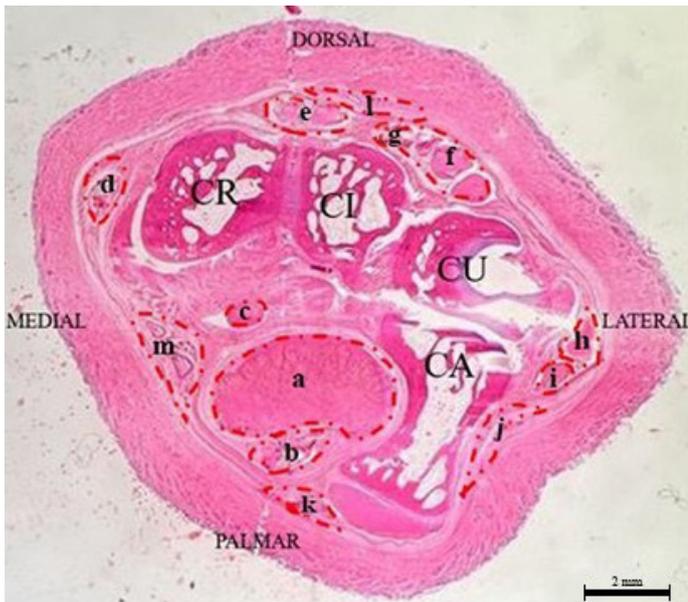


Figure 2: Image from histological section at the carpal tunnel inside (2. Level) (H&E, x20 magnification). CR: radial carpal bone, CI: intermediate carpal bone, CU: ulnar carpal bone, CA: accessory carpal bone, a; deep digital flexor tendon, b; superficial digital flexor tendon c; radial carpal flexor tendon, d; abductor pollicis longus tendon, e; radial carpal extensor tendon, f; common digital extensor tendon, g; primum and secundum digital extensor tendon, h; ulnar carpal extensor tendon, i; lateral digital extensor tendon, j; dorsal ramus of ulnar nerve, ulnar artery and ulnar vein, k; ulnar nerve bundle (palmar ramus of ulnar nerve, arteriole and venule), l: radial artery, accessory cephalic vein and radial nerve, m; median nerve bundle (median nerve, median artery, caudal interosseus artery, median vein and caudal interosseus vein)

of the median artery was measured by aligning the epithelial layer was measured. The wall thickness, including the epithelial and muscular layers of the median artery, was measured. These measurements were taken from images at three levels. The lengths of both layers of the flexor retinaculum were measured. Furthermore, the carpal tunnel area was measured between the flexor retinaculum and the bones. Since the flexor retinaculum was located at the entrance and inside, the carpal tunnel area was measured at both levels. The connective tissue area was calculated by subtracting the area of these anatomical structures from the area of the carpal tunnel (Figure 2, 3).

Statistical evaluation

The statistical analysis of the data was performed using the SPSS Statistics 21.0 software. The tables present the data as Mean value \pm Standard deviation (Mean \pm Std).

Results

The carpal tunnel was investigated on three different levels. These were the entrance of the carpal tunnel (1. Level), the inside of the carpal tunnel (2. Level), and the exit of the carpal tunnel (3. Level) (Figure 1).

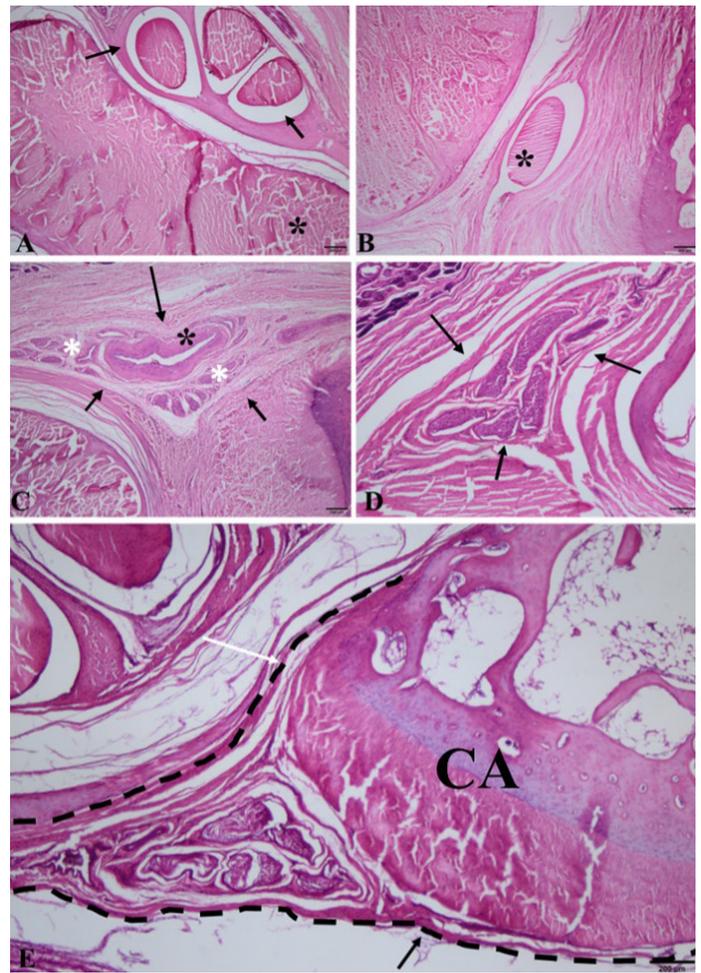


Figure 3: Images from histological sections of tendons and vascular nerve bundles in the carpal tunnel (H&E, A, B, C, E, x40 magnification and scale 200 μ m, D, x100 magnification and scale 100 μ m, Olympus-BX51, Light microscope). A; deep digital flexor tendon (black star), superficial digital flexor tendons (black arrow), B; radial carpal flexor tendon (black star), C; median nerve bundle (black arrow), median artery (black star), median nerve (white star) D: ulnar nerve bundle (black arrow), E: superficial (black arrow) and deep (white arrow) layers of the flexor retinaculum, CA; accessory carpal bone

It was found that the deep digital flexor, the superficial digital flexor and the radial carpal flexor tendons were within the carpal tunnel. It was observed that the median nerve bundle included the median nerve, the median artery, the median vein, the caudal interosseus artery and the caudal interosseus vein. The palmar ramus of the ulnar nerve, arteriole, and venule were located in the ulnar nerve bundle. The flexor retinaculum was bilayered as superficial and deep. These bundles were located between these two layers within the carpal tunnel at both sides. In the carpal tunnel, the deep digital flexor tendon and the radial carpal flexor tendon were observed to be in a single piece, whereas the superficial digital flexor tendon was observed to be in three separate parts (Figure 2, 3).

It was found that the area of deep flexor tendons decreased from level 1 to level 3, while the area of the radial carpal flexor tendon increased. It was observed that the area of

Table 1: The measurements of tendons in the carpal tunnel in histological sections (HS) at the three levels. PA; deep digital flexor tendon area, SA; superficial digital flexor tendon area, RA; radial carpal flexor tendon area

	1. Level		2. Level		3. Level	
	n	Mean±Std	n	Mean±Std	n	Mean±Std
PA(mm ²)	9	6.16±0.74	10	6.03±0.39	6	5.66±0.55
SA(mm ²)	9	1.91±0.44	10	1.86±0.25	6	2.10±0.29
RA(mm ²)	8	0.17±0.03	9	0.21±0.03	5	0.23±0.03

Table 2: Comparison of the measurements of vascular nerve bundles in the carpal tunnel in histological sections (HS) all levels. MNBA; median nerve bundle area, MAA; median artery area, MAWT; median artery wall thickness, UNBA; ulnar nerve bundle area

	1. Level		2. Level		3. Level	
	n	Mean±Std	n	Mean±Std	n	Mean±Std
MNBA(mm ²)	8	0.91±0.32	10	0.97±0.28	7	0.75±0.29
MAA(mm ²)	7	0.17±0.02	10	0.19±0.09	4	0.14±0.03
MAWT(mm)	6	0.04±0.01	10	0.05±0.01	4	0.04±0.01
UNBA(mm ²)	7	0.26±0.04	10	0.30±0.04	6	0.35±0.04

Table 3: Comparison of the measurements in histological sections (HS) two levels. CTA; carpal tunnel area, SFRL; superficial flexor retinaculum length, DFRL; deep flexor retinaculum length

	1. Level		2. Level	
	n	Mean±Std	n	Mean±Std
CTA (mm ²)	10	18.34±1.14	10	17.50±0.49
SFRL (mm)	10	9.14±0.30	9	8.33±0.24
DFRL (mm)	10	6.37±0.34	9	5.92±0.19

Table 4: Percentage changes in the areas of the anatomical structures within the carpal tunnel and the proportional differences of these changes during the transition from level 1 to level 2. CTA; carpal tunnel area, PA; deep digital flexor tendon area, SA; superficial digital flexor tendons area, RA; radial carpal flexor tendon area, MNBA; median nerve bundle area, UNBA; ulnar nerve bundle area

	Area%	Ratio
CTA	4.58 ↓	
PA	2.11 ↓	0.87 ↑
SA	2.62 ↓	0.22 ↑
RA	23.53 ↑	0.27 ↑
MNBA	6.59 ↑	0.58 ↑
UNBA	15.38 ↑	0.29 ↑
Connective Tissue	8.96 ↓	2.23 ↓

superficial flexor tendons was decreased at the inside of the carpal tunnel (Table 1).

It was observed that the measurements of MNBA (median nerve bundle area), MAA (median artery area), and MAWT (median artery wall thickness) were increased at the inside of the carpal tunnel. It was found that the area of ulnar nerve bundle increased from level 1 to level 3 (Table 2).

Measurements of the flexor retinaculum and carpal tunnel were taken from the 1st and 2nd levels, where the carpal tunnel was formed. The lengths of both the superficial and profound layers of the flexor retinaculum were decreased in the transition from level 1 to level 2. A similar reduction in carpal tunnel area was also seen (Table 3).

While passing through from level 1 to level 2 in the carpal tunnel, the percentage changes in the areas of the anatomical structures and the proportional differences of these changes in the carpal tunnel are shown in Table 4. In both levels, the connective tissue was calculated by taking out these anatomic structures from the carpal tunnel area. Based on this data, while the percentage changes in areas of the radial carpal flexor tendon and both nerve bundles were increasing, other structures' were decreasing. However, in terms of proportionality (the ratio of these structures' areas to the carpal tunnel area), the areas of all tendons and both nerve bundles have shown an increase, whereas the amount of connective tissue has seen a reduction.

Discussion

The carpal tunnel is a groove between the carpal bones and the flexor retinaculum. In humans, the entrance to the carpal tunnel is formed by the proximal row of carpal bones, especially the accessory carpal bone. The exit is shaped by the distal carpal bone row and especially the hook of the hamate bone (22-24). Since the hamate bone has no hook in dogs, the carpal tunnel is formed only by the accessory carpal bone (25). Since the bony roof of the carpal tunnel in rabbits is similar to that in dogs, in this study the carpal tunnel was examined at two levels, as in dogs.

In the morphology of the carpal tunnel in rabbits, unlike humans, it has been determined that one deep digital flexor tendon instead of four, three superficial digital flexor tendons instead of four, and the flexor carpi radialis tendon instead of the flexor pollicis longus tendon pass through this tunnel. In addition, it has been determined that the median nerve bundle in rabbits, unlike humans, is located more superficially between the two layers of the flexor retinaculum. It has been observed that the ulnar nerve package is located between the two layers of the flexor retinaculum, which resembles the Guyon canal in humans (23, 26). It has been reported that in dogs, unlike rabbits, the flexor retinaculum consists of two layers, and the superficial digital flexor tendon is located between these two layers. Additionally, it was observed that the ulnar and median nerve packages were located under the deep layer of the flexor retinaculum differently in rabbits (25). It should be emphasized that there is radial carpal flexor tendon but no flexor pollicis longus tendon in the carpal tunnel in rabbits do not agree with the previous studies (1, 2, 27).

The carpal tunnel area of rabbits was noticed to be approximately 1/9 that of humans (4, 6, 28). In dogs, taking the boundary of the deep layer of the flexor retinaculum, the carpal tunnel area of rabbits was seen to be approximately 1/7 that of dogs, and the superficial layer boundary of it was 1/13 that of dogs (25).

In humans, the ratio of the area of the tendons passing through the carpal tunnel to the carpal tunnel area has been reported to be approximately 58–59% (28). This rate was found to be slightly low and 46 % in this study conducted on rabbits at carpal tunnel inlet. It is thought that the discrepancies in these rates may be due to different tendons and numbers of tendons passing through the carpal tunnel.

The morphological profile of the median nerve (cross-sectional area, flattening ratio and circularity) has been proven to be the most effective parameter for evaluating compression neuropathy other than nerve conduction tests. Additionally, the assessment of flexor retinaculum bowing is also regarded as a significant criterion in the diagnosis of carpal tunnel syndrome (CTS) (29-31). In the determination of the flattening ratio of the median nerve in the human, the long and short diameters of the nerve bundle are used.

In humans, the median nerve normally presents in the carpal tunnel as a whole, with all nerve fibers surrounded by a sheath. However, in rabbits, the median nerve fibers are dispersed, and present as a vascular nerve bundle together with the median artery and median vein. Therefore, it is thought that it is more appropriate to measure the area of the vascular nerve bundle in rabbits instead of the flattening ratio.

It has been reported that the area of the median nerve is an average of 8 mm² (7.16-8.7 mm²) in the carpal tunnel in humans by ultrasonography (29-35). When calculating the ratio of the median nerve area to the carpal tunnel area, it is found to be approximately 5% in humans (29). Rodríguez et al. (2022) found that the area is 12 mm² with a digital caliper in human cadavers, and the ratio is approximately 7% in humans. In this study, it has been observed that the median nerve area of rabbits is approximately 1/9, according to the data of Rodríguez et al. (2022) (28), this ratio is 1/13 the size of humans. The ratio of the median nerve area to the carpal tunnel area in this study was found to be 5% in rabbits and showed similarity with the data of Potuznik et al. (2023) (29). It is thought that the differences in these rates may be due to the measurement method used and population differences.

According to Ginanneschi et al. (2018) (36), it appears that carpal tunnel syndrome can have an effect on the morphology of the Guyon canal as well as the morphology and function of the ulnar nerve at the wrist. The area of the ulnar nerve within the Guyon canal was reported to be 4.54 mm² (4.05–5.11 mm²) on average in humans by ultrasonography (34, 35, 37-39). In this study, it was determined that the area of the ulnar nerve in rabbits was approximately 1/15 of that of humans. According to the data above, the ratio of the ulnar nerve area to the carpal tunnel area is found to be approximately 2.75% in humans. This rate was found to be slightly low and 1.7% in this study conducted on rabbits. It is thought that this difference arises from the fact that the Guyon canal is not considered within the carpal tunnel area in humans.

It was reported that the length of the flexor retinaculum, when measured with a digital caliper, was 26.65 mm in humans, 13.03 mm in dogs, 8.46 mm in rabbits, and 2.46 mm in rats (17). The given length of the flexor retinaculum in rabbits is comparable to the length of the superficial flexor retinaculum. In this study, it has been showed similarity with the length in rabbits and was approximately 1/4 that of humans.

With this study, it was determined that there was a narrowing in the area from the carpal tunnel entrance to the inside of the carpal tunnel in rabbits. Although there is a decrease in the areas of the deep and superficial tendons in this region, it is noteworthy that the decrease in the connective tissue is greater. Although there is a proportional increase in the areas of anatomical structures passing through the

carpal tunnel, the decrease in the connective tissue tries to compensate this situation. However, the narrowing in the carpal tunnel and the decrease in connective tissue bring the anatomical structures here closer together. This clearly shows that the carpal tunnel is an entrapment neuropathy area, especially for the median nerve.

One of the study limitations is the rabbit cadavers utilized, which had been stored in a formaldehyde solution for an extended duration. Prolonged fixation in formalin may result in secondary shrinkage on soft tissues (40). As a consequence of the subsequent decalcification procedure, tissue detachment occurred, resulting in a reduction in the quantity of histological sections available for analysis.

Conclusions

The carpal tunnel anatomy of rabbits was examined in detail in this study, and the insufficient and erroneous data in previous studies on the carpal tunnel in rabbits were tried to be clarified. In this study, we tried to explain more clearly with the measurements taken that the carpal tunnel is the site of entrapment neuropathy for the median nerve. Additionally, this study emphasizes the validation of the rabbit model in carpal tunnel syndrome research. By establishing the closer resemblance of the rabbit carpal canal to humans, this research paves the way for more accurate investigations into the condition.

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Conflict of interest statement. The authors declare no conflicts of interest.

Data availability statement. The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Ettema AM, Zhao C, An KN, Amadio PC. Comparative anatomy of the subsynovial connective tissue in the carpal tunnel of the rat, rabbit, dog, baboon, and human. *Hand (N Y)* 2006; 1(2): 78–84. doi: 10.1007/s11552-006-9009-z
2. Craigie EH. Bensley's practical anatomy of the rabbit. 5th ed. Philadelphia: The Blakiston Company, 1948.
3. Schrier VJMM, Vrieze A, Amadio PC. Subsynovial connective tissue development in the rabbit carpal tunnel. *Vet Med Sci* 2020; 6(4): 1025–33. doi: 10.1002/vms3.281
4. Bower JA, Stanisz GJ, Keir PJ. An MRI evaluation of carpal tunnel dimensions in healthy wrists: implications for carpal tunnel syndrome. *Clin Biomech (Bristol, Avon)*. 2006; 21(8), 816–25. doi: 10.1016/j.clinbiomech.2006.04.008
5. Diao E, Shao F, Liebenberg E, Rempel D, Lotz JC. Carpal tunnel pressure alters median nerve function in a dose-dependent manner: a rabbit model for carpal tunnel syndrome. *J Orthop Res* 2005; 23(1): 218–23. doi: 10.1016/j.orthres.2004.05.014
6. Kamolz LP, Schröngendorfer KF, Rab M, Girsch W, Gruber H, Frey M. The precision of ultrasound imaging and its relevance for carpal tunnel syndrome. *Surg Radiol Anat* 2001; 23(2): 117–21. doi:10.1007/s00276-001-0117-8
7. Stapleton MJ. Occupation and carpal tunnel syndrome. *ANZ J Surg* 2006; 76(6): 494–6. doi:10.1111/j.1445-2197.2006.03770.x
8. Wright AR, Atkinson RE. Carpal tunnel syndrome: an update for the primary care physician. *Hawaii J Health Soc Welf* 2019; 78(11, suppl. 2): 6–10.
9. Zuniga AF, Keir PJ. Diagnostic and research techniques in carpal tunnel syndrome. *Crit Rev Biomed Eng* 2019;4 7(6): 457-71. doi: 10.1615/CritRevBiomedEng.2020030827
10. Aleksenko D, Varacallo M. Guyon canal syndrome. In: StatPearls. StatPearls Publishing, 2023. <https://www.ncbi.nlm.nih.gov/books/NBK431063/>
11. Georgiew F, Maciejczak A, Florek J, Kotela, I. Ulnar and median nerve abnormalities in carpal tunnel syndrome. *Ortop Traumatol Rehabil* 2020; 22(5): 311–8. doi: 10.5604/01.3001.0014.4214
12. Levy SM, Reid M, Montgomery AM, et al. Do magnetic resonance imaging abnormalities of the non-dominant wrist correlate with ulnar-sided wrist pain in elite tennis players?. *Skeletal Radiol* 2020; 49(3): 407–15. doi: 10.1007/s00256-019-03285-y
13. Vij N, Traube B, Bisht R, et al. An update on treatment modalities for ulnar nerve entrapment: a literature review. *Anesth Pain Med* 2020; 10(6): e112070. doi: 10.5812/aapm.112070
14. Yamamoto R, Izumida M, Sakuraya T, Emura K, Arakawa T. The ulnar nerve is surrounded by the tendon expansion of the flexor carpi ulnaris muscle at the wrist: an anatomical study of Guyon's canal. *Anat Sci Int* 2021; 96(3): 422–6. doi: 10.1007/s12565-021-00607-w
15. Moriya T, Zhao C, Cha SS, Schmelzer JD, Low PA, An KN, Amadio PC. Tendon injury produces changes in SSCT and nerve physiology similar to carpal tunnel syndrome in an in vivo rabbit model. *Hand (NY)* 2011; 6(4): 399–407. doi: 10.1007/s11552-011-9356-2
16. Werthel JD, Zhao C, An KN, Amadio PC. Carpal tunnel syndrome pathophysiology: role of subsynovial connective tissue. *J Wrist Surg* 2014; 3(4): 220–6. doi: 10.1055/s-0034-1394133
17. Tung WL, Zhao C, Yoshii Y, Amadio PC, Su FC, An KN. . A comparative study of carpal tunnel compliance in the human, dog, rabbit, and rat. *J Orthop Res* 2014; 28(5), 652–6. doi: 10.1002/jor.21037
18. Yoshii Y, Zhao C, Schmelzer JD, Low PA, An KN, Amadio PC. Effects of multiple injections of hypertonic dextrose in the rabbit carpal tunnel: a potential model of carpal tunnel syndrome development. *Hand (N Y)* 2014;9(1): 52–7. doi: 10.1007/s11552-013-9599-1
19. Oh S, Ettema AM, Zhao C, et al. Dextrose-induced subsynovial connective tissue fibrosis in the rabbit carpal tunnel: A potential model to study carpal tunnel syndrome?. *Hand* 2008; 3(1): 34–40. doi: 10.1007/s11552-007-9058-y

20. Yamaguchi T, Osamura N, Zhao C, Zobitz ME, An KN, Amadio PC. The mechanical properties of the rabbit carpal tunnel subsynovial connective tissue. *J Biomech* 2008; 41(16): 3519–22. doi: 10.1016/j.jbiomech.2007.06.004
21. Luna LG. *Manual of histologic staining methods of the armed forces Institute of pathology*. 3rd ed., Mc Graw - Hill Book Company, New York: Blakiston Division McGraw-Hill, 1968.
22. Park JS, Won HC, Oh JY, Kim DH, Hwang SC, Yoo JI. Value of cross-sectional area of median nerve by MRI in carpal tunnel syndrome. *Asian J Surg* 2020; 43(6): 654–59. doi: 10.1016/j.asjsur.2019.08.001
23. Presazzi A, Bortolotto C, Zacchino M, Madonia L, Draghi F. Carpal tunnel: normal anatomy, anatomical variants and ultrasound technique. *J Ultrasound* 2011; 14(1): 40–6. doi: 10.1016/j.jus.2011.01.006
24. Sora MC, Genser-Strobl B. The sectional anatomy of the carpal tunnel and its related neurovascular structures studied by using plastination. *Eur J Neurol* 2005; 12(5): 380–4. doi: 10.1111/j.1468-1331.2004.01034.x
25. Turan E, Erden H. Computed tomography and morphometry of the carpal canal in the dog. *Ann Anat* 2003; 185(2): 173–8. doi: 10.1016/S0940-9602(03)80084-5
26. Deak N, Black AC, Bordoni B. Anatomy, shoulder and upper limb, wrist flexor retinaculum. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing, 2024. <https://www.ncbi.nlm.nih.gov/books/NBK545198/> (12. 2. 2024)
27. Ekim O, Oto C, Algin O. Magnetic resonance (MR) imaging of the carpal tunnel and related structures in New Zealand Rabbit (*Oryctolagus cuniculus*): an anatomic and radiologic evaluation on an animal model. *Ankara Univ Vet Fak Derg* 2014; 61(1): 1–7. doi: 10.1501/Vetfak_0000002597
28. Rodríguez P, Casado A, Potau JM. Quantitative anatomical analysis of the carpal tunnel in women and men. *Ann Anat* 2022; 243: 151956. doi: 10.1016/j.aanat.2022.151956
29. Potuznik P, Hosek P, Kotas R. Median nerve ultrasonography examination correlates with electrodiagnostic studies for the diagnosis of moderate to severe carpal tunnel syndrome. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.* 2023; 167(2): 192–8. doi:10.5507/bp.2021.068
30. Ulaşlı AM, Duymuş M, Nacir B, Rana Erdem H, Koşar U. Reasons for using swelling ratio in sonographic diagnosis of carpal tunnel syndrome and a reliable method for its calculation. *Muscle Nerve* 2013; 47(3): 396–402. doi:10.1002/mus.23528
31. Asghar A, Naaz S, Ansari S, Kumar A, Singh V. The cross-sectional morphology of median nerve in carpal tunnel of healthy, adult population: a systematic review and meta-analysis. *Morphologie* 2023; 107(356): 99–115. doi: 10.1016/j.morpho.2022.05.005
32. Fu T, Cao M, Liu F, et al. Carpal tunnel syndrome assessment with ultrasonography: value of inlet-to-outlet median nerve area ratio in patients versus healthy volunteers. *PloS One* 2015; 10(1): e0116777. doi: 10.1371/journal.pone.0116777
33. Bueno-Gracia E, Malo-Urriés M, Ruiz-de-Escudero-Zapico A, et al. Reliability of measurement of the carpal tunnel and median nerve in asymptomatic subjects with ultrasound. *Musculoskeletal Sci Pract* 2017; 32: 17–22. doi: 10.1016/j.msksp.2017.08.001
34. Kerasnoudis A, Pitarokoili K, Behrendt V, Gold R, Yoon MS. Cross sectional area reference values for sonography of peripheral nerves and brachial plexus. *Clin Neurophysiol* 2013; 124(9): 1881–8. doi: 10.1016/j.clinph.2013.03.007
35. Won SJ, Kim, BJ Park, Yoon JS, Choi H.. Reference values for nerve ultrasonography in the upper extremity. *Muscle Nerve* 2013; 47(6): 864–71. doi: 10.1002/mus.23691
36. Ginanneschi F, Mondelli M, Cioncoloni D, Rossi A. Impact of carpal tunnel syndrome on ulnar nerve at wrist: systematic review. *J Electromyogr Kinesiol* 2018; 40: 32–8. doi: 10.1016/j.jelekin.2018.03.004
37. Darwish HS, Habash WY, Kamel H. A. Ultrasound reference values estimation for the upper limb peripheral nerves in adult females; a hospital-based Study. *Suez Canal Uni Med J* 2022; 25(3): 48–55. doi: 10.21608/scumj.2022.261000
38. Bedewi MA, Abodonya A, Kotb M, et al. Estimation of ultrasound reference values for the upper limb peripheral nerves in adults: a cross-sectional study. *Medicine(Baltimore)* 2017; 96(50): e9306. doi: 10.1097/MD.0000000000009306
39. Qrimli M, Ebadi H, Breiner A, et al. Reference values for ultrasonography of peripheral nerves. *Muscle Nerve* 2016; 53(4): 538–44. doi:10.1002/mus.24888
40. Chatterjee S. Artefacts in histopathology. *J Oral Maxillofac Pathol* 2014; 18(suppl. 1): S111–6. Doi: 10.4103/0973-029X.14134

Anatomske strukture v karpalnem kanalu kunca: primerjava s človekom

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Izveček: Cilj te raziskave je bil raziskati spremembe v morfologiji in morfometriji anatomskih struktur v karpalnem kanalu, natančneje na mestu ukleščanja medianega živca. Poleg tega smo želeli oceniti primernost kuncev kot živalskega modela za študije karpalnega kanala. V ta namen smo uporabili trupla novozelandskih kuncev in opravili histološki pregled njihovih zapestij. Histološke rezine, obarvane s hematoksilinom in eozinom, smo analizirali s programom ImageJ za pridobitev meritev. Naš pregled je bil osredotočen na tri nivoje karpalnega kanala: vhod, notranjost in izhod. Karpalni kanal je obdan z dvema plastema flektornega retinakuluma na palmarni strani. Znotraj karpalnega kanala smo opazovali globoke in površinske kite upogibalk prstov in radialnih upogibalk zapestja, ne pa tudi tetiv dolge upogibalke. Žilni živčni snopi medianega in ularnega živca se nahajata med obema plastema flektornega retinakuluma znotraj karpalnega kanala. Da bi ocenili velikost teh anatomskih struktur, smo izmerili njihove površine na podlagi slik, posnetih na treh prej omenjenih ravneh.

Ob upoštevanju prisotnosti flektornega retinakuluma smo meritev površine karpalnega kanala izvedli tako na vhodu kot znotraj kanala. Pri kuncih zožitev območja od vhoda do notranjosti karpalnega kanala skupaj z zmanjšanjem vezivnega tkiva povzroči, da se anatomske strukture približajo. To zagotavlja jasen dokaz, da je karpalni kanal mesto, kjer se pojavi utesnitvena nevropatija, ki posebej vključuje mediani živec. S primerjavo anatomije karpalnega kanala pri kuncih in psih je bilo ugotovljeno, da so kunci bolj podobni ljudem, zlasti v smislu flektornega retinakuluma. Ta raziskava poudarja pomen uporabe kunčjega modela za preučevanje sindroma karpalnega kanala, saj prikazuje podobnosti med kunčjo in človeško anatomijo ter poudarja pomen tega živalskega modela za prihodnje preiskave.

Ključne besede: karpalni kanal; morfometrija; histologija; mediani živec; ularni živec; flektorni retinaculum

Hemodynamic Changes of the Uterine Artery in Mares With Different Uterine Pathologies

Key words

Doppler ultrasonography;
endometritis;
intrauterine cyst;
notch

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Abstract: Cysts, fluid accumulation, inflammatory changes and tumors are common uterine pathologies in mares. It was aimed to investigate the change of uterine hemodynamics in mares according to uterine pathologies (endometritis or intrauterine cysts). The study contained 28 mares and the groups formed as 9 gynaecologically healthy diestrus mares (Group H), 9 mares with endometritis (Group E) and 10 mares with intrauterine cyst (Group C). The mean uterine diameter (UD) in Group H (2.29 ± 0.18 cm) was significantly lower than in Group C (2.97 ± 0.19 cm) ($P < 0.05$) and in Group E (3.47 ± 0.29 cm) ($P < 0.01$). The diameters of the intrauterine cysts ranged between 0.51 cm and 1.83 cm in Group C. The highest diameter of the uterine artery (UA) was observed in Group E. Hemodynamic indices of UA (PI and RI) in Group E were not significantly different from those in Group C ($P > 0.05$). However, PI and RI values in Group H were significantly lower than both in Group E and Group C ($P < 0.001$). The UA waveform in Group E had an early diastolic notch while systolic notches and small diastolic peaks were observed in the UA waveforms of the mares in group C. Doppler USG is considered as a useful tool to analyze the waveform and hemodynamics of the UA related to the different pathologies (cyst or endometritis). It was concluded that not only Doppler indices but also characteristics of the waveforms of the UA should be evaluated to understand the hemodynamic effect of the uterine pathologies.

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Introduction

Doppler ultrasonography is a non-invasive technique that enables the examination of physiological and pathological changes in the blood supply of the female reproductive system (1). Pulsatility index (PI) and resistance index (RI) values, which are angle-independent, are used to identify abnormal waveforms (2). The PI characterizes blood flow of the uterine artery (UA) and exhibits a wave-shaped profile throughout the estrous cycle in mares, which are negatively correlated with plasma estrogen levels during estrus (3). In humans, decreased uterine blood flow and poor uterine perfusion during the luteal phase are associated with reduced chances of conception and infertility (4).

The most common forms of uterine pathology detected by ultrasonography in the non-pregnant mare are accumulations of intrauterine fluid, inflammatory changes (increase in uterine wall thickness, edema of the endometrial folds, presence of luminal content, etc.), uterine cysts (5). Uterine

cysts are fluid-filled and they can be glandular or lymphatic in origin. Glandular cysts, which are typically microscopic features, originate from the endometrial glands. These cysts are commonly undetectable by reproductive ultrasonography and are usually < 10 mm in diameter (6). The second form of uterine cysts is lymphatic in origin and generally is larger than endometrial cysts. Most uterine cysts detected by ultrasonography that protrude into the uterine lumen are lymphatic. Lymphatic cysts can be measured from as small as a few millimeters to as large as several centimeters and they are pedunculated or sessile. The establishment of pregnancy may be impaired in mares with multiple large (> 3 cm) cystic areas (single cyst or cluster of multiloculated cysts) (7).

Rectal palpation and ultrasound examinations identify the uterine fluids that may be indicative of endometritis. An endometrial swab or endometrial biopsy provides the

microscopic analysis of the uterine pathology (8). Although different methods have also been used to diagnose endometrial diseases, the most effective and practical technique used to collect endometrial material has been the commercial cytobrush/swab collector (9). Rahawy and colleagues (10) have recommended ultrasound and cytological techniques for the diagnosis of endometritis in mares. Doppler ultrasound improved the diagnosis of endometritis as it showed alterations in uterine vascularization (11). Da Silva-Álvarez and colleagues (12) recommended power Doppler ultrasound in combination with computerized image analysis which has the potential to be a very useful tool in the diagnosis of endometritis in mares. In addition, real-time characterization of uterine blood flow and changes in uterine vascular perfusion and mesometrial PI in response to semen infusion in mares were evaluated (13).

Considering all the above, we hypothesize that pulsed Doppler ultrasound could detect uterine hemodynamic alterations associated with uterine pathologies (endometritis or intrauterine cysts) in equines. It was aimed to determine if the indices of UA are a good marker of uterine pathologies in mares.

Materials and methods

General design

In total, 50 mares were examined to get the number of 28 mares in this study. The study groups were formed as 9 gynaecologically healthy mares (group H), 9 mares with endometritis (group E) and 10 mares with intrauterine cysts (group C). The mares both with cysts and endometritis were not included in the study. The mares were not mated or inseminated during the study. The cytological examinations were performed luteal phase of the estrous cycle. All ultrasound evaluations were performed in mid-diestrus, 10 to 12 days after ovulation.

Animals and experimental groups

Twenty eight Arabian mares (7-14 years old; 350-435 kg) were enrolled for the study between March and June 2021 in the Northern Hemisphere (latitude 41° N). The body condition scores of the mares were between 5 and 7 (14). All mares were from the same herd. Mares were kept in an open shed and outdoor grass paddock under natural light with access to water and mineral salt (*ad libitum*). They received 2 kg of concentrated balanced grains daily.

Eight of 10 mares in group C and all mares in group H have foaled in the last year. However, only 2 of 9 mares in group E have foaled in the last year. At the end of the study, the mares in Group C were naturally mated at the next estrus and their pregnancy status was recorded. Also, the routine breeding protocol was continued for healthy mares, and they did not have any problems in conceiving. The first examination for

pregnancy diagnosis of mares mated in both group H and group C was performed on the 14th day after mating. Also, the vitality of the mare and the fetus were followed by ultrasonography from the diagnosis of pregnancy until delivery.

B-mode and Doppler USG

The internal genital tract (ovaries and uterus) was examined transrectally by the same operator in all groups with B-mode ultrasonography (Esaote MyLab One Vet, Esaote Pie Medical, Genova, Italy) equipped with linear veterinary transducer (SV 3513 Vet; Esaote Pie Medical, Genova, Italy). Follicles larger than 35mm were visualized daily by ultrasonography until ovulation occurs. The ovulation (day 0) was confirmed by the disappearance of the largest follicle and visualization of the newly forming corpus luteum (CL) in ultrasonography examination. The date of ovulation was noted for each mare. Ovaries in all groups were examined for the presence of CL and small follicles (< 20 mm). The widest cross-sectional uterine diameter (UD), edema of the endometrial folds, presence of luminal content, number, localization and diameter of uterine cysts were visualized on left and right uterine horns. Related diameters were calculated automatically by the USG equipment software. Grading of the uterine inflammatory fluid echo pattern was performed by grayscale echogenicity (15). The researchers (15) have defined this classification as: Grade 1: The intrauterine fluid is very echogenic (almost white) and represents large amounts of suspended debris. On occasion, it may be difficult to see the junction of the fluid and the uterine wall. Grade 2: The intrauterine fluid is echo dense (light gray) but less so than grade 1. Grade 3: The intrauterine fluid is dark gray due to a few hypoechoic foci suspended in an anechoic medium. Grade 4: The intrauterine fluid is black or anechoic. Subjective evaluation of the uterine edema was classified according to Samper and Pycocok (16). The related classification was defined as follows: 0: No edema, 1: Minimal detectable uterine edema, 2: Moderate uterine edema, 3: Significant edema, 4: The highest level of normal edema, little free fluid can be detected in the lumen, 5: Abnormal uterine edema characterized by an irregular and disorganized appearance.

The UA was found the way Bollwein and colleagues (1) described. Uterine blood supply was studied by the investigation of the left and right uterine arteries. The UA was examined as published previously (1). The color Doppler was used to visualize UA by using a 5 MHz transducer. Three UA diameters were determined at the same location using a linear array ultrasound transducer. The hemodynamic changes of the UA were visualized by using pulsed wave Doppler USG using a 5 MHz transducer. The angle of insonation was approximately 60° during spectral measurements. At least three consecutive systolic peaks with similar amplitude and velocity were included for analysis. The PI and RI values of UA were automatically calculated by the Doppler machine software. To minimize the variations, settings were kept constant (B mode frequency 10 MHz, color

flow Doppler frequency 5 MHz, gain 28%, pulse repetition frequency 1 KHz, Pulsed wave Doppler frequency 5 MHz, gain 70%, pulse repetition frequency 2 KHz).

Cytological examination

Cytobrush (CB) technique was performed using a cytology brush instrument (Equine Cytology Brush, Ref. 17214/2960; Minitube GmbH, Tiefenbach, Germany) as reported before (17). The slides were stained with MGG Diff Quick stain set (ADR Group, Mediko Kimya, Istanbul, Türkiye) according to the manufacturer's instructions. Slides were examined by a light microscope (Olympus CX41, Tokyo, Japan) at x400 magnification. A digital camera (Olympus SC30, Tokyo, Japan) combined with a software package program allowed digital documentation and further analysis. In total,

200 cells were counted to determine the percentage of PMNs in each slide (17). The ratios of PMNs less than 0.5%, between 0.5% and 5%, between 5% and 30%, and higher than 30% were used to determine the classifications as 0, +1, +2 and +3; respectively (18, 19).

Statistical analysis

Statistical analyses were performed with the SPSS 13.0 package program. The normal distribution of the data was checked by using Shapiro-Wilk test. The comparison of the groups about USG measurements were applied with One-way ANOVA and Duncan tests. Values were given as mean \pm standard error of the mean (SEM). The significance level was accepted as $P < 0.05$.

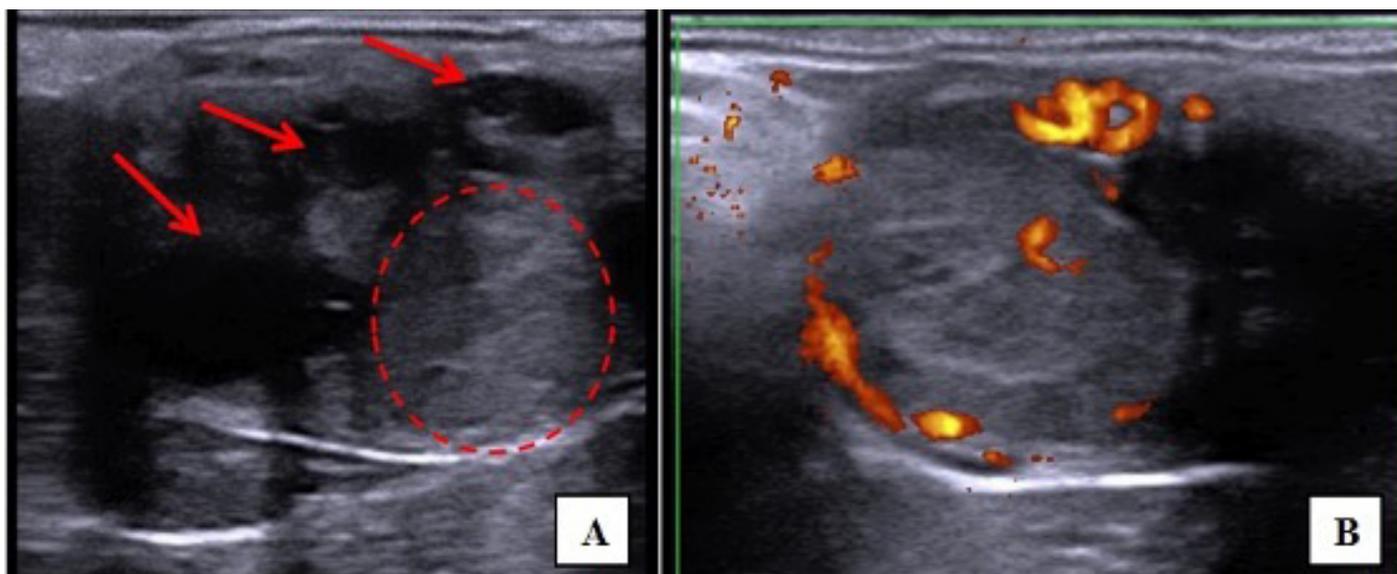


Figure 1: A. The presence of CL and small follicles (< 20 mm). Red circle: CL. Red arrows: Small follicles. B. Power Doppler image of an active CL

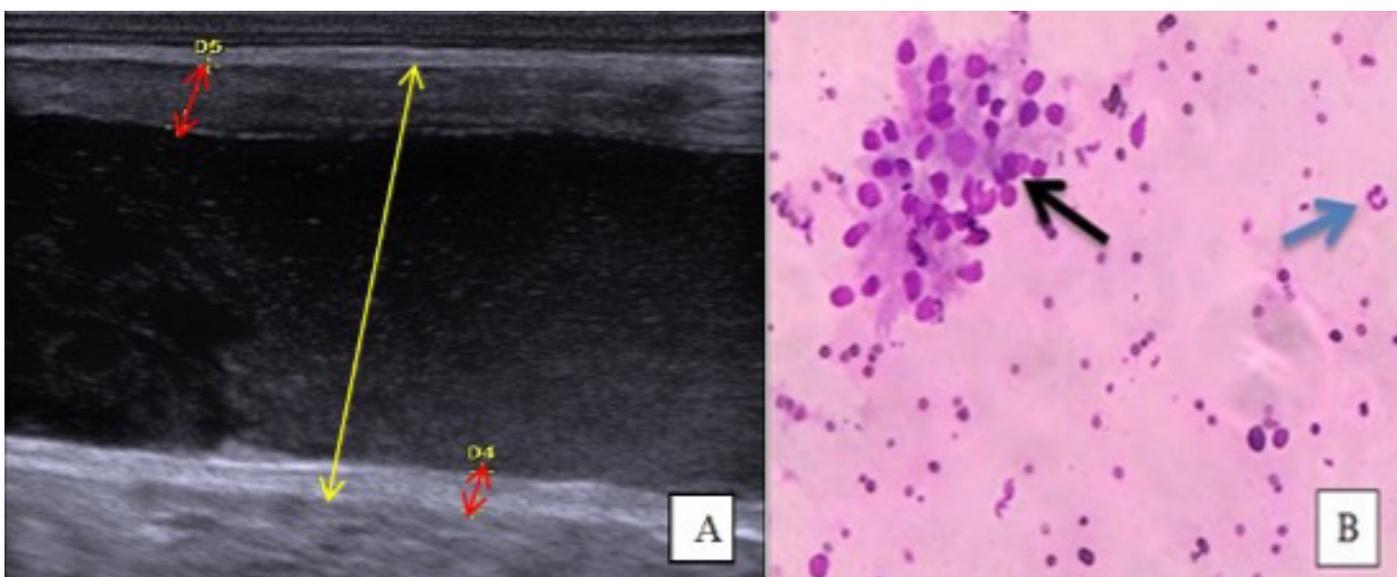


Figure 2: A. Transrectal USG image of the uterus in a mare with endometritis and presence of the intrauterine fluid. Red arrows: Excessive edema of the endometrial folds (D_4 : 3.69 mm and D_5 : 6.08 mm). Yellow arrow: Increased uterine diameter (4.02 cm). B. Cytology smear obtained by CB in a mare with +2 endometritis. Black arrow: PMNs. Blue arrow: Neutrophil

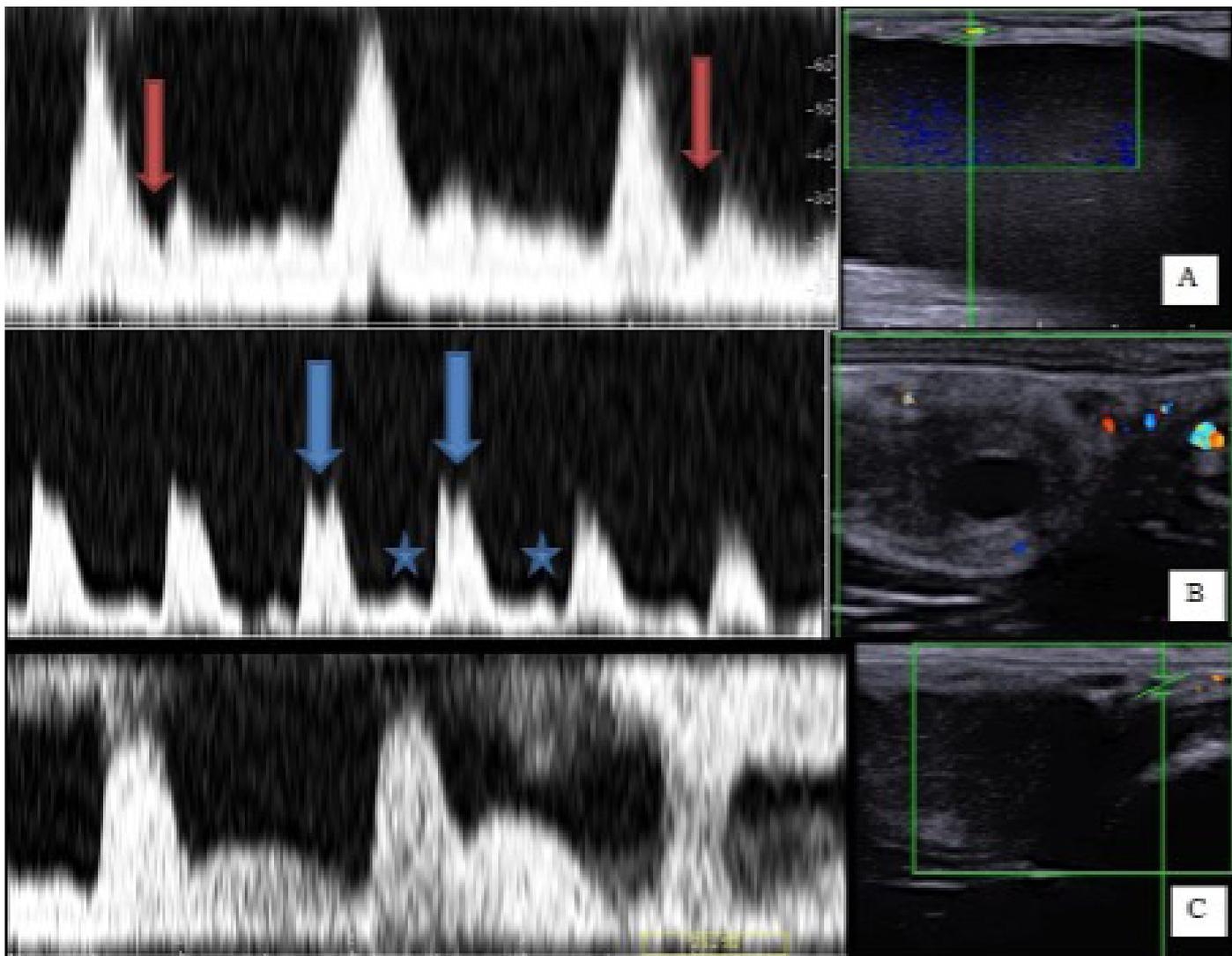


Figure 3: Waveforms and color mapped of UA in all groups. A. Early diastolic notches (red arrows). Color mapped of the UA and the presence of the intrauterine fluid accumulation. B. Waveform evident with systolic notches (blue arrows) followed by a small diastolic peak (blue stars). Color mapped of the UA and the presence of an intrauterine cyst. C. Spectral Doppler graph and typical pattern of UA waveform. Color mapped of the UA and transversal section of the healthy uterus in the luteal phase

Results

The mean ages of the mares related to groups were 8.4 ± 0.50 years in Group H, 12.25 ± 1.03 years in Group E, and 11.2 ± 0.37 years in Group C. Significant rises were determined in the mean ages of the mares both in Group E and Group C than in Group H ($P < 0.01$) while the ages between Group E and Group C were not significantly different ($P > 0.05$). In B-mode ultrasonography, all mares which were not mated/inseminated, had follicles less than 20mm and corpora lutea on the ovaries (Figure 1).

In nine mares in Group E, endometritis was diagnosed by transrectal palpation, CB, and transrectal USG (Figure 2). All mares in Group E had chronic endometritis. In each mare of group E, an excessively toned, partially pendulous, and enlarged uterus was detected by rectal palpation. Cytology smears of Group E were classified as PMN +2 (Table 1).

However, none or less than 0.5% PMNs was observed in Group H and Group C.

In Group E, the mean UD and uterine wall thickness were 3.47 ± 0.29 cm and 4.30 ± 0.28 mm; respectively. The amount and echogenicity of the intrauterine fluid in Group E was Grade 2 (Table 1). Subjective evaluation of uterine edema, 2 of the 9 mares in Group E exhibited Grade 3, while the remainder presented with Grade 2 (Table 1). Edema of the endometrial folds, presence of intrauterine fluid, and rise in UD was visualized in Group E by transrectal USG whereas absence of edema of the endometrial folds, absence of the intrauterine fluid, and UD were 2.29 ± 0.18 cm in Group H. The mean UD of all groups is presented in Table 2. The mean UD in Group H was significantly lower than in Group C ($P < 0.05$) and in Group E ($P < 0.01$). According to trans-rectal ultrasonography, single ($n=6$ mares) and double intrauterine cysts ($n=4$ mares) were observed in Group C. The cysts were located on the right uterine horn ($n=5$ mares) and on the left

Table 1: Uterine edema, echogenicity of intrauterine fluid, PMN grades in mares with endometritis

Mares in Group E	Uterine edema					Echogenicity of intrauterine fluid				PMN (%)				
	0	1	2	3	4	5	Grade 1	Grade 2	Grade 3	Grade 4	(0) <0.5 %	(+1) 0.5-5%	(+2) 5-30%	(+3) >30%
	No.1			+							+			
No.2			+							+				+
No.3			+							+				+
No.4				+						+				+
No.5			+							+				+
No.6			+							+				+
No.7			+							+				+
No.8				+						+				+
No.9			+							+				+

uterine horn (n=5 mares). The diameters of the intrauterine cysts ranged between 0.51 cm and 1.83 cm (1.27±0.10 cm). The diameter of UA (DUA) was measured on the color image of UA in all groups. Accordingly, the mean DUA in Group C (3.85±0.04 mm) was significantly lower than in Group E (3.94±0.02 mm) (P<0.05). In Group H, DUA (3.79±0.03 mm) was significantly lower than in Group E (P<0.01) while significant differences in DUAs were not observed between Group H and Group C (P>0.05) (Table 2).

Blood flow in UA was analyzed by calculation of PI and RI. The PI and RI values of all groups are presented in Table 3. Both PI and RI values in Group E were not significantly different from those in Group C (P>0.05). However, PI and RI values in Group H were significantly lower than both in Group E and Group C (P<0.001).

The waveform of the UA belong to the groups were presented at Figure 3. In Group E, the waveform was characterized by a systolic peak followed by diastolic flow, extending throughout the cardiac cycle with prominent early diastolic notch. Additionally, systolic notches and small diastolic peaks were observed in the UA waveforms of the mares in Group C. In Group H mares, the biphasic waveform was

Table 2: Mean and standard error of the UD and DUA in all groups

	Group E (n=9)	Group C (n=10)	Group H (n=9)
UD (cm)	3.47±0.29 ^a	2.97±0.19 ^a	2.29±0.18 ^b
DUA (mm)	3.94±0.02 ^a	3.85±0.04 ^b	3.79±0.03 ^b

^{a,b} Different letters in the same line indicate the significant difference as P<0.05

characterized by a short fall in peak systolic flow and was continuous with diastolic flow. The diastolic component continued with the previous systolic component and presented throughout the cardiac cycle which indicated good uterine perfusion in Group H.

When the measurements of the study were completed, 10 mares in Group C were naturally mated at the next estrus. One mare had pregnancy loss on Day 45 due to the localization of the embryonic vesicle next to the intrauterine cyst. All but 1 of the rest of 8 mares became pregnant and gave birth to a healthy foal. Also, all mares in Group H had healthy foals in the next breeding.

Discussion

Infertility problems increase in parallel with the mare's age. A decrease in fertility is seen in mares older than 15 years (20). Leidl and colleagues (21) reported that there was no evidence of cysts in mares under 10 years of age. Consistent with the previous report, the mean age of the mares in group C was greater than 10 years. Carnevale and

Table 3: Mean and standard error of the UA resistance index (RI), and pulsatility index (PI) of the mares

	Group E (n=9)	Group C (n=10)	Group H (n=9)
PI	2.34±0.09 ^a	2.22±0.10 ^a	1.65±0.06 ^b
RI	0.85±0.00 ^a	0.84±0.01 ^a	0.76±0.01 ^b

^{a,b} Different letters in the same line indicate the significant difference as P<0.001

Ginther (22) indicated that old age was associated with increased endometrial inflammation, reduced pregnancy rate, increased embryo-loss rate, increased severity of endometrial fibrosis, increased uterine vascular dysfunction, and reduced fertility in mares. Also, a progressive increase in the frequency of mares with cysts with the advancing of age has been reported (23). Although the mean ages of the mares in Group E and Group C were not older than 15 years in this study, they were significantly higher than in Group H. The similar results emphasized that uterine problems become common when the age progresses.

Uterine cysts had two additional origins: Lymphatic cysts from obstruction of lymphatic channels and pooling of lymph in multiparous mares, glandular cysts from endometrial glandular distension resulting from periglandular fibrosis (24, 6). Glandular cysts range from a few millimeters to 1 cm in diameter and are frequently seen in pregnant mares but mean diameter of lymphatic cysts varying from 2 to 48 mm (23, 24). Most uterine cysts detected by ultrasonography that protrude into the uterine lumen are lymphatic (7). In line with the previous reports, the mean diameter of the uterine cysts in our study was 1.27 ± 0.10 cm and the mares were multiparous but not pregnant, which supports the lymphatic nature of the intrauterine cysts in Group C. Tannus and Tunn (23) observed that uterine cysts were most commonly located at the junction of both uterine horns and the body of the uterus, which is also the site of vesicle fixation. Additionally, the pregnancy rates were found lower at Days 14 and 40 in mares with uterine cysts (23). Consistent with the previous report, one mare in our study had an embryonic loss at Day 45 due to the localization of the vesicle next to the intrauterine cyst. Early embryonic losses could be explained by inadequate blood flow and nutrient provision for the conceptus due to improper implantation adjoining a cyst (6). However, Leidl and colleagues (21) reported that endometrial cysts can be recognized together with embryonic vesicles and pregnancies can continue without any problems. Similar to Leidl and colleagues (21), 8 of 10 mares in Group C became pregnant and had healthy foals in this study.

Transrectal palpation, ultrasonography and uterine cytology are the non-invasive, easy, and rapid methods for the diagnosis of endometritis (25). Similarly, three diagnostic methods were used to identify endometritis in mares in this study. The researchers reported that mares with endometritis classified as +2 and +3 had a mean uterine diameter of 4.16 ± 0.28 cm (26). Although the mean uterine diameter in Group E (3.47 ± 0.29 cm) was significantly higher than in Group H (2.29 ± 0.18 cm), it was lower than previously reported. Different results may have arisen due to differences in the cytological stage of endometritis (only +2 PMN) in this study. Rahawy and colleagues (10) stated that the mean uterine fluid echogenicity in cases of endometritis is proportional to the increased endometrial wall thickness. Grading of the uterine inflammatory fluid echo pattern, performed by gray scale echogenicity as the researchers

reported (15), identified a moderate amount of slightly echogenic, light gray echodense intrauterine fluid in Group E. This group also demonstrated a marked increase in uterine wall thickness, consistent with previous reports (10,15).

The researchers reported that DUA ranges between 2 to 6 mm in cyclic mares (1). Two maiden mares had a uterine artery diameter of 0.29 and 0.25 cm which was significantly smaller compared with the mean uterine artery diameter of the multiparous mares (0.4 ± 0.07 cm) in a study by Klewitz and colleagues (27). In line with the previous reports, DUA was ranged between 3.79 to 3.94 mm in this study. Similar results were thought to be obtained because all mares were multiparous in this study. Lüttgenau and colleagues (28) observed greater uterine perfusion in mares with endometritis. In addition, it had been reported that the DUA before treatment in mares with acute endometritis was significantly larger than that at the end of treatment (29). Abdelnaby and colleagues (11) stated that uterine artery showed an increase in circumference and area in mares with endometritis. Similarly, in the presented report, the highest DUA was detected in Group E but no difference was observed in DUA between Group C and Group H. This result could be explained by the increase of uterine perfusion in endometritis as Lüttgenau and colleagues (28) indicated.

Uterine artery notches are another sign of the blood flow spectrum. An early diastolic notch is considered to be present when there is a visibly defined upturn of the flow velocity waveform at the beginning of diastole. The presence of an early diastolic notch in uterine artery flow velocity waveforms has been reported to be a good predictor of poor pregnancy outcome. Early diastolic notch depth, double notches and reversed diastolic flow may be accepted as different uterine artery waveforms related to the severity of adverse effects and outcomes (30). Although poor pregnancy outcome was not presented in this study, early diastolic notches were observed in mares with endometritis which also had the highest DUA. The presence of early diastolic notches in Group E can be explained by the researchers (31) who reported that larger uterine vessels and myometrial resistance are highly associated with UA notching. A systolic notch is considered to be present when there is a defined upturn or horizontal flow velocity waveform in the systolic wave (30). If Doppler waves have significant magnitude and are delayed concerning the incident waveform, notching occurs (32). Thaler and colleagues (33) observed that fetal growth retardation rates were significantly higher in pregnant patients with systolic or diastolic notches. Lau and colleagues (34) hypothesized that reverse diastolic flow or double notches in the uterine artery may be the result of progressive deterioration in the underlying vasculopathy. Though pregnant mares were not included in this study, systolic notches were observed in waveforms of the mares with intrauterine cysts. It is thought to occur due to the deterioration in the vasculopathy and the delay concerning the waveform (32,34).

A significant increase was reported in Doppler velocities and blood flow rate accompanied by a significant decrease in Doppler indices in mares with endometritis (11). Significantly higher PI and RI were reported in cows diagnosed with mild degree in comparison with cows diagnosed with moderate and severe degrees of clinical endometritis (35). In contrast with Abdelnaby and colleagues (11), significantly higher PI and RI were determined in Group E and Group C than in Group H in this study. Ferreira and colleagues (36) reported that mares with cysts had lower PI and RI of the mesometrial vessels than the controls. Also, they demonstrated reduced uterine vascular perfusion in mares with uterine cysts and a positive association between the size of the cystic area and disturbed uterine hemodynamics. Contradictory to the previous report (36), the rise in PI and RI in Group C than in healthy controls was thought that less disturbed uterine hemodynamics due to smaller sizes of the intrauterine cysts in the present study. Thaler and colleagues (33) reported that notches had significantly higher rates of fetal growth retardation and the presence of both in systolic or diastolic notch, the RI in the UAs on both sides of the uterus were significantly higher than in subjects without a notch. Clark and colleagues (32) indicated that high RI in UA, combined with notching, reflects an abnormal remodeling of the entire uterine vasculature. In line with the findings of the researchers (32, 33), the second reason for the rise in Doppler indices (PI and RI) in Group E and Group C could be the occurrence of the notches (diastolic or systolic) on the spectral waveforms which reflect the abnormal uterine vasculature.

Conclusions

Doppler USG is considered a useful tool to analyze the waveform and hemodynamics of the UA related to the different pathologies (cyst or endometritis). It was concluded that not only Doppler indices but also characteristics of the waveforms of the UA should be evaluated to understand the hemodynamic effect of the uterine pathologies.

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References

1. Bollwein H, Maierl J, Mayer R, Stolla R. Transrectal color Doppler sonography of the A. uterina in cyclic mares. *Theriogenology* 1998; 49(8): 1483–8. doi: 10.1016/s0093-691x(98)00094-6
2. Evans DH, McDicken WN. Doppler ultrasound: physics, instrumentation and signal processing. 2nd ed. Chichester: Wiley, 1999.
3. Bollwein H, Weber F, Kolberg B, Stolla R. Uterine and ovarian blood flow during the estrous cycle in mares. *Theriogenology* 2002; 57(8): 2129–38. doi: 10.1016/s0093-691x(02)00703-3
4. Goswamy RK, Williams G, Steptoe PC. Decreased uterine perfusion--a cause of infertility. *Hum Reprod* 1988; 3(8): 955–9. doi: 10.1093/oxford-journals.humrep.a136825
5. McKinnon AO, McCue PM. Uterine abnormalities. In: *Tercer Congreso Argentino de Reproducción Equina*. Río Cuarto: UniRío Editora, 2013: 78–95.
6. Stanton MB, Steiner JV, Pugh DG. Endometrial cysts in the mare. *J Equine Vet Sci* 2004; 24(1): 14-19. doi: 10.1016/j.jevs.2003.12.003
7. Pinto C, de Aguiar LH. Endometrial Cysts. In: Orsini JA, eds. *Comparative veterinary anatomy*. London: Academic Press, 2021; 801–7.
8. Assad NI, Pandey AK. Different approaches to diagnose uterine pathology in mares: a review. *Theriogenology Insight* 2015; 5(3): 157–82. doi: 10.5958/2277-3371.2015.00018.2
9. Teixeira-Soares CM, Viana AGA, Ribeiro IM, Silva KDP, Sancler-Silva YFR, Machado-Neves M. Comparison between gynecological examination methods and sample collection techniques for the diagnosis of endometritis in subfertile mares. *J Equine Vet Sci* 2022; 119: 104147. doi: 10.1016/j.jevs.2022.104147
10. Rahawy MA, Al-Haak AG, Hayawy EH. Detection of endometritis using uterine cytobrush and ultrasonography in mares. *Iraqi J Vet Sci* 2022; 36(1): 39–44. doi: 10.33899/ijvs.2021.128858.1608
11. Abdelnaby EA, Emam IA, Salem NY, et al. Uterine hemodynamic patterns, oxidative stress, and chromoendoscopy in mares with endometritis. *Theriogenology* 2020; 158: 112–20. doi: 10.1016/j.theriogenology.2020.09.012
12. Da Silva-Álvarez E, Gómez-Arrones V, Martín-Cano FE, et al. Endometrial area of the blood flow as a marker of endometritis in equine. *Reprod Domest Anim* 2022; 57 (Suppl. 5): 98–102. doi: 10.1111/rda.14132
13. Ferreira JC, Ignácio FS, Rocha NS, Thompson DL, Pinto CR, Meira C. Real-time characterization of the uterine blood flow in mares before and after artificial insemination. *Anim Reprod Sci* 2015; 160: 90–6. doi: 10.1016/j.anireprosci.2015.07.008
14. Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurement, and body fat percentage in mares. *Equine Vet J* 1983; 15(3): 371–2. doi: 10.1111/j.2042-3306.1983.tb01826.x
15. McKinnon AO, Voss JL, Squires EL. Diagnostic ultrasonography. In: McKinnon AO, Voss JL, eds. *Equine reproduction*. Philadelphia: Lea & Febiger, 1993: 281.
16. Samper JC, Pycocock JF, McKinnon AO. *Current Therapy in Equine Reproduction*. St. Louis: Saunders Elsevier, 2007: 492.
17. Rua MAS, Quirino CR, Riberio RB, et al. Diagnostic methods to detect uterus illnesses in mares. *Theriogenology* 2018; 114: 285–92. doi: 10.1016/j.theriogenology.2018.03.042
18. Perez-Marin CC, Vizuete G, Borge C, Galisteo J. Cytological and bacteriological sampling from filters used for embryo recovery to evaluate the uterine status of donor mares. *Acta Vet Hung* 2018; 66(3): 462–73. doi: 10.1556/004.2018.041
19. Ricketts SW, Mackintosh ME. Role of anaerobic bacteria in equine endometritis. *J Reprod Fertil Suppl* 1987; 35: 343–51.
20. Kouider ZE, Benallou B, Houari H. Ultrasonographic and cytological diagnosis of endometritis in the mare. *Global Vet* 2017; 19(4): 586–9. doi: 10.5829/idosi.gv.2017.586.589

21. Leidl W, Kaspar B, Kähn W. Endometrial cysts in the mare. 2. Clinical studies: occurrence and significance. *Tierarzt Prax* 1987; 15(3): 281–9.
22. Carnevale EM, Ginther OJ. Relationships of age to uterine function and reproductive efficiency in mares. *Theriogenology* 1992; 37(5): 1101–15. doi: 10.1016/0093-691X(92)90108-4
23. Tannus RJ, Thun R. Influence of endometrial cysts on conception rate of mares. *J Vet Med Series A* 1995; 42(4): 275–83. doi: 10.1111/j.1439-0442.1995.tb00378.x
24. Kenney RM, Ganjam VK. Selected pathological changes of the mare uterus and ovary. *J Reprod Fertil* 1975; 23: 335–9.
25. Overbeck W, Witte TS, Heuwieser W. Comparison of three diagnostic methods to identify subclinical endometritis in mares. *Theriogenology* 2011; 75(7): 1311–8. doi: 10.1016/j.theriogenology.2010.12.002
26. Uçmak ZG, Kurban I, Uçmak M. Evaluation of vascularization in the walls of preovulatory follicles in mares with endometritis. *Theriogenology* 2020; 157: 79–84. doi: 10.1016/j.theriogenology.2020.07.024
27. Klewitz J, Struebing C, Rohn K, et al. Effects of age, parity, and pregnancy abnormalities on foal birth weight and uterine blood flow in the mare. *Theriogenology* 2015; 83(4): 721–9. doi: 10.1016/j.theriogenology.2014.11.007
28. Lüttgenau J, Imboden I, Wellnitz O, et al. Intrauterine infusion of killed semen adversely affects uterine blood flow and endometrial gene expression of inflammatory cytokines in mares susceptible to persistent breeding-induced endometritis. *Theriogenology* 2021; 163: 18–30. doi: 10.1016/j.theriogenology.2020.12.029
29. Farghali HA, AbdelKader NA, Fathi M, et al. The efficiency of intrauterine infusion of platelet-rich plasma in the treatment of acute endometritis as assessed by endoscopic, Doppler, oxidative, immunohistochemical, and gene expression alterations in jennies. *Theriogenology* 2022; 181: 147–60. doi: 10.1016/j.theriogenology.2022.01.023
30. Polat I, Gedikbasi A, Kiyak H, et al. Double notches: association of uterine artery notch forms with pregnancy outcome and severity of preeclampsia. *Hypertens Pregnancy* 2015; 34(1): 90–101. doi: 10.3109/10641955.2014.982330
31. Talbert D. Uterine flow velocity waveform shape as an indicator of maternal and placental development failure mechanisms: a model-based synthesising approach. *Ultrasound Obstet Gynecol* 1995; 6(4): 261–71. doi: 10.1046/j.1469-0705.1995.06040261.x
32. Clark AR, James JL, Stevenson GN, Collins SL. Understanding abnormal uterine artery Doppler waveforms: a novel computational model to explore potential causes within the utero-placental vasculature. *Placenta* 2018; 66: 74–81. doi: 10.1016/j.placenta.2018.05.001
33. Thaler I, Weiner Z, Itskovitz J. Systolic or diastolic notch in uterine artery blood flow velocity waveforms in hypertensive pregnant patients: relationship to outcome. *Obstet Gynecol* 1992; 80(2): 277–82.
34. Lau WL, Lam HS, Leung WC. Reversed diastolic flow in the uterine artery – a new Doppler finding related to placental insufficiency? *Ultrasound Obstet Gynecol* 2007; 29(2): 232–5. doi: 10.1002/uog.3872
35. Sharma A, Singh M, Abrol A, Soni T. Doppler sonography of uterine blood flow at mid-oestrus during different degree of clinical endometritis in dairy cows. *Reprod Domest Anim* 2019; 54(9): 1274–8. doi: 10.1111/rda.13512
36. Ferreira JC, Gastal EL, Ginther OJ. Uterine blood flow and perfusion in mares with uterine cysts: effect of the size of the cystic area and age. *Reproduction* 2008; 135(4): 541–50. doi: 10.1530/REP-07-0447.

Hemodinamične spremembe maternične arterije pri kobilah z različnimi patologijami maternice

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Izvleček: Ciste, nabiranje tekočine, vnetne spremembe in tumorji so pogoste patologije maternice pri kobilah. Naš namen je bil preučiti hemodinamične spremembe maternice glede na patologijo maternice (endometritis ali intrauterine ciste). V študijo je bilo vključenih 28 kobil, razdeljenih v skupine: 9 ginekološko zdravih kobil v diestrusu (skupina H), 9 kobil z endometritisom (skupina E) in 10 kobil z intrauterino cisto (skupina C). Povprečni premer maternice (UD) v skupini H (2.29 ± 0.18) je bil bistveno manjši kot v skupini C (2.97 ± 0.19 cm) ($P < 0.05$) in skupini E (3.47 ± 0.29 cm) ($P < 0.01$). Premeri intrauterinih cist v skupini C so se gibali med 0.51 cm in 1.83 cm. Največji premer maternične arterije (UA) je bil opažen v skupini E. Hemodinamični indeksi UA (PI in RI) v skupini E se niso bistveno razlikovali od tistih v skupini C ($P > 0.05$). Vrednosti PI in RI v skupini H pa so bile bistveno nižje kot v skupini E in C ($P < 0.001$). Pri skupini E je bil v krivulji UA prisoten zgodnji diastolični vrez, medtem ko so bile v krivulji UA kobil iz skupine C opazne sistolične zarezne in majhni diastolični vrhovi. Dopplerjev USG velja za uporabno orodje za analizo valovanja in hemodinamike UA, povezanih z različnimi patologijami (cista ali endometritis). Ugotovljeno je bilo, da je za razumevanje hemodinamskega učinka patologij maternice poleg dopplerskih indeksov potrebno vrednotiti tudi značilnosti valovnih oblik UA.

Ključne besede: Dopplerjeva ultrazvočna preiskava; endometritis; intrauterina cista; zarezna

Prevalence and Potential Risk Factors Associated With Ketosis in Dairy Farms in Egypt

Key words

dairy cow;
ketosis;
risk factors;
BHBA

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Abstract: Ketosis is an energy-related metabolic disease that primarily occurs during the early lactation period in dairy cattle. Ketosis adversely affects production, animal health, and reproduction. The present study determines the prevalence and risk factors associated with ketosis in dairy cattle during early lactation in Egypt. A total of 1179 dairy cows from 37 dairy farms were screened for ketosis using a semi-quantitative cow-side milk strip test. A questionnaire was constructed to include the hypothesized risk factors. Risk factors were assessed on both the cow and herd levels using logistic regression model. The findings showed that the prevalence of ketosis was 6.35% (75/1179 cases). On the cow level, the final logistic regression model revealed a significant association between ketosis and parity ≥ 4 ($P = 0.040$, OR: 1.74, CI 95%: 1.025–2.95), cows with a dry period length ≥ 65 days ($P = 0.02$, OR: 1.88, CI 95%: 1.1–3.18), calving season ($P = 0.037$, OR: 1.73, CI 95%: 1.03–2.89), BCS > 3.5 ($P = 0.010$, OR: 2.03, CI 95%: 1.19–3.47), milk yield ≥ 25 L/day ($P = 0.033$, OR 2.04, CI 95%: 1.06–3.92), dystocia ($P < 0.001$; OR: 3.18, CI 95%: 1.75–5.77), retained placenta ($P = 0.006$, OR: 2.85, CI 95%: 1.35–6.032), and displaced abomasum ($P < 0.001$, OR: 26.28; CI 95%: 7.20–95.90). On the herd level, there was an association between ketosis and insufficient prepartum feeding of a total mixed ration ($P = 0.021$, OR: 6.29, CI 95%: 1.325–29.86), and in herds deficient-lactation supplementation propylene glycol ($P = 0.047$, OR: 4.86, CI 95%: 1.020–23.19). In conclusion, ketosis is an existing problem in dairy farms in Egypt; therefore, identification of risk factors may provide a useful approach for the prevention and control such metabolic problems.

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Introduction

Ketosis is a metabolic disease in dairy cattle defined by high concentrations of ketone bodies in body fluids. Ketosis occurs mainly near peak milk production (1), but ketosis has also been documented in late pregnancy (2). The differences in energy requirements for milk production and energy intake

due to reduced feed intake during the transition period result in a negative energy balance state (3). Fat mobilization occurs in response to increased energy demands and concomitant deficiency in blood glucose, resulting in high ketones production in the liver (4). These ketone bodies, including acetone,

acetoacetate, and beta-hydroxybutyrate are utilized peripherally as an energy source (5). A blood serum concentration of BHB ≥ 1.2 mmol/L (6) and ketosis thresholds of 100 $\mu\text{mol/L}$ milk BHB using point-of-care meter indicate a positive case for ketosis (7)

In dairy cows, ketosis may appear either in clinical or subclinical form (8). Decreased milk production and increased risk for other diseases are the main economic losses due to ketosis (9), but bodyweight loss, culling, and increased mortality are additional implications (10).

The detection of ketone bodies in serum, urine, or milk is used to diagnose ketosis (11). Several techniques have been employed to measure the concentration of ketone bodies in blood, urine, or milk in order to diagnose bovine ketosis (12). As BHB is the standard indicator of ketosis in ruminants and is more stable than other ketone bodies, colorimetric detection of blood BHB using an ultraviolet spectrometer is the gold standard method for detecting ketosis (13). Fourier Transform Infrared (FTIR) Spectrometry is used also for measurement of milk ketone bodies (14). This method is fast, inexpensive, and easy to implement on a large scale (15). Fluorometric detection of BHBA levels in milk and blood was recommended by Larsen (16) because it produces results that are closely correlated with those of the standard spectrophotometric method, are not affected by blood sample hemolysis, and do not require the preparation of whole milk samples. Gas liquid chromatography can also be used for diagnostic purposes (17). Recently, metabolomics analysis using nuclear magnetic resonance (NMR) spectroscopy mass spectrometry (MS) has been introduced for diagnosis of different blood metabolite associated with ketosis (18). Cow-side tests, a field technique has been developed to provide rapid evaluation and overcome the cost of other diagnostic tests (1). PortaBHB is a semi-quantitative cow-side test that may be used for screening cows for ketosis by measuring BHBA in milk (19).

Several studies have highlighted the association between ketosis and other diseases, including displacement of abomasum, which is strongly associated with ketosis (20). In addition, mastitis (21), and metritis (22) are more likely to occur in ketotic cows. Ketotic cows' reproductive performance was hindered since their conception rate was lower than that of non-ketotic cows (23). Several animal risk factors for ketosis of which a higher dairy cow body condition score (BCS) at calving (24, 25), and increased parity (26) have been identified.

Clinical and biochemical investigation on subclinical and clinical ketosis have been conducted in dairy cows (27, 28) and buffalo (29, 30). In addition, limited trials on treatment of ketosis in dairy cattle has been presented (31). However, to the best of the authors knowledge, studies on risk factors associated with ketosis in Holstein dairy cows are scarce. Therefore, the objective of this study was to determine the prevalence and the associated risk factors of ketosis at both cow and farm levels in Egypt.

Materials and methods

Study area

The present study was carried out at the middle and north-eastern Delta region of River Nile, Egypt. Eight main governorates namely Qalyubia, Dakahlia, Menofia, Gharbia, Ismailia, Al Sharqia, Damietta, and Kafr Elsheikh were included in this study. According to the Köppen-Geiger climate classification (32), the climate of these governorates is a hot desert climate (Figure 1).



Figure 1: Study area (7 governorates) for prevalence of ketosis in dairy cows in Egypt. The red dots are sampling locations

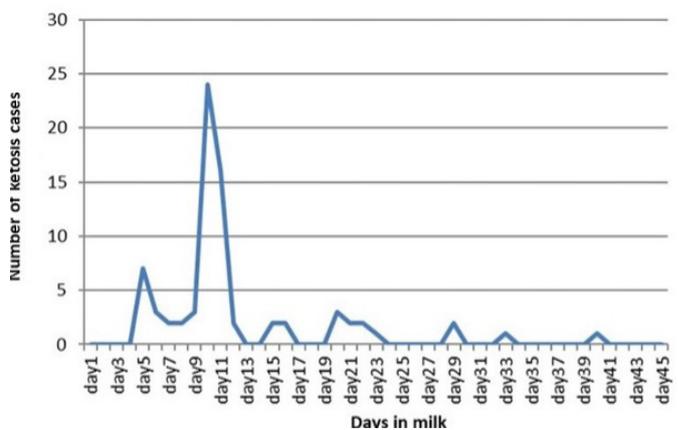


Figure 2: Frequency of ketosis cases across the first 42 days after parturition

Study Animals

The present study has been approved by Mansoura university Animal Care and use committee (MU-ACUC:VM.R.23.01.43). Firstly, farms from the basic geographic and administrative units in the Delta region were visited. Owners were asked to participate in the study and to give consent and thirty-seven owners out of 93 (39.8%) accepted to be enrolled in the study. In farms under

investigation, all parturient cows at risk of ketosis during 42 days after parturition are included in the study (1,179 cows). All the dairy farms that participated in this survey had Holstein-Friesian cows in free-stall barns. The cows were 3-9 years of age with a mean body weight of 575 ± 78 kgm. Each farm was sampled once, and all cows were screened during 42 days after parturition. A questionnaire was constructed to explore the hypothesized risk factors based on previous studies (33). The questionnaire included data concerning each cow and the farming system. Risk factors on both cow and herd level were proposed (Table 1). On the cow level, the hypothesized risk factors were parity, dry period length, BCS at parturition (34), the season of parturition, calving assistance, milk yield, and post-parturient diseases (dystocia, retained placenta, metritis, lameness, displaced abomasum, and clinical mastitis) (Table 2). However, on the herd level, the hypothesized risk factors were herd size, amount of prepartum total mixed ration, and lactation supplementation with glucose precursors.

Samples and test for BHBA in Milk

A composite milk sample was collected from each cow participating in this study AM. To quantify β-hydroxybutyric acid (BHBA) in milk, a semi-quantitative cow-side milk strip test (PortaBHBTM, PortaCheck, USA) was used. Each milk sample was tested by dipping a Porta BHBTM milk strip into it. The results were read after one minute by comparing the color of the test strip to the color chart on the test bottle. The values of the color charts indicated 0, 50, 100, 200, and 500 μmol/L based on the color density. The positive cows for ketosis were identified at a level of 200 μmol of BHBA/L.

Statistical Analysis

For statistical analysis, SPSS, commercial statistical software, was used (SPSS for Windows, version 16.0, SPSS Inc, USA). First, descriptive statistics and the distribution of risk factors for cases of ketosis were performed. At animal levels, the logistic regression analysis was conducted to test the association between ketosis and the possible risk factors. In an initial step, univariate logistic regression statistics were conducted. In such a process, the category of the cattle (ketotic or non-ketotic) was the dependent dichotomous variable, but the proposed risk factors were the independent variables. Then, independent factors with a significant association ($P < 0.1$) were included in the multivariate backward stepwise logistic regression analysis. The goodness of the fit statistical test greater than 0.05, was used by Hosmer and Lemeshow to suggest that the results of the model match the data in the multivariate analysis were at an appropriate level. The regression coefficient (β), odds ratio (OR), confidence interval (CI: 95%), standard error, and P value were the parameters in the results for each variable. Results were considered significant at $P < 0.05$ in every statistical analysis.

Results

The prevalence of ketosis in cows under investigation was 6.35% (75/1179) using the PortaBHB milk ketone test at a cut-off point of 200 μmol of BHBA /L (Figure 2). The herd prevalence of ketosis varied from 0 to 11.55%. Most ketosis cases (65.3%) were observed during the second postpartum week, whereas the remaining instances (16%) and (18.7%) were observed during the first and other weeks, respectively.

Table 2 and 3 show the descriptive statistics and the results of univariate analysis of factors associated with ketosis. On cow level, univariate statistical analysis showed a significant association between ketosis and parity ≥ 4 ($P = 0.017$, OR: 1.82, CI 95%: 1.11–2.097). Where cows with parity 1 was (35.96%), parity 2 (20.36%); parity 3 (19.25%), and parity ≥ 4 was (24.43) %. A significant association was also recorded between ketosis and cows with a dry period length ≥ 65 days ($P = 0.019$, OR: 1.8, CI 95%: 1.1–2.9). Thus, 75.07 % of cows had a dry period length < 65 days, whereas 24.93% of the cows had a dry period length ≥ 65 days. Cows with BCS > 3.5 are significantly associated with ketosis ($P = 0.001$, OR: 2.25, CI 95%: 1.37–3.68). Where, cows with BCS of ≤ 3.5 represented 78.97% and those with a high BCS > 3.5 represented 21.03%. Calving in winter season was significantly associated with high prevalence of ketosis ($P = 0.013$, OR: 1.84, CI 95%: 1.14–2.96). Thus, 28.6 % of dairy cows calved in winter, 36.64 % of cows calved in autumn, 19.92 % of cows calved in summer, and 14.84 % of cows calved in spring. Regarding the milk yield, ketosis was prevalent in cows with milk yield ≥ 25 kg ($P = 0.012$, OR: 2.24, CI 95%: 1.20–4.2). Cows with a milk yield ≥ 25 kg/day represented 71% and cows with < 25 kg/day represented 29%. Concerning postpartum events, a significant association between ketosis and the following disorders was documented: dystocia ($P < 0.001$; OR: 2.92, CI: 1.68–5.08), retained placenta ($P = 0.001$; OR: 3.1, CI 95%: 1.6–6.03), displaced abomasum ($P < 0.001$; OR: 37.5, CI 95%: 11.25–124.97). Where calving assistance was required for (11.37%) of cows. Placenta was retained in (6.45%) of cows. Moreover, the most frequently identified postpartum disorders were clinical mastitis (21.9%), lameness (7.12%), metritis (3.81%), and left displaced abomasum (1.1%). However, metritis, mastitis, and lameness showed non-significant associations.

Multivariate logistic regression model revealed a significant association between ketosis and parity ≥ 4 ($P = 0.040$; OR: 1.74; CI 95%: 1.025–2.95), calving in winter season ($P = 0.037$; OR: 1.73, CI 95%: 1.033–2.89), BCS > 3.5 ($P = 0.010$, OR: 2.03; CI 95%: 1.19–3.47), milk yield ≥ 25 L/day ($P = 0.033$, OR: 2.04, CI 95%: 1.06–3.92), dystocia ($P < 0.001$; OR: 3.18, CI 95%: 1.75–5.77), retained placenta ($P = 0.006$; OR: 2.85, CI 95%: 1.35–6.032), and displaced abomasum ($P < 0.000$, OR: 26.28, CI 95%: 7.20–95.90) (Table 4).

On herd level risk factors, risks of ketosis increased in herds fed a prepartum total mixed ration < 12 kg/day ($P = 0.021$;

Table 1: Description and levels of hypothesized risk factors associated with ketosis in dairy cows

	Risk factors	Level and description
1	Calving Season	According to calving season, categories were winter calving =1, spring calving =2, summer calving =3, and autumn calving =4
2	Cow Parity	cows with parity 1 =1, parity 2 =2, parity 3=3, parity≥4=4
3	Body Condition Score	According to the body condition at calving, dairy cows were categorized into cows with low and moderate BCS ≤3.5 =1, and cows with high BCS > 3.5 =2.
4	Milk Production	According to average daily milk production, cows were categorized into: cows with low milk production <25Kg =1, and cows with high milk production ≥ 25kg =2
5	Dry Period Length	According to length of dry period, cows were categorized into: cows with short dry period < 65 days =1, and cows with long dry period ≥65 days =2
6	Dystocia	Cows had dystocia=1, and cows with normal birth =2
7	Retained Placenta	cows with retained placenta=1, and cows without placental retention=2
8	Metritis	Cows were categorized into cows with metritis=1, and cows without metritis=2
9	Left Displaced Abomasum (LDA):	Cows with LDA=1, and cows without LDA=2
10	Mastitis	Cows with clinical mastitis =1, and cows without clinical mastitis=2
11	Lameness	Cows with lameness =1, and cows without lameness=2
1	Herd Size	Small dairy herds with lactating cows <100 =1, and large dairy herds with lactating cows ≥ 100
2	Amount of Prepartum Total Mixed Ration	According to the amount of prepartum, TMR, dairy herds were categorized into: herds receive a sufficient TMR (≥12kg)=1, and herds did not receive adequate TMR (<12)=2
3	Lactation supplementation with propylene glycol	Herds were supplemented with propylene glycol=1, and herds were not supplemented with propylene glycol=2

OR: 6.29; CI 1.325-29.858), and in the herds with a lack of lactation supplementation with glucose precursors (P= 0.047; OR: 4.86; CI 1.020-23.19) (Table 5-6).

Discussion

Dairy cows are at risk of a period of negative energy balance (NEB) during the transition from late gestation to early lactation, caused by insufficient nutritional intake to fulfill the animal's maintenance and milk production requirements (35). To meet energy demand, dairy cows' body fat is being used for energy and protein for gluconeogenesis, leading to an increase in non-esterified fatty acids (NEFA) and BHBA (36). It has been concluded that the time in which early-lactation dairy cows are at risk for hyperketonemia lasts at least until 42 day in milk (37). The design of the present study fulfills the objectives to determine the prevalence and risk factors of ketosis in dairy cows. It has been stated that the cross-sectional studies are the best choice when the aim of the research is to estimate the prevalence of a characteristic in a specific population, they may also be useful if the aim is to evaluate factors associated with a disease or condition (38). In the present study, the prevalence of ketosis was 6.35% with peak prevalence during the first

two weeks of lactation. A higher prevalence was reported in North America (18.8%) (39), Europe 29.3-39% (22, 40), Asia 9.6%-17.9% (41, 42), and Africa 16.9 (22). This finding may be attributed to differences between geographic regions which affect dairy cows' husbandry and farm management. It has been stated that country differences, cultural backgrounds, climate, dairy farming structures, increased milk production and herd size seem to have been considerably associated with dairy farm development (22, 43). Moreover, feeding strategies, genetic variation, and other diseases may also affect the prevalence (44). McArt et al. (45) stated that using the incidence of hyperketonemia has benefits over using its prevalence for the recognition of optimal testing and treatment policies at the herd level.

Many techniques have been used to quantify or semi-quantify ketone bodies in blood, milk, and urine have been used to diagnose bovine ketosis (12), of which the detection of BHBA in serum and plasma is the gold standard test for ketosis since BHBA is the primary ketone body in ruminants and is more stable than other ketones, although this test is costly and time-consuming (46). A variety of cow-side tests are used to screen herds for ketosis in body fluids (blood, milk, and urine). In the present study, PortaBHB milk ketone test, is a simple, non-invasive, and quick field test

Table 2: Categorization of cows as ketotic or non-ketotic with relation to different risk factors

Variable	Ketotic cows		Non- ketotic cows	
	Number (75)	%	Number (1104)	%
Seasons				
Winter:	31	41.3	306	27.72
Autumn:	24	32	408	36.95
Summer:	12	16	223	20.20
Spring:	8	10.7	167	15.13
Parity:				
1:	25	33.33	399	36.1
2:	12	16.00	228	20.7
3:	11	14.67	216	19.6
≥4:	27	36.00	261	23.6
Body condition score:				
≤ 3.5	48	64.00	883	80.00
>3.5	27	36.00	221	20.00
Milk production:				
<25kg	12	16.00	330	29.00
≥25 kg	63	84.00	774	71.00
Long dry period:				
Short < 65	48	64.00	841	76.20
Long ≥ 65	27	36.00	263	23.80
Dystocia:				
Present	19	25.30	115	10.40
Absent	56	74.70	989	89.60
Retained placenta:				
Present	12	16	64	5.80
Absent	63	84	1040	94.20
Metritis:				
Present	02	2.70	43	3.90
Absent	73	97.30	1061	99.80
Lef displaced abomasum:				
Present	09	12	04	.40
Absent	66	88	1100	99.60
Mastitis :				
Present	18	24	240	21.7
Absent	57	76	864	78.3
Lameness:				
Present	8	10.7	76	6.9
Absent	67	89.3	1028	93.1

Table 3: Univariate logistic regression model for animal level risk factors associated with ketosis

Variable	β	S.E.	OR	95.0% C.I. Lower-Upper	P
Parity	.59	.251	1.82	1.11-2.097	.017
Dry Period Length	.58	.251	1.8	1.1-2.9	.019
BCS: >3.5	.81	.252	2.25	1.37-3.68	.001
Calving season	.61	.244	1.84	1.14-2.96	.013
Milk Yield \geq 25kg/day	.81	.32	2.24	1.2-4.2	.012
Dystocia	1.07	.28	2.92	1.68 - 5.08	0.001
Retained placenta	1.10	.34	3.1	1.6-6.03	.001
Metritis	-.392	.733	.68	0.16-2.9	.59
Abomasal displacement (LDA)	3.62	.614	37.5	11.25-124.97	0.000
Mastitis	.128	.28	1.14	0.66-1.97	0.647
Lameness	.479	.392	1.62	0.75-3.5	0.222

β : Regression coefficient; SE: Standard error; OR: Odds ratio; 95% CI: Confidence interval; P: P value

Table 4: Multivariate logistic regression model for animal level risk factors associated with ketosis

Variable	β	S.E.	OR	95.0% C.I. Lower-Upper	P
Parity \geq 4	.553	.269	1.74	1.025-2.95	.040
Calving in the winter season	.546	.262	1.73	1.033-2.89	.037
Dry period length >65 days	.629	.27	1.88	1.1-3.18	.020
BCS>3.5	.707	.274	2.03	1.19-3.47	.010
Milk Yield \geq 25L/day	.712	.334	2.04	1.06-3.92	.033
Dystocia	1.16	.304	3.18	1.75-5.77	.000
Retained Placenta	1.05	.382	2.85	1.35-6.03	.006
LDA	3.27	.661	26.28	7.20-95.90	.000
Constant	-4.241	.866	-	-	.000

β : Regression coefficient; SE: Standard error; OR: Odds ratio; 95% CI: Confidence interval; P: P value

with good specificity for the detection of ketones in milk is used, but it has a low sensitivity (50.7%) at a cut-off point of 200 μ mol/L (47). A Study on accuracy of PortaBHB milk strip cow-side test for diagnosis of hyperketonemia suggested that this cow-side test has good accuracy (28). The prevalence of subclinical ketosis has been found to be relatively high using different diagnostic techniques to assess BHB concentrations. However, this may be due to the accuracy of various diagnostic thresholds for SCK detection

(48). Commercial automatic devices have become widely available for practical measurements of blood BHB concentrations and SCK diagnosis in dairy cows to predict outcomes with reasonable accuracy (49). Likewise, according to a recent meta-analytical study, the handheld glucometer has the greatest sensitivity and specificity (94.8 and 97.5%, respectively), and milk BHB TSs have low Se (39.7%) (48). Some BHB-detecting devices also had low sensitivity and specificity, which could be responsible for false negative or

Table 5: Classification of cattle herds as ketotic or non-ketotic in respect to different exposure factors

Risk Factor	Category	+ve (n =16)	-ve (n =21)	OR	95.0% C.I. Lower-Upper	*P
Herd size (lactating cows)	Small<100	6	13	2.7	0.70-10.3	0.146
	Large≥100	10	8			
Amount of prepartum total mixed ration /day (TMR)	Sufficient (≥ 12 kg TMR)	5	15	5.5	1.33-22.73	0.019
	Insufficient (< 12 kg TMR)	11	6			
Supplementation of propylene glycol (200 gm/cow daily)	Yes	6	15	4.1	1.042-16.66	0.044
	No	10	6			

*P-values are for comparisons of data for each factor evaluated

Table 6: Final logistic regression model for positive risk factors associated with ketosis in cattle on herd level

Variable	β	S.E.	OR	95.0% C.I. Lower-Upper	P
Insufficient prepartum TMR	1.84	0.795	6.291	1.32-29.85	.021
Lactation supplementation with glucose precursors	1.582	0.797	4.864	1.02 -23.19	.047
Constant	-1.88	0.720	0.153	-	.009

β: Regression coefficient; SE: Standard error; OR: Odds ratio; 95% CI: Confidence interval; P: P value

false positive rates in subclinical ketosis diagnosis (50, 51) FTIR spectroscopy for routine milk BHB analysis measuring in early lactation has been used (52). Though, FTIR threshold differences can affect various sensitivity and specificity and influencing the diagnostic accuracy (53).

On the cow level, the final logistic regression model revealed a significant association between ketosis and cows of parity ≥4. This finding is consistent with that reported by Vanholder et al. (26), who concluded that cows of parity ≥ 3 were at a higher risk of ketosis (2.8 times) than heifers. The increased likelihood of ketosis with increased parity has been attributed to a significant depletion of energy reserves in multiparous cows compared with primiparous ones (21).

Regarding the dry period, the significant association between ketosis and cows with a long dry period in the present study may be attributed a greater BCS during parturition and higher lipid mobilization at the start of lactation in cows at risk (54). It has been concluded that A 4-wk DP improved metabolic status, as reflected by lower plasma NEFA concentration in early lactation compared with an 8-wk dry period (55). However, the increased risk of ketosis in high milk-yielding cows described in this study may be explained by greater energy requirements and inadequate dry matter intake (DMI) to meet metabolic lactation demands, resulting in NEB and hyperketonemia (56). In addition, Roche (57) stated that inadequate nutritional management of the cow in the dry period and after calving has a significant negative

impact on subsequent conception rate, services per conception and interval from calving to conception.

In respect to the BCS, there was a significant association between ketosis and BCS >3.5. Similar finding has been presented by Garzón-Audor et al. (58), who reported that the relative risk of ketosis in cows with BCS more than 3.5 was 3.3 times greater than in cows with BCS less than 3. The association between increased BCS and ketosis has been attributed to the reduction in DMI that occurs around calving in obese cows and increased insulin resistance (59, 60). Several studies highlighted the association between BCS and milk yield, metabolic disorders, and reproductive performance in dairy cows (61-63).

The significant association between ketosis and calving in the winter season reported in this study is consistent with the results of Asrat et al. (64), who observed an increase in the prevalence of ketosis in winter in Ethiopia. In addition, it has been reported that cows with parturition during the hottest seasons had a lower risk of ketosis (8). Whereas, Ha, Seungmin et al 2023 (65) found that cows who calved in the summer had a greater risk of ketosis, which might be related to the increased warmth and humidity in Korea. The seasonality of ketosis may be related to several factors, including ambient temperature, forage quality, and pasture availability (66)

Regarding calving related-events, the identification of dystocia as a risk factor for ketosis in this study is in contrast to Berge and Vertenten (21), who did not find an increased risk of ketosis after dystocia and is similar to Duffield et al. (67), who linked ketosis to loss of appetite caused by dystocia-related traumatic pain. The association between ketosis and retained placenta identified in this study contradicts Vanholder et al. (26), who did not record an association between ketosis and retained placenta, and agrees with Garzón-Audor and Oliver-Espinosa (58) who attributed that to the reduction in DMI. This is also consistent with (68), who reported that daily feed intake in the cows with the RP decreased on average by 0.8 kg/day on average.

Several postpartum conditions have been identified as potential risk factors for ketosis (8, 69). The significant association between displaced abomasum and ketosis reported in this study is consistent with the findings of Suthar et al. (70). Cows with a displaced abomasum developed ketosis as a result of a negative energy balance due to their decreased appetite (71). In this study, metritis, clinical mastitis, and lameness were not identified as risk factors for ketosis. These findings contrast with those reported by Vince et al. (72), Steeneveld et al. (56), and Berge and Vertenten (21) who reported an association between ketosis and endometritis, mastitis, and lameness, respectively, and agree with (58) where they reported no association between ketosis and clinical mastitis, and (73) who concluded that there was no clear association between lameness and the development of severe NEB, even though lameness has previously been shown to affect feeding behavior with diminished feeding time and lower DMI (74).

On the herd level, fed on an insufficient prepartum total mixed ration and insufficient supplementation with glucose precursors were the main risk factors. This association could be explained by enhanced lipolysis of deposited fat and the release of NEFA into the circulation (75). An increased NEFA concentration in the blood results in the accumulation of triglycerides in the liver and a considerable rise in ketonic compound synthesis (76).

In the present study, supplementation with propylene glycol has been found to reduce the prevalence of ketosis. It has been postulated that glucose precursors promote glucose metabolism and reduce lipolysis during the periparturient period (77). McArt et al. (78) find positive effects of bolus administrations of propylene glycol on resolution and further outcomes of hyperketonemia. Similarly, the incidence of subclinical ketosis was reduced with improvement of reproductive performance has been recorded after long period supplementation with propylene glycol and glycerol (27). In a study carried out by Lomander et al. (79), it has been found that feeding of dairy cows on concentrates supplemented with 450 gm of glycerol did not affect on BHBA concentration in early-lactation. On the contrary, Hoedemaker et al. (80) stated that despite improvement of metabolic indicators after periparturient supplementation with

concentrate enriched at 10% propylene glycol to cows, milk production, animal health, and fertility were not influenced.

The limitations of the present study should be recognized. First, this study included farms related to governorates of Delta region of River Nile (Lower Egypt). Because most of well-constructed dairy farms are found in such localities, the study was performed here. However, further studies need to be done to include other localities of Upper Egypt. Second, the present study was conducted on large scale farms, which doesn't provide concrete conclusion about smallholder farms in Egypt. But, because it is difficult to count and to have owners' consent in small-holder farms, we conducted our study in large dairy farms. Moreover, PortaBHB milk strip cow-side test may be more suitable for large scale farms than smallholders. Third, the use of PortaBHB, a semi-quantitative cow side milk strip test only, without use of BHBA test kits, standard test for diagnosis of ketosis. However, PortaBHB milk ketone test, has been reported to be a simple, non-invasive, and quick field test with good specificity for the detection of ketosis in milk (47). Interestingly, a Study on accuracy of PortaBHB milk strip cow-side test for diagnosis of hyperketonemia suggested that this cow-side test has good accuracy, and that there is no benefit of collecting a composite milk sample compared to sampling a single quarter (28). Consequently, the on farm application of this milk strip may be helpful to distinguish cows with hyperketonemia, or to measure herd-level incidence of hyperketonemia (81). Fourth, there was a variation in the management strategies and feeding programs for transition cows organized and performed by the farms' personnel, veterinarians, and nutrition consultants. Therefore, concrete conclusions about the prevalence of hyperketonemia may be influenced. Fifth, we mentioned only the frequency of cows with hyperketonemia during the first 42 post calving only. Definite association between DIM and incidence of hyperketonemia has been presented (37, 82). But in our study, we obtained snapshot about the hyperketonemia lactating cows.

Conclusions

The results of the present investigation indicate considerable variation in the prevalence of ketosis in dairy herds in Egypt. Factors associated with ketosis could help to identify cows at risk and to improve management strategies to limit their effects.

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References

1. Lei MAC, Simões J. Invited review: ketosis diagnosis and monitoring in high-producing dairy cows. *Dairy* 2021; 2(2): 303–25. doi:10.3390/dairy2020025
2. Viña C, Fouz R, Camino F, Sanjuán M, Yus E, Diéguez F. Study on some risk factors and effects of bovine ketosis on dairy cows from the Galicia region (Spain). *J Anim Physiol Anim Nutr (Berl)* 2017; 101(5): 835–45. doi: 10.1111/jpn.12471
3. Wankhade PR, Manimaran A, Kumaresan A, et al. Metabolic and immunological changes in transition dairy cows: a review. *Vet World* 2017; 10(11): 1367–77. doi: 10.14202/vetworld.2017.1367-1377
4. Sun F, Cao Y, Cai C, Li S, Yu C, Yao J. Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. *PLoS One* 2016; 11(8): e0160659. doi: 10.1371/journal.pone.0160659
5. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes Metab Res Rev* 1999; 15(6): 412–26.
6. Soares GSL, Ribeiro ACS, Paula J, et al. Cardiac biomarkers and blood metabolites in cows with clinical ketosis. *Semin: Ciências Agrárias* 2019, 40(6): 3525–40.
7. Serrenho RC, Williamson M, Berke O, et al. An investigation of blood, milk, and urine test patterns for the diagnosis of ketosis in dairy cows in early lactation. *J Dairy Sci* 2022; 105(9): 7719–27. doi: 10.3168/jds.2021-21590
8. Mellado M, Dávila A, Gaytán L, Macías-Cruz U, Avendaño-Reyes L, García E. Risk factors for clinical ketosis and association with milk production and reproduction variables in dairy cows in a hot environment. *Trop Anim Health Prod* 2018; 50(7): 1611–16. doi: 10.1007/s11250-018-1602-y
9. Mostert P, Bokkers E, Van Middelaar C, Hogeveen H, De Boer I. Estimating the economic impact of subclinical ketosis in dairy cattle using a dynamic stochastic simulation model. *Animal* 2018, 12(1): 145–54. doi: 10.1017/S1751731117001306
10. Marutsova V, Binev R, Marutsov P. Comparative clinical and haematological investigations in lactating cows with subclinical and clinical ketosis. *Mac Vet Rev* 2015; 38(2): 159–66. 10.14432/j.macvetrev.2015.04.042
11. Puppel K, Gołębiewski M, Solarczyk P, et al. The relationship between plasma β -hydroxybutyric acid and conjugated linoleic acid in milk as a biomarker for early diagnosis of ketosis in postpartum Polish Holstein-Friesian cows. *BMC Vet Res* 2019; 15(1): 367. doi: 10.1186/s12917-019-2131-2
12. Zhang G, Ametaj BN. Ketosis an old story under a new approach. *Dairy* 2020, 1(1): 42–60. doi:10.3390/dairy1010005
13. Đoković R, Ilić Z, Kurćubić V, et al. Diagnosis of subclinical ketosis in dairy cows. *Biotechnol Anim Husb* 2019; 35(2): 111–25.
14. Pralle R, Weigel K, White H. Predicting blood β -hydroxybutyrate using milk Fourier transform infrared spectrum, milk composition, and producer-reported variables with multiple linear regression, partial least squares regression, and artificial neural network. *J Dairy Sci* 2018, 101(5): 4378–87. Doi: 10.3168/jds.2017-14076
15. Hansen PW. Screening of dairy cows for ketosis by use of infrared spectroscopy and multivariate calibration. *J Dairy Sci* 1999; 82(9): 2005–10. doi: 10.3168/jds.S0022-0302(99)75437-8
16. Larsen T, Nielsen N. Fluorometric determination of β -hydroxybutyrate in milk and blood plasma. *J Dairy Sci* 2005; 88(6): 2004–9. doi: 10.3168/jds.S0022-0302(05)72876-9
17. Enjalbert F, Nicot M, Bayourthe C, Moncoulon. Ketone bodies in milk and blood of dairy cows: Relationship between concentrations and utilization for detection of subclinical ketosis. *Journal of dairy science* 2001, 84(3): 583–9. doi: 10.3168/jds.S0022-0302(01)74511-0
18. Tran H, McConville M, Loukopoulos P. Metabolomics in the study of spontaneous animal diseases. *J Vet Diagn Invest* 2020; 32(5): 635–47. Doi: 10.1177/1040638720948505
19. Ghanem M, Mahmoud M, Abd El-Raof Y, El-Attar H. Alterations in biochemical parameters and hepatic ultrasonography with reference to oxidant injury in ketotic dairy cows. *Banha Vet Med J* 2016; 31(2): 231–40.
20. McArt J, Nydam D, Oetzel G. Epidemiology of subclinical ketosis in early lactation dairy cattle. *J Dairy Sci* 2012; 95(9): 5056–66. doi: 10.3168/jds.2012-5443
21. Berge AC, Vertenten G. A field study to determine the prevalence, dairy herd management systems, and fresh cow clinical conditions associated with ketosis in western European dairy herds. *J Dairy Sci* 2014; 97(4): 2145–54. doi: 10.3168/jds.2013-7163
22. Brunner N, Groeger S, Canelas Raposo J, Bruckmaier RM, Gross JJ. Prevalence of subclinical ketosis and production diseases in dairy cows in Central and South America, Africa, Asia, Australia, New Zealand, and Eastern Europe. *Transl Anim Sci* 2019, 3(1): 84–92. doi: 10.1093/tas/txy102
23. Rutherford AJ, Oikonomou G, Smith RF. The effect of subclinical ketosis on activity at estrus and reproductive performance in dairy cattle. *J Dairy Sci* 2016; 99(6): 4808–15. doi: 10.3168/jds.2015-10154
24. Gillund P, Reksen O, Gröhn Y, Karlberg K. Body condition related to ketosis and reproductive performance in Norwegian dairy cows. *J Dairy Sci* 2001; 84(6): 1390–96. doi: 10.3168/jds.S0022-0302(01)70170-1
25. McArt J, Nydam D, Overton M. Hyperketonemia in early lactation dairy cattle: a deterministic estimate of component and total cost per case. *J Dairy Sci* 2015; 98(3): 2043–54. Doi: 10.3168/jds.2014-8740
26. Vanholder T, Papen J, Bemers R, Vertenten G, Berge A. Risk factors for subclinical and clinical ketosis and association with production parameters in dairy cows in the Netherlands. *J Dairy Sci* 2015; 98(2): 880–8. doi: 10.3168/jds.2014-8362
27. El-Kasrawy NI, Swelum AA, Abdel-Latif MA, et al. Efficacy of different drenching regimens of gluconeogenic precursors during transition period on body condition score, production, reproductive performance, subclinical ketosis and economics of dairy cows. *Animals (Basel)* 2020; 10(6): 937. doi: 10.3390/ani10060937
28. Ghanem M, Mahmoud M, Abd El-Raof Y, El-Attar H. Efficacy of different cow side tests for diagnosis of ketosis in lactating cows. *Benha Vet Med J* 2016; 31(2): 225–30.

29. Ghanem MM, El-Deeb WM. Lecithin cholesterol acyltransferase (LCAT) activity as a predictor for ketosis and parturient haemoglobinuria in Egyptian water buffaloes. *Res Vet Sci* 2010; 88(1): 20–5. doi: 10.1016/j.rvsc.2009.07.004
30. Youssef MA, El-Khodery SA, El-deeb WM, Abou El-Amaiem WE. Ketosis in buffalo (*Bubalus bubalis*): clinical findings and the associated oxidative stress level. *Trop Anim Health Prod* 2010; 42(8): 1771–7. Doi: 10.1007/s11250-010-9636-9
31. Elmeligy E, Oikawa S, Mousa SA, et al. Role of insulin, insulin sensitivity, and abomasal functions monitors in evaluation of the therapeutic regimen in ketotic dairy cattle using combination therapy with referring to milk yield rates. *Open Vet J* 2021; 11(2): 228–37. doi: 10.5455/OVJ.2021.v11.i2.732
32. Lasantha V, Oki T, Tokuda D. Data-driven versus Köppen–geiger systems of climate classification. *Hindawi Adv Meteorol* 2022; 2022: 3581299. doi:10.1155/2022/3581299
33. Garro CJ, Mian L, Cobos Roldán M. Subclinical ketosis in dairy cows: prevalence and risk factors in grazing production system. *J Anim Physiol Anim Nutr (Berl)* 2014; 98(5): 838–44. doi: 10.1111/jpn.12141
34. Edmonson A, Lean I, Weaver L, Farver T, Webster G. A body condition scoring chart for Holstein dairy cows. *J Dairy Sci* 1989; 72(1): 68–78. doi: 10.3168/jds.S0022-0302(89)79081-0
35. Seymour D, Cánovas A, Baes CF, et al. Invited review: determination of large-scale individual dry matter intake phenotypes in dairy cattle. *J Dairy Sci* 2019; 102(9): 7655–63. doi: 10.3168/jds.2019-16454
36. Moore S, DeVries T. Effect of diet-induced negative energy balance on the feeding behavior of dairy cows. *J Dairy Sci* 2020; 103(8): 7288–301. doi: 10.3168/jds.2019-17705
37. Mahrt A, Burfeind O, Heuwieser W. Evaluation of hyperketonemia risk period and screening protocols for early-lactation dairy cows. *J Dairy Sci* 2015; 98(5): 3110–9. doi: 10.3168/jds.2014-8910
38. Hulley SB, eds. *Designing clinical research*. 3th ed. Philadelphia: Lippincott Williams & Wilkins; 2007.
39. Dubuc J, Denis-Robichaud J. A dairy herd-level study of postpartum diseases and their association with reproductive performance and culling. *J Dairy Sci* 2017; 100(4): 3068–78. doi: 10.3168/jds.2016-12144
40. Hejel P, Zechner G, Csorba C, Könyves L. Survey of ketolactia, determining the main predisposing management factors and consequences in Hungarian dairy herds by using a cow-side milk test. *Vet Rec Open* 2018; 5(1): e000253. doi: 10.1136/vetreco-2017-000253
41. Biswal S, Nayak DC, Sardar KK. Prevalence of ketosis in dairy cows in milk shed areas of Odisha state, India. *Vet World* 2016; 9(11): 1242–47. doi: 10.14202/vetworld.2016.1242-1247
42. Şentürk S, CİHAN H, MECİTOĞLU Z, et al. Prevalence of ketosis in dairy herds in Marmara, Aegean and Mediterranean regions of Turkey. *Ankara Üniv Vet Fak Derg* 2016; 63(3): 283–8.
43. Wenz JR, Solis TE, Moore DA. An Estimation of the cow-and herd-level prevalence of post-partum subclinical ketosis in large Washington state dairy herds and evaluation of mean β -hydroxybutyrate concentration for herd-level assessment. *Bov Pract* 2016; 50(2): 202–9. 10.21423/bovine-vol50no2p202-209
44. Van der Drift S, Van Hulzen K, Teweldemedhn T, Jorritsma R, Nielen M, Heuven H. Genetic and nongenetic variation in plasma and milk β -hydroxybutyrate and milk acetone concentrations of early-lactation dairy cows. *J Dairy Sci* 2012; 95(11): 6781–7.
45. McArt JA, Nydam DV, Oetzel GR, Overton TR, Ospina PA. Elevated non-esterified fatty acids and β -hydroxybutyrate and their association with transition dairy cow performance. *Vet J* 2013; 198(3): 560–70.
46. Madreseh-Ghahfarokhi S, Dehghani-Samani A, Dehghani-Samani A. Ketosis (acetonaemia) in dairy cattle farms: practical guide based on importance, diagnosis, prevention and treatments. *J Dairy Vet Anim Res* 2018; 7(6): 299–302. doi: 10.15406/jdvar.2018.07.00230
47. Denis-Robichaud J, DesCôteaux L, Dubuc J. Accuracy of a new milk strip cow-side test for diagnosis of hyperketonemia. *Bov Pract* 2011; 45(2): 97–100.
48. Tatone EH, Gordon JL, Hubbs J, LeBlanc SJ, DeVries TJ, Duffield TF. A systematic review and meta-analysis of the diagnostic accuracy of point-of-care tests for the detection of hyperketonemia in dairy cows. *Prev Vet Med* 2016; 130: 18–32. doi: 10.1016/j.prevetmed.2016.06.002
49. Macmillan K, Helguera IL, Behrouzi A, Gobikrushanth M, Hoff B, Colazo M. Accuracy of a cow-side test for the diagnosis of hyperketonemia and hypoglycemia in lactating dairy cows. *Res Vet Sci* 2017; 115: 327–31.
50. Carrier J, Stewart S, Godden S, Fetrow J, Rapnicki P. Evaluation and use of three cow-side tests for detection of subclinical ketosis in early postpartum cows. *J Dairy Sci* 2004; 87(11): 3725–35.
51. Faruk MS, Park B, Ha S, Lee S-S, Mamuad LL, Cho Y. Comparative study on different field tests of ketosis using blood, milk, and urine in dairy cattle. *Vet Med* 2020; 65(5): 199–206.
52. Santschi D, Lacroix R, Durocher J, Duplessis M, Moore R, Lefebvre D. Prevalence of elevated milk β -hydroxybutyrate concentrations in Holstein cows measured by Fourier-transform infrared analysis in dairy herd improvement milk samples and association with milk yield and components. *J Dairy Sci* 2016; 99(11): 9263–70. doi: 10.3168/jds.2016-11128
53. Denis-Robichaud J, Dubuc J, Lefebvre D, DesCôteaux L. Accuracy of milk ketone bodies from flow-injection analysis for the diagnosis of hyperketonemia in dairy cows. *Journal of dairy science* 2014; 97(6): 3364–70. doi: 10.3168/jds.2013-6744
54. Van Hoeij R, Dijkstra J, Bruckmaier R, Gross JJ, Lam T, Rummelink G, Kemp B, Van Knegsel A. The effect of dry period length and postpartum level of concentrate on milk production, energy balance, and plasma metabolites of dairy cows across the dry period and in early lactation. *J Dairy Sci* 2017; 100(7): 5863–79. Doi: 10.3168/jds.2016-11703
55. O'Hara EA, Båge R, Emanuelson U, Holtenius K. Effects of dry period length on metabolic status, fertility, udder health, and colostrum production in 2 cow breeds. *J Dairy Sci* 2019; 102(1): 595–606. doi: 10.3168/jds.2018-14873
56. Steeneveld W, Amuta P, van Soest FJ, Jorritsma R, Hogeveen H. Estimating the combined costs of clinical and subclinical ketosis in dairy cows. *PLoS One* 2020; 15(4): e0230448. doi: 10.1371/journal.pone.0230448
57. Roche JF. The effect of nutritional management of the dairy cow on reproductive efficiency. *Anim Reprod Sci* 2006; 96(3/4): 282–96. doi: 10.1016/j.anireprosci.2006.08.007
58. Garzón-Audor A, Oliver-Espinosa O. Incidence and risk factors for ketosis in grazing dairy cattle in the Cundi-Boyacencian Andean plateau, Colombia. *Trop Anim Health Prod* 2019; 51(6): 1481–7. doi: 10.1007/s11250-019-01835-z
59. Holtenius P, Holtenius K. A model to estimate insulin sensitivity in dairy cows. *Acta Vet Scand* 2007; 49(1): 29. doi: 10.1186/1751-0147-49-29
60. De Koster JD, Opsomer G. Insulin resistance in dairy cows. *Vet Clin North Am Food Anim Pract* 2013; 29(2): 299–322. doi: 10.1016/j.cvfa.2013.04.002

61. Domecq J, Skidmore A, Lloyd J, Kaneene J. Relationship between body condition scores and milk yield in a large dairy herd of high yielding Holstein cows. *J Dairy Sci* 1997; 80(1): 101–12.
62. Dechow C, Rogers G, Clay J. Heritability and correlations among body condition score loss, body condition score, production and reproductive performance. *J Dairy Sci* 2002; 85(11): 3062–70.
63. Wathes D, Cheng Z, Bourne N, Taylor V, Coffey M, Brotherstone S. Differences between primiparous and multiparous dairy cows in the inter-relationships between metabolic traits, milk yield and body condition score in the periparturient period. *Domest Anim Endocrinol* 2007; 33(2): 203–25. doi: 10.1016/j.domaniend.2006.05.004
64. Asrat M, Tadesse GH, Gounder RV, Nagappan R: Prevalence and treatment of ketosis in dairy cows in and around Addis Ababa, Ethiopia. *British Journal of Dairy Sciences* 2013, 3(3):26–30.
65. Ha S, Kang S, Jeong M, Han M, Lee J, Chung H, Park J. Characteristics of Holstein cows predisposed to ketosis during the post-partum transition period. *Vet Med Sci* 2023; 9(1): 307–14. doi: 10.1002/vms3.1006
66. Duffield T: Subclinical ketosis in lactating dairy cattle. *Vet Clin North Am Food Anim Pract* 2000; 16(2): 231–53.
67. Duffield T, Lissemore K, McBride B, Leslie K. Impact of hyperketonemia in early lactation dairy cows on health and production. *J Dairy Sci* 2009; 92(2): 571–80.
68. Bareille N, Beaudeau F, Billon S, Robert A, Faverdin P. Effects of health disorders on feed intake and milk production in dairy cows. *Livest Prod Sci* 2003; 83(1): 53–62.
69. Jeong J-K, Choi I-S, Moon S-H, et al. Risk factors for ketosis in dairy cows and associations with some blood metabolite concentrations. *J Vet Clin* 2017; 34(4): 255–60.
70. Suthar V, Canelas-Raposo J, Deniz A, Heuwieser W. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. *J Dairy Sci* 2013; 96(5): 2925–38.
71. Constable PD, eds. *Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats*. 11th ed. Amsterdam: Elsevier, 2016.
72. Vince S, Đuričić D, Valpotić H, et al. Risk factors and prevalence of subclinical ketosis in dairy cows in Croatia. *Vet Arh* 2017; 87(1): 13–24.
73. Mudroň P: Role of ketosis in lame dairy cows. *Bulg J Vet Med* 2019; 22(1): 70–3.
74. González L, Tolkamp B, Coffey M, Ferret A, Kyriazakis I. Changes in feeding behavior as possible indicators for the automatic monitoring of health disorders in dairy cows. *J Dairy Sci* 2008; 91(3): 1017–28.
75. Grummer RR. Nutritional and management strategies for the prevention of fatty liver in dairy cattle. *Vet J* 2008; 176(1): 10–20.
76. Rukkwamsuk T, Rungruang S, Choothesa A, Wensing T. Effect of propylene glycol on fatty liver development and hepatic fructose 1, 6 biphosphatase activity in periparturient dairy cows. *Livest Prod Sci* 2005; 95(1/2): 95–102.
77. Kabu M, Civelek T. Effects of propylene glycol, methionine and sodium borate on metabolic profile in dairy cattle during periparturient period. *Revue Med Vet* 2012; 163(8/9) :419-30.
78. McArt J, Nydam D, Oetzel G. A field trial on the effect of propylene glycol on displaced abomasum, removal from herd, and reproduction in fresh cows diagnosed with subclinical ketosis. *J Dairy Sci* 2012; 95(5): 2505–12. doi: 10.3168/jds.2011-4908
79. Lomander H, Frössling J, Ingvarsen K, Gustafsson H, Svensson C. Supplemental feeding with glycerol or propylene glycol of dairy cows in early lactation—Effects on metabolic status, body condition, and milk yield. *Journal of dairy science* 2012, 95(5): 2397–408.
80. Hoedemaker M, Prange D, Zerbe H, Frank J, Daxenberger A, Meyer H. Periparturient propylene glycol supplementation and metabolism, animal health, fertility, and production in dairy cows. *J Dairy Sci* 2004; 87(7): 2136–45.
81. Denis-Robichaud JD-R, DesCoteaux L, Dubuc J. Accuracy of a new milk strip cow-side test for diagnosis of hyperketonemia. *Bov Pract* 2011; 45(2): 97–100.
82. Ospina PA, McArt JA, Overton TR, Stokol T, Nydam DV. Using non-esterified fatty acids and β -hydroxybutyrate concentrations during the transition period for herd-level monitoring of increased risk of disease and decreased reproductive and milking performance. *Vet Clin North Am Food Anim Pract* 2013; 29(2): 387–412.

Razširjenost in potencialni dejavniki tveganja, povezani s ketozo na mlečnih kmetijah v Egiptu

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Izvelek: Ketoza je z energijo povezana presnovna bolezen, ki se pojavlja predvsem v zgodnjem obdobju laktacije pri kravah molznicah. Ketoza negativno vpliva na proizvodnjo, zdravje živali in reprodukcijo. V tej študiji smo določali razširjenost in dejavnike tveganja, povezane s ketozo pri mlečnem govedu v zgodnji laktaciji v Egiptu. Na ketozo je bilo pregledanih 1179 krav molznic iz 37 mlečnih farm z uporabo semikvantitativnega testa na mlečnem traku. Sestavili smo vprašalnik, ki je vključeval domnevne dejavnike tveganja. Dejavnike tveganja smo ocenili na ravni krave in črede z uporabo logističnega regresijskega modela. Ugotovitve so pokazale, da je bila razširjenost ketoze 6,35 % (75/1179 primerov). Na ravni krave je končni logistični regresijski model pokazal pomembno povezavo med ketozo in pariteto ≥ 4 ($P=0,040$, OR: 1,74, CI 95 %: 1,025-2,95), kravami z dolžino sušnega obdobja ≥ 65 dni ($P=0,02$, OR: 1,88, CI 95 %: 1,1-3,18), sezono telitve ($P=0,037$, OR: 1,73, CI 95 %: 1,03-2,89), BCS $>3,5$ ($P=0,010$, OR: 2,03, CI 95 %: 1,19-3,47), mlečnostjo ≥ 25 L/dan ($P=0,033$, OR 2,04, CI 95 %: 1,06-3,92), distociji ($P<0,001$; OR: 3,18, CI 95 %: 1,75-5,77), zadržani posteljici ($P=0,006$, OR: 2,85, CI 95 %: 1,35-6,032) in dislokacijo siriščnika ($P<0,001$, OR: 26,28; CI 95 %: 7,20-95,90). Na ravni črede smo ugotovili povezavo med ketozo in nezadostnim krmljenjem s skupnim mešanim obrokom pred porodom ($P=0,021$, OR: 6,29, CI 95 %: 1,325-29,86), v čredah s pomanjkljivo laktacijo pa z dodatkom propilenglikola ($P=0,047$, OR: 4,86, CI 95 %: 1,020-23,19). Zaključimo lahko, da je ketoza obstoječa težava na mlečnih kmetijah v Egiptu, zato lahko opredelitev dejavnikov tveganja predstavlja uporaben pristop za preprečevanje in nadzor teh presnovnih težav.

Ključne besede: krava molznica; ketoza; dejavniki tveganja; BHBA

Primary Pulmonary Mycobacteriosis in a Cat: Immunohistochemical and Histopathological Evaluation

Key words

feline mycobacteriosis;
immunohistochemistry;
histopathology;
pulmonary

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Abstract: In this case report, primary pulmonary mycobacteriosis in a domestic cat was assessed using histopathological and immunohistochemical methods. The cat was admitted to a private veterinary clinic and presented with hyporexia, lethargy, and dyspnea with an abdominal component. For diagnostic purposes, thorax radiography was performed. Radiographic lung imaging showed the presence of opaque foci that were widely distributed throughout the lung. The cat's condition gradually deteriorated, and the cat died 2 hours after being brought to the clinic. Following owner consent, gross post-mortem examination was performed immediately after death at the same clinic. White foci measuring 2–10 mm in diameter covered the surface of the lungs and were present throughout the parenchyma, mostly multifocally and occasionally coalescing. No other macroscopical pathological findings were observed in other organs or on the skin. Some of the lung tissue with macroscopic lesions underwent further histopathological and immunohistochemical examination. Multifocal to coalescing areas of caseous necrosis (without a fibrous capsule) with karyorrhectic nuclei, and numerous macrophages were observed. Ziehl-Neelsen staining revealed many acid-fast bacteria. Immunohistochemical staining revealed positive immunostaining for *Mycobacterium* spp. This case study highlights the importance of considering zoonotic risks in cats diagnosed with primary pulmonary mycobacteriosis.

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Introduction

Mycobacteria are nonsporulating, acid-fast, weakly Gram-positive, and straight to slightly curved bacilli that measure 0.3–0.6 μm in width and 1–4 μm in length (1-4). They are associated with local and disseminated infections in a range of host species (1-4) and include the *Mycobacterium avium* and *Mycobacterium tuberculosis* complexes as well as many other nontuberculous species. The *M. tuberculosis* complex includes *M. tuberculosis*, *M. bovis*, and *M. microti* (1, 4, 5).

Mycobacteria are phagocytosed by macrophages after entering the body through the alimentary tract, lungs, and skin. In the event of an insufficient cell-mediated immune response, they can persist and replicate within phagocytes by inhibiting phagosome-lysosome fusion, among other defence mechanisms. Mycobacteria cause some of the most difficult and significant infections in the world due to these defence mechanisms (6, 7).

Granulomatous inflammation occurs not only in cases of tuberculosis but also in other mycobacterial infections (8). Mycobacteriosis is usually characterized by a proliferative lesion type, in contrast to the rarely observed exudative lesion type (9). Exudative lesions, in which neutrophils are the major cell type, are both triggered by and induce high local bacillary load and tend to enlarge and progress toward liquefaction and cavitation. By contrast, proliferative lesions are triggered by low bacillary loads, mainly comprise epithelioid cells and fibroblasts, and tend to fibrose, encapsulate, and calcify (10).

Infected cats typically present with localised cutaneous disease with possible local lymph node involvement, which may become systemic, but can also present with primary gastrointestinal or respiratory disease as well as other primary disorders (11-16). Pulmonary involvement most likely arises via haematogenous spread from distant sites to the lungs,

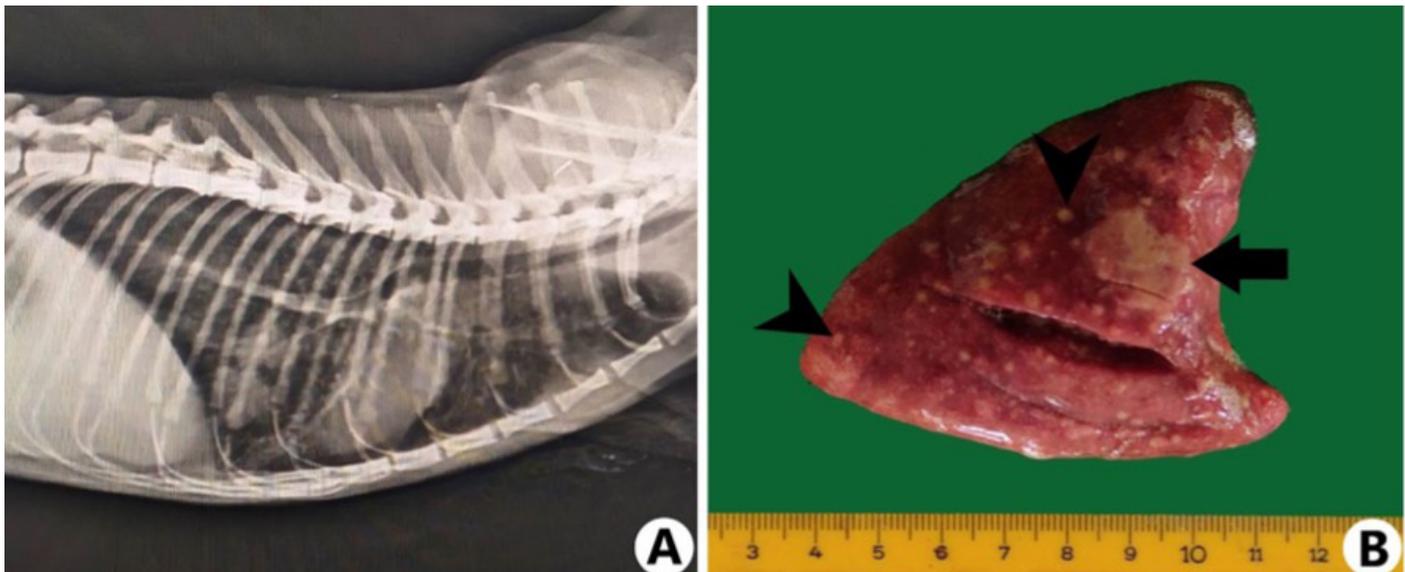


Figure 1: Radiographic and macroscopic findings. A: Lateral thoracic radiograph showing the presence of opaque foci that were widely distributed throughout the lungs. B: Medium-soft white foci measuring 2–10 mm in diameter (arrowheads) covered the surface of the lung and were present throughout the parenchyma, either multifocally or coalescing over larger extensive areas (arrow)

and pulmonary lesions are typically interstitial and extensive, causing dyspnoea and rarely progressing to coughing (4, 17).

Mycobacterial infections are recognised as a global health concern, both for humans and animals (18-20). One species known to be infected by a range of different mycobacteria is the domestic cat (12). Mycobacterial disease in domestic cats can cause many different syndromes, including tuberculosis, feline leprosy, and nontuberculous mycobacteriosis caused by nontuberculous mycobacteria (4, 21-24). A study in Great Britain included 339 cases of mycobacterial diseases in cats, from which the following bacteria were cultured: *M. microti* (19%), *M. bovis* (15%), *M. avium* (7%), and non-*M. avium* nontuberculous mycobacteria (6%); no bacterial growth in culture occurred in 53% of the cases (12). Different species cause feline mycobacterial infections, including *M. avium*, *M. tuberculosis*, *M. bovis*, and *M. microti*, of which the latter two are the most important (25, 26).

This report describes a case of primary pulmonary feline mycobacteriosis using histopathological and immunohistochemical techniques. Furthermore, this report aims to attract the attention of veterinarians and cat owners regarding this disease with zoonotic potential in terms of public health.

Case presentation

A 2-year-old, neutered, female, domestic shorthair Mackerel tabby cat was presented to a private veterinary clinic with reported hyporexia, lethargy, and dyspnea with an abdominal component. According to the anamnesis, the cat had no previous chronic disease or outdoor access, and there was no other cat in the house. For diagnostic purposes, thoracic radiography was performed. Multifocal ill-defined and sometimes confluent lesions with soft tissue opacity were

widely distributed throughout the lung parenchyma (Figure 1A). While the cat was being treated for respiratory problems, its condition gradually deteriorated, and it died 2 hours after being brought to the clinic. Following owner consent, gross post-mortem examination was performed immediately after death at the same private veterinary clinic. Macroscopic examination revealed firm white foci measuring 2–10 mm in diameter on the surface of the lung and throughout the parenchyma, which occasionally coalesced over larger extensive areas (Figure 1B). The trachea lumen was filled with a foamy serous liquid. No gross pathological findings were observed in other organs or skin. After identifying macroscopic lesions in the lung, a sample of the fresh lung tissue was submitted to the Department of Pathology, Faculty of Veterinary Medicine, Selçuk University, for further histopathological examination.

Five samples of the lesioned lung tissue were fixed in 10% neutral buffered formaldehyde solution. After routine histopathological tissue processing, tissues were embedded in paraffin and cut into 5- μ m-thick sections. These sections underwent haematoxylin and eosin and Ziehl-Neelsen staining (27) and immunohistochemical staining with an anti-*M. tuberculosis* antibody (1:100, Abcam, ab214721) in a staining device (Leica, Bondmax) using the Bond™ polymer refine detection system (Leica DS9800) according to a previously reported method (28). Sections treated with phosphate buffer saline instead of primary antibodies served as negative controls. Slides were examined by light microscopy (Olympus BX51, Tokyo, Japan) and photographed (Olympus EP50, Tokyo, Japan).

Multifocal to coalescing nodular areas consisting of caseous necrosis with karyorrhectic nuclei were widely observed in the lung. Around the necrotic areas, no fibrous capsules were observed but many lymphohistiocytic cell infiltrations and alveolar oedema were seen (Figure 2A). Mineralisation

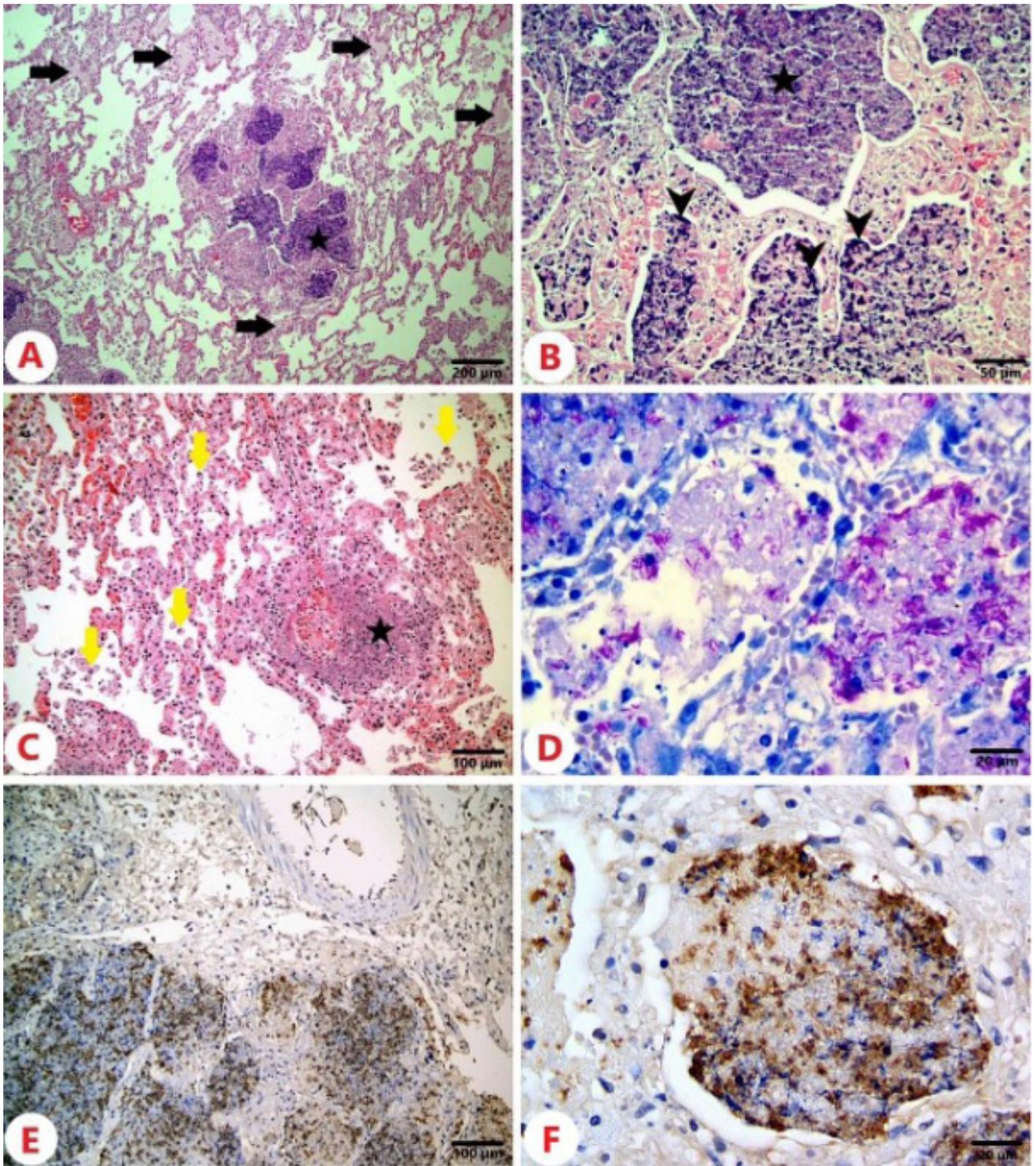


Figure 2: Histopathological and immunohistochemical findings of the lungs. A: Multifocal and coalescing nodular areas consisting of caseous necrosis without fibrous capsules (asterisk) and oedemas (arrows). Haematoxylin-Eosin staining; scale bar: 200 μm . B: High magnification of nodular lesion: caseous necrosis (asterisk), mineralisation (arrowheads), and lymphohistiocytic cell infiltration. Haematoxylin-Eosin staining; scale bar: 50 μm . C: Necrotic areas (asterisk) and numerous macrophages (arrows) around the vessel and necrotic vasculitis. Haematoxylin-Eosin staining; scale bar: 100 μm . D: Acid-fast bacteria in areas of caseous necrosis. Ziehl-Neelsen staining; scale bar: 20 μm . E: Positive immunostaining for *Mycobacterium* spp. in areas of caseous necrosis. IHC; scale bar: 100 μm . F: High magnification of *Mycobacterium* spp. immunostaining. IHC; scale bar: 20 μm

was observed in the areas of caseous necrosis (Figure 2B). Around the vessel, similar necrotic areas and necrotic vasculitis were observed (Figure 2C). Local haemorrhagic areas were also identified. Although there were very few epithelioid macrophages, no giant cell types were found. In the areas of caseous necrosis and in the cytoplasm of macrophages, Ziehl-Neelsen staining revealed numerous acid-fast bacilli (Figure 2D), and anti-*M. tuberculosis* immunostaining was positive (Figure 2E, F).

Discussion

This case report presents primary pulmonary mycobacteriosis in a domestic cat diagnosed using histopathological and immunohistochemical techniques. Radiography can detect pulmonary involvement in many cases of mycobacteriosis; however, the radiographic appearance of lesions is variable and nonspecific. In feline mycobacteriosis, pulmonary involvement is commonly interstitial, but may progress to bronchial changes in chronic cases (17). In the current case, diffuse interstitial changes were observed.

Mycobacterial lesions in carnivores differ from those in other species. Typical tuberculous granulomas are not as common, and when they occur, caseous necrosis is not a prominent gross feature. More often there is nonspecific granulation tissue in which macrophages are scattered at random, and giant cells are rare or absent (29). Necrosis associated with feline mycobacteriosis is called caseous necrosis and is defined as areas where cellular and structural details are lost due to the accumulation of eosinophilic and basophilic (karyorrhectic) residues (30). In the current case, mycobacterial lesions displayed caseous necrosis, in which karyorrhectic nuclei were observed, whereas giant cells were not. Few epithelioid macrophages and no evidence of a fibrous capsule were observed, which suggests a more acute infection. Macroscopic lesions were only observed in the lungs, which indicates that the route of infection was via inhalation of infectious particles.

The current case was of a domestic cat without access to the outdoors or other animals, indicating the possibility of human-to-animal transmission. Mycobacterial diseases are considered both zoonoanthropotic and zoonotic (31). If an immunocompromised individual has an active untreated mycobacterial infection, there is a potential risk of transmission to others, including cats (32). There is a theoretical risk of transmission of infection from cats to humans via infectious aerosols. However, if the cat has untreated productive respiratory disease, the greatest risk to humans is exposure to infectious fluids (33). In the current case, the presence of respiratory tract symptoms increased the zoonotic risk. In terms of public health, cats infected with mycobacteria should be examined, and necessary measures should be taken.

Conclusions

This study characterized the radiographic, pathological, and immunohistochemical appearance of primary pulmonary mycobacteriosis lesions in a cat. The purpose of this case report was to draw attention to feline mycobacteriosis, which is under-reported but increasingly recognised. Suspected or infected cats with respiratory symptoms should be examined on a regular basis because they can pose a zoonotic risk to cat owners, children, and veterinarians.

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References

1. Sakamoto K. The pathology of *Mycobacterium tuberculosis* infection. *Vet Pathol* 2012; 49(3): 423–39. doi: 10.1177/0300985811429313
2. O'Reilly LM, Daborn C. The epidemiology of *Mycobacterium bovis* infections in animals and man: a review. *Tuber Lung Dis* 1995; 76(Suppl. 1): 1–46. doi: 10.1016/0962-8479(95)90591-x
3. Schrenzel M, Nicolas M, Witte C, et al. Molecular epidemiology of *Mycobacterium avium* subsp. *avium* and *Mycobacterium intracellulare* in captive birds. *Vet Microbiol* 2008; 126(1/3): 122–31. doi: 10.1016/j.vetmic.2007.06.016
4. Gunn-Moore D, Dean R, Shaw S. Mycobacterial infections in cats and dogs. *In Pract* 2010; 32(9): 444–52. doi: 10.1136/inp.c5313
5. Rufenacht S, Bogli-Stuber K, Bodmer T, et al. *Mycobacterium microti* infection in the cat: a case report, literature review and recent clinical experience. *J Feline Med Surg* 2011; 13(3): 195–204. doi: 10.1016/j.jfms.2011.01.012
6. Palmer M. *Mycobacterium bovis*: characteristics of wildlife reservoir hosts. *Transbound Emerg Dis* 2013; 60(Suppl. 1): 1–13. doi: 10.1111/tbed.12115
7. Monies R, Cranwell M, Palmer N, Inwald J, Hewinson RG, Rule B. Bovine tuberculosis in domestic cats. *Vet Rec* 2000; 146(14): 407–8. doi: 10.1136/vr.146.14.407
8. Oztomurcuk D, Terzi O, Demirci C, Kılıçaslan Z. Investigation of granulomatous inflammations in terms of tuberculosis diagnosis: A 5-year multi-center laboratory study. *Turk Thorac J* 2022; 23(1): 11–6. doi: 10.5152/TurkThoracJ.2022.20314
9. Maxie G ed. Jubb, Kennedy & Palmer's Pathology of Domestic Animals Pathology of domestic animals. Vol. 1. St. Louis: Elsevier, 2016: 639–41.
10. Cardona P-J. The key role of exudative lesions and their encapsulation: lessons learned from the pathology of human pulmonary tuberculosis. *Front Microbiol* 2015; 6: 612. doi: 10.3389/fmicb.2015.00612
11. Jennings AR. The distribution of tuberculous lesions in the dog and cat, with reference to the pathogenesis. *Vet Rec* 1949; 61: 380–4.
12. Gunn-Moore DA, McFarland SE, Brewer JI, et al. Mycobacterial disease in cats in Great Britain: I. Culture results, geographical distribution and clinical presentation of 339 cases. *J Feline Med Surg* 2011; 13(12): 934–44. doi: 10.1016/j.jfms.2011.07.012

13. Miller M, Fales W, McCracken W, et al. Inflammatory pseudotumor in a cat with cutaneous mycobacteriosis. *Vet Pathol* 1999; 36(2): 161–3. doi: 10.1354/vp.36-2-161
14. O'Halloran C, Ioannidi O, Reed N, et al. Tuberculosis due to *Mycobacterium bovis* in pet cats associated with feeding a commercial raw food diet. *J Feline Med Surg* 2019; 21(8): 667–81. doi: 10.1177/1098612X19848455
15. Madarame H, Saito M, Ogihara K, et al. *Mycobacterium avium* subsp. *hominissuis* meningoencephalitis in a cat. *Vet Microbiol* 2017; 204: 43–5. doi: 10.1016/j.vetmic.2017.04.008
16. Stavinohova R, O'Halloran C, Newton JR, et al. Feline ocular mycobacteriosis: clinical presentation, histopathological features, and outcome. *Vet Pathol* 2019; 56(5): 749–60. doi: 10.1177/0300985819844819
17. Bennett AD, Lalor S, Schwarz T, Gunn-Moore DA. Radiographic findings in cats with mycobacterial infections. *J Feline Med Surg* 2011; 13(10): 718–24. doi: 10.1016/j.jfms.2011.06.006
18. Glaziou P, Floyd K, Raviglione M. Global burden and epidemiology of tuberculosis. *Clinics in Chest Medicine* 2009; 30: 621–36.
19. LoBue P, Enarson D, Thoen C. Tuberculosis in humans and animals: an overview. *Int J Tuberc Lung Dis* 2010; 14(9): 1075–8.
20. Shiloh MU, Champion PAD. To catch a killer. What can mycobacterial models teach us about *Mycobacterium tuberculosis* pathogenesis? *Curr Opin Microbiol* 2010; 13(1): 86–92. doi: 10.1016/j.mib.2009.11.006
21. McIntosh D. Feline leprosy: a review of forty-four cases from Western Canada. *Can Vet J* 1982; 23(10): 291–5.
22. Baral RM, Metcalfe SS, Krockenberger MB, et al. Disseminated *Mycobacterium avium* infection in young cats: overrepresentation of Abyssinian cats. *J Feline Med Surg* 2006; 8: 23–4. doi: 10.1016/j.jfms.2005.06.004
23. Horne KS, Kunkle GA. Clinical outcome of cutaneous rapidly growing mycobacterial infections in cats in the south-eastern United States: a review of 10 cases (1996–2006). *J Feline Med Surg* 2009; 11(8): 627–32. doi: 10.1016/j.jfms.2008.10.008
24. Smith N, Crawshaw T, Parry J. *Mycobacterium microti*: more diverse than previously thought. *J Clin Microbiol* 2009; 47(8): 2551–9.
25. Barry M, Taylor J, Woods JP. Disseminated *Mycobacterium avium* infection in a cat. *Can Vet J* 2002; 43(5): 369–71.
26. Pesciaroli M, Alvarez J, Boniotti M, et al. Tuberculosis in domestic animal species. *Res Vet Sci* 2014; 97(Suppl.): S78–85. doi: 10.1016/j.rvsc.2014.05.015
27. Luna L. *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. 3rd ed. New York: McGraw-Hill Book Company, 1968: 32.
28. Ates MB, Ciftci MK, Oruc E, et al. *Mycobacterium bovis* tuberculosis in a neonatal Holstein calf. *Eurasian J Vet Sci* 2019; 35(3): 175–9. 10.15312/EurasianJVetSci.2019.242
29. Maxie G ed. *Jubb, Kennedy & Palmer's Pathology of Domestic Animals Pathology*
30. Mitchell J, Del Pozo J, Woolley C, Dheendsa R, Hope JC, Gunn-Moore DA. Histological and immunohistochemical (muco) cutaneous feline tuberculosis lesions. *J Small Anim Pract* 2022; 63(3): 174–87. doi: 10.1111/jsap.13386
31. Lloret A, Hartmann K, Pennisi MG, et al. Mycobacterioses in cats: ABCD guidelines on prevention and management. 10.1177/1098612X13489221
32. Ramdas KE, Lyashchenko KP, Greenwald R, Robbe-Austerman S, McManis C, Waters R. *Mycobacterium bovis* infection *Emerg Infect Dis* 2015; 21(3): 480–3. doi: 10.3201/eid2103.140715
33. O'Connor CM, Abid M, Walsh AL, et al. Cat-to-human transmission of *Mycobacterium bovis*, United Kingdom. *eid2512.190012*

Primarna pljučna mikobakterioza pri mački: imunohistokemična in histopatološka ocena

Osman Dağar, Mehmet Burak Ateş, Mustafa Ortatlatli, Ertan Oruc

Izveček: V tem poročilu o primeru je bila ocenjena primarna pljučna mikobakterioza pri domači mački z uporabo histopatoloških in imunohistokemičnih metod. Mačka je bila sprejeta v zasebno veterinarsko kliniko zaradi simptomov hiporeksije, letargije in dispneje z abdominalno komponento. Za diagnostične namene je bila opravljena rentgenska preiskava prsnega koša, ki je pokazala prisotnost motnih žarišč, široko razporejenih po pljučih. Stanje mačke se je postopoma slabšalo, umrla je dve uri po prihodu v kliniko. Po privolitvi lastnika je bila takoj po smrti opravljena razteslba. Bela žarišča, premera od 2 do 10 mm, so prekrivala površino pljuč. Prisotna so bila po celotnem parenhimu večinoma multifokalno in mestoma zlivajoče. V drugih organih in na koži ni bilo opaznih drugih makroskopskih patoloških sprememb. Nekateri dele pljučnega tkiva z makroskopskimi spremembami smo dodatno histopatološko in imunohistokemično pregledali. Opazili smo multifokalna do zlivajoča se območja kazeozne nekroze (brez fibrozne kapsule) s karioznimi jedri in številnimi makrofagi. Ziehl-Neelsenovo barvanje je razkrilo številne acidofilne bakterije. Imunohistokemično barvanje je pokazalo pozitivno imunobarvanje za *Mycobacterium* spp. To poročilo o primeru poudarja pomen upoštevanja zoonotskih tveganj pri mačkah z diagnozo primarne pljučne mikobakterioze.

Ključne besede: mikobakterioza mačk; imunohistokemija; histopatologija; pljuča

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Primary Pulmonary Mycobacteriosis in a Cat: Immunohistochemical and Histopathological Evaluation

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