Cover

University of Zagreb

Faculty of Veterinary Medicine

Lea Grbavac

SEROPREVALENCE AND RISK FACTORS FOR TOXOPLASMOSIS IN PIGS IN CROATIA

DOCTORAL THESIS

Zagreb, 2026

First inner page (text in English language)

University of Zagreb

Faculty of Veterinary Medicine

Lea Grbavac

SEROPREVALENCE AND RISK FACTORS FOR TOXOPLASMOSIS IN PIGS IN CROATIA

DOCTORAL THESIS

Supervisors:
Prof. Tatjana Živičnjak, PhD
Prof. Delphine Le Roux, PhD

Zagreb, 2026

Second inner page (text in Croatian language)

Sveučilište u Zagrebu

Veterinarski fakultet

Lea Grbavac

SEROPREVALENCIJA I RIZIČNI ČIMBENICI TOKSOPLAZMOZE KOD SVINJA U HRVATSKOJ

DOKTORSKI RAD

Mentori:
Prof. dr. sc.Tatjana Živičnjak
Prof. dr. sc. Delphine Le Roux

Zagreb, 2026.

Sveučilište u Zagrebu

VETERINARSKI FAKULTET

I Z J A V A

Ja, Lea Grbavac, potvrđujem da je moj doktorski rad izvorni rezultat mojega rada te da se u njegovoj izradi nisam koristio/-la drugim izvorima do onih navedenih u radu.

_____

(potpis studenta)

Zagreb, 2026

This doctoral thesis was conducted in the laboratory of the Department of Parasitology and Parasitic Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, under the supervision of Professor Tatjana Živičnjak, and at the UMR BIPAR, Laboratoire de Santé Animale, L'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses), Maisons-Alfort, France, under the supervision of Professor Delphine Le Roux.

About the mentors

Professor Tatjana Živičnjak works in the Department of Parasitology and Parasitic Diseases with Clinic, at the Faculty of Veterinary Medicine, University of Zagreb. She earned her PhD at the Faculty of Veterinary Medicine in Zagreb; her dissertation focused on a sero-epizootiological investigation of leishmaniosis in asymptomatic dogs in an enzootic area of the Dalmatia region. Her research focuses on epizootiology, diagnostic methods, prevention, treatment, and monitoring of parasitic diseases in animals, with an emphasis on zoonoses. As a mentor, she guided research design, identified risk factors, and oversaw all aspects required for dissertation preparation. She has published 49 scientific and professional papers, with 1062 citations according to Scopus and an h-index of 19 and has participated in more than 50 scientific conferences.

Recent publications

GRBAVAC, L., F. DÁMEK, S. THOUMIRE, A. MERCIER, K. PASSEBOSC-FAURE, N. KONSTANTINOVIĆ, M. KIŠ, Ž. MIHALJEVIĆ, **T. ŽIVIČNJAK**, R. BLAGA, D. LE ROUX (2025): Seroprevalence and genetic characterization of *Toxoplasma gondii* in hunted wild boars (*Sus scrofa*) from Croatia. Parasitol. Res. 124, 130, 1-11. DOI: 10.1007/s00436-025-08590-1.

GRBAVAC, L., A. ŠIKIĆ, P. KOSTEŠIĆ, I. C. ŠOŠTARIĆ-ZUCKERMANN, V. MOJČEC PERKO, J. BORAS, I. BATA, A. MUSULIN, T. KOSTANJŠAK, **T. ŽIVIČNJAK** (2024): Comprehensive Diagnosis, Treatment, and Outcome of *Taenia crassiceps* Cysticercosis in a Ring-Tailed Lemur (*Lemur catta*) from a Croatian Zoo: No Longer Unusual? Pathogens 13, 1-13. DOI: 10.3390/pathogens13040283.

Professor Delphine Le Roux is Professor of Veterinary Immunology at the National Veterinary School of Alfort, France. She obtained her PhD in Cellular Biology and Immunology in 2006. After several years of postdoctoral fellowships in cellular biology and immunology, she joined the BIPAR Joint Research Unit (Maisons-Alfort, France) in 2012. She became Deputy Director of the BIPAR JRU in 2021. Her main research focuses on the circulation of *Toxoplasma gondii* in different animal species, with a recent emphasis on cats. She investigates host-pathogen interactions at the feline intestinal level by developing different feline cellular models to study the parasite's sexual cycle and the host response to infection. She has published 25 articles as first, last, or co-author and has an h-index of 12.

Recent publications

GRBAVAC, L., F. DÁMEK, S. THOUMIRE, A. MERCIER, K. PASSEBOSC-FAURE, N. KONSTANTINOVIĆ, M. KIŠ, Ž. MIHALJEVIĆ, T. ŽIVIČNJAK, R. BLAGA, **D. LE ROUX** (2025): Seroprevalence and genetic characterization of *Toxoplasma gondii* in hunted wild boars (*Sus scrofa*) from Croatia. Parasitol Res. 124, 130. DOI: 10.1007/s00436-025-08590-1.

FRIESEMA, I.H., H. WAAP, A. SWART, A. GYÖRKE, **D. LE ROUX**, F. M. EVANGELISTA, F. SPANO, G. SCHARES, G. DEKSNE, M. J. GARGATÉ, R. CALERO-BERNAL, P. JOKELAINEN, F. SEEBER, J. SROKA, A. LUNDÉN, O. VAN DEN BERG, S. JORE, H. J. WISSELINK, F. DÁMEK, L. S. VESTERGAARD, M. OPSTEEGH (2025): Systematic review and modelling of *Toxoplasma gondii* seroprevalence in humans, Europe, 2000 to 2021. Euro Surveill. 30, 34. DOI: 10.2807/1560 7917.

A. Y. BELLATRECHE, R. BOUZID, A. BLAIZOT, D. AUBERT, R. BLAGA, K. AIT-OUDHIA, **D. LE ROUX** (2022): Comparison of a Commercial Enzyme-Linked Immunosorbent Assay (ELISA) with the Modified Agglutination Test (MAT) for the Detection of Antibodies against *Toxoplasma gondii* in a Cohort of Hunting Dogs. Animals 18, 12. DOI: 10.3390/ani12202813.

I sincerely extend my deepest gratitude to Professor Radu Blaga, PhD, and to my dear colleague and friend Vitomir Djokić, PhD, who together made this research idea possible.

I would like to express my deep gratitude to the entire Toxo team, especially Filip Dámek, PhD and Sandra Thoumire, for generously sharing their knowledge, providing expert guidance in sample processing, and offering thoughtful advice. I am also grateful for the warm hospitality and incredible kindness shown to me during my stay in Maisons-Alfort, and for the generous time you all so selflessly devoted to me. I will always remember these days with appreciation.

I express my most sincere gratitude to my mentors, Professor Tatjana Živičnjak, PhD and Professor Delphine Le Roux, PhD for their invaluable guidance throughout this research, their unwavering and selfless support and their wise leadership during my entire doctoral journey.

My sincere gratitude goes to my colleagues at Veterinarska stanica Velika Gorica d.o.o., Veterinarska stanica Prelog d.o.o., Veterinarska stanica Slatina d.o.o., Veterinarska stanica Valpovo d.o.o., Veterinarska stanica Vrbovec d.o.o., and Veterinarska stanica Sveti Ivan Zelina d.o.o., as well as to Ivica Hader, Goran Galić, Professor Goran Bačić, PhD, Professor Sven Menčik, PhD, and Tomislav Bosanac, who generously gave their time to collect the samples essential for this research.

I also warmly and gratefully thank the staff of the Croatian Veterinary Institute – Davor Balić, PhD, Marica Lolić, PhD, Dragan Brnić, PhD, and Anđelka Borošak – for their dedicated efforts in sample collection, and Željko Mihaljević, PhD for his invaluable help with the statistical analysis. I would further like to thank to Žaklin Acinger-Rogić, PhD from the Directorate of Veterinary and Food Safety of the Croatian Ministry of Agriculture, for her kind support and valued advice.

A very special thank you to my dear friends Lucija, Marta, Antonija, Dajana, Keti, Jasmina, Ana, and Marija for your understanding and constant encouragement.

I am immensely grateful to my parents and my parents-in-law, who made it possible for me to write this dissertation by lovingly caring for my children. Your support was essential.

The greatest and most profound thank you goes to my beloved husband for your boundless love and unwavering support, which gave me strength and belief even in my weakest moments on this long and challenging journey. To our wonderful boys, Toma and Ivan, who fill every day with joy and light – I dedicate this work to you with all my love.

ABSTRACT

Toxoplasmosis is among the most widespread and significant parasitic foodborne zoonosis worldwide, representing a major public health concern due to its cosmopolitan distribution and its role in causing miscarriages in pregnant women, disease in newborns and children, and life-threatening conditions in immunocompromised individuals. The main routes of human infection are the consumption of undercooked meat from livestock and the ingestion of food or water contaminated with sporulated oocysts from cat faeces. Meat-borne transmission accounts for most reported human cases, with pork considered one of the most common sources. However, reliable serological surveillance systems for *Toxoplasma gondii* (*T. gondii*) during routine meat inspection are still lacking.

This study analysed 1621 pig serum samples from fattening pigs and sows collected in 2021 and 2022 in Croatia for the presence of immunoglobulin G (IgG) antibodies against *T. gondii* using the modified agglutination test (MAT). Additionally, a risk factor analysis for toxoplasmosis in the domestic pig population was conducted based on existing questionnaire data from the biosecurity categorisation of farms. The overall seroprevalence was estimated at 19.68%, with higher seroprevalence of 30.96% in sows compared to 12.21% in fattening pigs. Titre analysis showed a significant difference in the antibody titres against *T. gondii* between fattening pigs and sows, with sows exhibiting 1.87 times higher titres and a stronger average humoral immune response to the parasite. The variables identified as significant risk factors increasing the likelihood of toxoplasmosis infection were older age, smaller herd size, keeping pigs outdoors or indoors with outdoor access, and on-farm feed production.

The main risk factors identified in this study can inform targeted interventions to reduce or eliminate toxoplasmosis risks on farms, thereby lowering seroprevalence and improving pork safety for human consumption. They may also be useful in planning a monitoring system for toxoplasmosis in pigs. The results of this study make a valuable contribution to understanding swine toxoplasmosis in Croatia as part of the epidemiological chain of human toxoplasmosis, and highlight the need for, and usefulness of, future research on other production animals using the same methodology to obtain reliable and comparable results.

Keywords: *Toxoplasma gondii*, pigs, seroprevalence, risk factors, MAT, biosecurity

PROŠIRENI SAŽETAK

Uvod

Toksoplazmoza je jedna od najraširenijih i najznačajnijih zoonoza koje se prenose hranom i vodom. Invaziju uzrokuje protozoon *Toxoplasma gondii*, obligatni unutarstanični parazit svih toplokrvnih životinja i čovjeka koji služe kao posrednici, dok su domaće i divlje mačke jedini nositelji. Zbog svog zoonotskog karaktera, ova parazitoza predstavlja važan javno zdravstveni problem jer pored asimptomatskog tijeka bolesti koji je najčešći, prate ju vrlo ozbiljne komplikacije u trudnica, novorođenčadi, djece i imunokompromitiranih osoba.

Najčešći putevi invazije za posrednike su ingestija termički nedovoljno obrađenog mesa sa tkivnim cistama, unos hrane i vode kontaminirane sporuliranim oocistama iz mačjeg izmeta, te transplacentarni prijenos. Prema podacima Europske agencije za sigurnost hrane (*engl.* EFSA), oko 60% slučajeva toksoplazmoze u ljudi u Europi povezano je s konzumacijom termički nedovoljno obrađenog mesa, pri čemu su svinjetina, govedina i janjetina identificirane kao glavni izvori, a svinjetina se ističe kao najvažniji. U zakonodavstvu Europske unije, toksoplazmoza je prepoznata kao važna zoonoza koju treba pratiti ovisno o epidemiološkim prilikama u državama članicama. Međutim kako ne postoji ustaljeni sustav praćenja kongenitalne toksoplazmoze u ljudi u većini država EU, epidemiološku situaciju je teško procijeniti. Osim toga, učinkovit sustav nadzora mesa pozitivnog na *T. gondii* koje je namijenjeno ljudskoj prehrani također ne postoji. Brojna istraživanja u Europi procijenila su seroprevalenciju u svinja i u drugim domaćim životinjama namijenjenih ljudskoj prehrani, uključujući identifikaciju čimbenika rizika za invaziju s *T. gondii*. Cilj je bilo prepoznati ključne rizične čimbenike, poboljšati zoohigijenske mjere, čime bi se smanjila seroprevalencija u životinja i posljedično rizik za invaziju ljudi.

Za detekciju IgG protutijela na *T. gondii* u životinja koristi se više seroloških metoda, od kojih su najraširenij komercijalno dostupni enzimski imunosorbentni testovi (ELISA), a zatim modificirani aglutinacijski test (MAT) i indirektni imunofluorescentni test (IFAT). Serološka dijagnostika toksoplazmoze općenito je izazovna zbog nedostatka standardiziranog testa u većini zemalja, a to otežava usporedbu rezultata između različitih istraživanja i između različitih vrsta životinja. Iako su neka istraživanja pokazala dobru sukladnost između ovih testova, MAT se smatra referentnom serološkom metodom jer nije specifičan za pojedine vrste životinja, što omogućuje pouzdanu usporedbu između različitih životinjskih vrsta.

Svinjetina je među najčešće konzumiranim vrstama mesa u Hrvatskoj na što ukazuje prosječna potrošnja svježeg svinjskog mesa u kućanstvima koja je za 2022. godinu iznosila 17,6 kg po stanovniku. Osim toga u Hrvatskoj je u svinjogojstvu 2024. godine zabilježen porast proizvodnje od 13,3% u odnosu na prethodnu godinu. Ovi podaci jasno ukazuju na važnost svinjogojstva u Hrvatskoj. Stoga je razumijevanje epidemiologije toksoplazmoze na hrvatskim svinjogojskim farmama, kao i uloge rizičnih čimbenika, ključno za učinkovitu kontrolu ove zoonoze. Do danas je u Hrvatskoj obavljeno samo jedno istraživanje o epidemiologiji toksoplazmoze u svinja, a ono je provedeno na vrlo malom uzorku. Ovo istraživanje predstavlja prvo veliko, reprezentativno ispitivanje seroprevalencije toksoplazmoze koristeći MAT te donosi sveobuhvatnu procjenu rizika invazije u populaciji svinja u Hrvatskoj.

Materijali i metode

U ovom istraživanju ispitivani su serumi tovljenika i krmača prikupljeni tijekom 2021. i 2022. godine na prisutnost IgG protutijela parazita *T. gondii*. Krv tovljenika i krmača je uzorkovana na liniji klanja prilikom iskrvarenja, dok je dio uzoraka seruma krmača preuzet iz arhive Hrvatskog veterinarskog instituta. Svi uzorci seruma su nakon pripreme transportirani u Laboratoire de Santé Animale (ANSES, Maisons-Alfort, Francuska) gdje se za detekciju protutijela koristio MAT za čiju izvedbu je antigen ustupio Centre National de Référence de la Toxoplasmose (Reims, Francuska). Aglutinacija je očitana pod povećalom, a uzorak je smatran pozitivnim ako je aglutinat prekrivao više od polovice dna jažice mikrotitracijske ploče. Dva su ispitivača neovisno očitavala ploče, a svi uzorci reaktivni pri razrjeđenju ≥1/6 klasificirani su kao pozitivni.

Procijenjena je ukupna seroprevalencija u svinja, a vrijednosti seroprevalencije su izračunate i za svaku kategoriju mogućeg rizičnog čimbenika. Zatim je provedena deskriptivna statistika kako bi se ispitala raspodjela seropozitivnih i seronegativnih životinja unutar kategorija svakog mogućeg rizičnog čimbenika. Potom je ispitana povezanost između svakog pojedinog čimbenika i seroprevalencije. Kako bi se odabrali potencijalni čimbenici rizika relevantni za epidemiologiju toksoplazmoze, korištena su odabrana pitanja i dostupni rezultati iz Upitnika za kategorizaciju (Prilog 1), zajedno s ostalim podacima o svinjama i gospodarstvima. U analizu su kao mogući čimbenici rizika za invaziju *T. gondii* uvrštene sljedeće varijable: kategorija svinje (tovljenik ili krmača), dobna kategorija (<12 mjeseci, ≥12 mjeseci), spol (muški ili ženski), ukupan broj svinja na farmi (≤20, 21–100, >100), biosigurnosna kategorija (0, 1, 2, 3, 4), prisutnost kućnih ljubimaca na farmi (da/ne), zaštita od ulaska drugih životinja (da/ne), držanje u zatvorenom bez vanjskog ispusta (da/ne), vanjsko držanje (da/ne), dezinfekcija obuće

pri ulasku u objekt (da/ne), postojanje dezinfekcijske barijere na ulazu u gospodarstvo (da/ne), proizvodnja hrane na gospodarstvu (da/ne) te pravilno skladištenje hrane uz zaštitu od štetnika (da/ne). Za procjenu povezanosti pojedinačnih čimbenika rizika na izglede za seropozitivnost u svinja učinjena je univarijatna logistička regresija. U završnom koraku izrađen je multivarijatni logistički model, kako bi se utvrdili najvažniji čimbenici rizika seropozitivnosti. Naposljetku je provedena statistička analiza titra koja je uključivala usporedbu između skupina tovljenika i krmača i procjenu veličine i značaja razlike između navedenih skupina.

Rezultati

Tijekom razdoblja istraživanja 2021. i 2022. godine prikupljeno je ukupno 1693 seruma svinja. Zbog nepotpunih podataka u analizu je uključeno 1621 uzoraka. Ukupna seroprevalencija procijenjena je na 19,68%. Svi ispitivani čimbenici rizika pokazali su statistički značajnu povezanost sa seropozitivnošću ($p < 0,05$).

Rezultati pokazuju da krmače imaju višu seroprevalenciju toksoplazmoze (30,96%) u usporedbi s tovljenicima (12,21%). Također, svinje starije od 12 mjeseci pokazuju veću seroprevalenciju (30,94%) u odnosu na mlađe životinje (7,26%), dok su ženke češće seropozitivne nego mužjaci (22,17% naspram 13,17%). Nadalje gospodarstva bez dezbarijere na ulazu ili bez dezinfekcije obuće pri ulasku u objekt imaju višu seroprevalenciju, 35,45% i 38,46% u usporedbi s gospodarstvima koja te mjere provode (14,88% i 16,71%). Svinje držane na otvorenom ili u zatvorenom sustavu s vanjskim ispustom imaju gotovo dvostruko višu seroprevalenciju (33,96% i 31,30%) u odnosu na svinje držane u potpuno zatvorenom sustavu (17,53% i 15,96%). Slično tome, prisutnost kućnih ljubimaca na gospodarstvima povezana je s gotovo dvostruko većom seroprevalencijom (28,20%) nego na gospodarstvima bez kućnih ljubimaca (14,54%). Nadalje, gospodarstva s vlastitom proizvodnjom hrane imaju više nego trostruko veću seroprevalenciju (24,51%) u odnosu na gospodarstva koja ne proizvode hranu (7,42%), a gospodarstva koja pravilno skladište hranu i štite je od štetnika bilježe dvostruko nižu seroprevalenciju (19,06%) u usporedbi s gospodarstvima koje to ne čine (40,43%). Na kraju, veličina krda pokazuje obrnut učinak: mala krda (≤20 svinja) imaju seroprevalenciju od 36,67%, srednja krda (21–100 svinja) 26,29%, dok velika krda (>100 svinja) bilježe 11,59% seropozitivnih svinja.

Deskriptivna statistika biosigurnosnih kategorija je pokazala da je u kategoriji 1 i 4 utvrđena seroprevalencija od 32,48% i 33,15%, dok je u kategorijama 2 i 3 seroprevalencija bila 22,14%

i 11,57%. Također je utvrđena neujednačena veličina uzoraka između biosigurnosnih kategorija.

Univarijatna logistička regresija pokazala je da su svi ispitivani čimbenici značajno povezani s invazijom s *T. gondii*. Završni multivarijantni model pokazao je da su dob, veličina uzgoja, način držanja i vlastita proizvodnja hrane na gospodarstvu neovisni čimbenici rizika. Svinje starije od 12 mjeseci imaju više nego dvostruko veće izglede za seropozitivnost (OR = 2,32). Mala gospodarstva (≤20) imaju više od četiri puta veći rizik od velikih (>100) te dvostruko u odnosu na srednja gospodarstva. Unutarnje držanje bez vanjskog ispusta djeluje zaštitno (OR = 0,55), dok vanjsko držanje udvostručuje rizik (OR = 2,09). Svinje na gospodarstvima koja proizvode vlastitu hranu imaju 1,63 puta veće izglede za seropozitivnost.

Analiza titra je pokazala da krmače imaju statistički značajno viši titar od tovljenika.

Rasprava

U analizi 1621 uzorka seruma svinja u Hrvatskoj utvrđena je seroprevalencija protutijela na *T. gondii* od 19,68%, pri čemu su krmače imale znatno višu seroprevalenciju (30,96%) od tovljenika (12,21%). Dobiveni rezultati razlikuju se od ranijeg manjeg istraživanja, vjerojatno zbog različitih načina držanja svinja, veličine uzorka i primjenjene dijagnostičke metode. Usporedba s međunarodnim istraživanjima pokazuje da je seroprevalencija toksoplazmoze u svinja u Hrvatskoj usporediva s globalnim prosjekom (oko 19%) te s rezultatima iz zemalja s razvijenim svinjogojstvom. Varijabilnost rezultata uglavnom proizlazi iz razlika u sustavu držanja, kategorijama svinja, dijagnostičkim metodama i primijenjenim *cut-off* vrijednostima.

Istraživanjem je utvrđena viša seroprevalencija u svinja s gospodarstava u biosigurnosnim kategorijama 1 i 4 (32,48% i 33,15%) u usporedbi s gospodarstvima u kategorijama 2 i 3 (22,14% i 11,57%). Ovo se može objasniti time što su u kategoriji 1 biosigurnosne mjere na nižoj razini nego u kategorijama 2 i 3, a u kategoriji 4 držanje svinja na otvorenom omogućuje mačkama direktan pristup i kontaminaciju okoline sporuliranim oocistama. Iako bi gospodarstva u kategoriji 3 trebala imati najvišu razinu zaštite, zabilježena je seroprevalencija od 11,57%, što upućuje na moguće propuste u provedbi mjera, npr. kretanje u istoj obući i pristup mačaka dijelovima gospodarstava što može dovesti do unosa oocisti u nastambe.

Svih 12 ispitivanih čimbenika rizika za invaziju s *T. gondii* su pokazala statistički značajnu povezanost sa seropozitivnošću svinja, pri čemu su se u konačnom multivarijatnom modelu najvažnijima pokazali dob, veličina krda, sustav držanja te vlastita proizvodnja hrane. U ovom je istraživanju utvrđeno da su svinje starije od 12 mjeseci imale više nego dvostruko veće

izglede (OR = 2,32) za seropozitivnost vjerojatno zbog dulje izloženosti parazitu. Veličina krda također je imala snažan utjecaj – svinje na malim gospodarstvima imale su višestruko veće izglede za invaziju zbog otvorenijeg sustava držanja i lakšeg pristupa mačaka koje kontaminiraju okoliš oocistama. Svinje držane na otvorenom imale su značajno viši rizik (OR = 2,09) za invaziju u odnosu na one držane u zatvorenom sustavu. Isto tako svinje držane u zatvorenom sustavu s pristupom vanjskom ispustu imale su značajno viši rizik, dok je potpuno zatvoreni sustav djelovao zaštitno (OR = 0,55), vjerojatno smanjujući mogućnost kontaminacije hrane i vode sporuliranim oocistama. Vlastita proizvodnja hrane također je povećavala rizik seropozitivnosti (OR = 1,63), najvjerojatnije zbog mogućnosti kontaminacije iste hrane ili dodataka prehrani privlačenjem mačaka i glodavaca skladištima hrane. Buduća istraživanja trebaju detaljnije ispitati proizvodnju i skladištenje hrane, te identificirati kritične točke.

Analiza titra pokazala je da krmače imaju 1,87 puta viši titar te da imaju i veću heterogenost titra (CV = 4,35) u odnosu na tovljenike (CV = 2,11). Visoki titar IgG protutijela teško je interpretirati, jer može ukazivati na akutnu, kroničnu invaziju ili reaktivaciju kronične. Veća heterogenost kod krmača najvjerojatnije je povezana sa starijom dobi, različitim fiziološkim i proizvodnim stadijima. Malo je poznato o dinamici specifičnih IgG protutijela na *T. gondii* u svinja i potrebna su daljnja istraživanja s longitudinalnim praćenjem titra, korištenjem više seroloških testova, uključujući detekciju IgM protutijela i indeks avidnosti IgG protutijela.

Zaključci

Seroprevalencija IgG protutijela na parazita *T. gondii* u svinja, utvrđena u ovom istraživanju, ukazuje na potencijalni rizik za invaziju ljudi, ako se svinjsko meso konzumira nedovoljno termički obrađeno. Identifikacija ključnih čimbenika rizika za toksoplazmozu kod svinja omogućuje provođenje ciljane prevencije, smanjenje seroprevalencije na razini gospodarstava i time poboljšanje sigurnosti svinjskog mesa za ljudsku potrošnju. Rizici utvrđeni u ovom istraživanju mogu se koristiti pri planiranju sustava praćenja toksoplazmoze. Preporučuje se i revizija postojećeg upitnika o kategorizaciji, s pitanjima koja detaljnije ispituju specifične čimbenike rizika. Praćenje, dijagnostika i prevencija ove zoonoze u skladu su s inicijativom Jedno zdravlje. Predlaže se standardizirati dijagnostičke metode za životinje (primjerice primjenom MAT testa), te provesti slična opsežna istraživanja i kod drugih vrsta domaćih životinja u Hrvatskoj koristeći MAT.

Ključne riječi: *Toxoplasma gondii*, svinja, seroprevalencija, rizični čimbenik, MAT, biosigurnost

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

ABBREVIATIONS AND SYMBOLS

AIDS	Acquired Immunodeficiency Syndrome
ASF	African Swine Fever
BABS	Bovine Albumin Buffer Solution
CI	Confidence Interval
CFT	Complement Fixation Test
CLIA	Chemiluminiscent Immunoassay
CV	Coefficient of Variation
DAT	Direct Agglutination Test
DNA	Deoxyribonucleic Acid
DTT	Dithiothreitol
DT	Dye Test
DF	Degrees of Freedom
EDTA	Ethylenediaminetetraacetic Acid
EFSA	European Food Safety Authority
ELFA	Enzyme Linked Fluorescent Assay
ELISA	Enzyme-Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GM	Geometric Mean
HIV	Human Immunodeficiency Virus
IFAT	Indirect Fluorescent Antibody Test
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHA	Indirect Hemagglutination Test
kg	kilogram
LAT	Latex Agglutination Test
MAT	Modified Agglutination Test
MPa	megapascal
µl	microlitre
ml	millilitre
NC	Negative Control
NaCl	sodium chloride

nm	nanometre
OD	Optical Density
OR	Odds Ratio
PC	Positive Control
PCR	Polymerase Chain Reaction
PBS	Phosphate-Buffered Saline
POD	Peroxidase
PP	Percentage of Positivity
RTE	Ready-to-eat
spp.	species
T.	*Toxoplasma*
TORCH	Toxoplasmosis, Other infections, *Rubella*, *Cytomegalovirus*, *Herpes simplex virus*
TRS	Treatment-Resistant Schizophrenia
UK	United Kingdom
USA	United States of America
WB	Western blot
WHO	World Health Organization

Table of Contents

1. INTRODUCTION

Toxoplasma gondii (T. gondii), a protozoan first described in the early 20th century, has since become the most intensively studied parasite worldwide and is widely regarded as the most successful, as it can infect all warm-blooded animals and humans, which serve as intermediate hosts, while domestic cats and wild felids are definitive hosts (DUBEY 2009).

Toxoplasmosis, the disease caused by *T. gondii* is classified among the most significant food- and waterborne parasitic zoonoses and ranks as the fourth most important foodborne parasitosis globally (FAO/WHO 2014). Although human infections are usually asymptomatic, the parasite can cause miscarriages in seronegative pregnant women and severe disease in children, the elderly, and immunocompromised individuals (DUBEY 2021; ROBERT-GANGNEUX et al. 2018). It is estimated that about one-third of the world's population is chronically infected with *T. gondii* (ROSTAMI et al. 2020).

The primary sources of human infection are undercooked meat containing viable tissue cysts, and food or water contaminated with sporulated oocysts from cat faeces (ALMERIA and DUBEY 2021). Data from the European Food Safety Authority (EFSA) indicate that meat-**b**orne infections account for approximately 60% of toxoplasmosis cases in humans in Europe, with pork, beef, and lamb identified as the main sources (EFSA 2018). Among these, pork is considered one of the main sources of infection for humans (DJURKOVIĆ-DJAKOVIĆ et al. 2013; DUBEY et al. 2020a). National studies in the United States of America (USA) and the Netherlands have shown that pork accounts for 41% and 50% of human cases of toxoplasmosis, respectively (EFSA 2018).

The European Union's (EU's) legislative framework on food safety has traditionally focused on identifying potential public health risks affecting consumers. Within this framework toxoplasmosis is recognised under the European Directive 2003/99/EC, which classifies it among zoonoses that require surveillance depending on the epidemiological conditions in member states. The absence of an effective surveillance programme for *Toxoplasma*-positive meat intended for human consumption has been emphasised (EFSA 2007). This lack of monitoring, together with the EFSA's recommendation to employ serology in pigs as an epidemiological marker for *T. gondii* infection in the context of food safety, has prompted considerable scientific investigations (EFSA 2011). As a result, numerous studies have been conducted to examine the seroprevalence and associated risk factors of *T. gondii* infection in pig populations. These studies aim to identify key predictors and mitigate them through

improved hygiene and animal husbandry practices, thereby reducing infection in pigs and consequently lowering the risk of zoonotic transmission.

Among the EU livestock sectors, the pig farming industry is dominant, with a total of 222 million pigs slaughtered in 2024 (EUROPEAN COMMISSION 2025). In 2023, the EU held the position of the second-largest producer of pigmeat globally, following China, with an annual production output approaching 21 million tonnes (EUROSTAT 2024). Specifically, in Croatia, the pig farming industry observed a production increase of 13.3% in 2024 compared to the previous year, resulting in roughly 1.6 million pigs slaughtered (CROATIAN BUREAU OF STATISTICS 2025a, 2025b). Pork is among most consumed meats in Croatia, alongside poultry. In 2022, average household consumption of pork (excluding smoked, salted and cured products) was 17.6 kg (CROATIAN BUREAU OF STATISTICS 2021). These data highlight the importance of pig farming in Croatia. A comprehensive understanding of the epidemiology of toxoplasmosis in Croatian pig farms, including the role of associated risk factors, is therefore essential for developing effective control strategies against this zoonosis. In the absence of commercial vaccines for pigs, reliable assessment of infection status can be achieved through serological detection of *T. gondii* specific antibodies in animal serum.

Various serological assays have been used to detect anti-*T. gondii* antibodies in pigs, with enzyme-linked immunosorbent assays (ELISAs), particularly commercially available ones, being the most common. These are followed in frequency by the modified agglutination test (MAT) and the indirect fluorescent antibody test (IFAT) (DUBEY et al. 2020a; HUERTAS-LÓPEZ et al. 2023). Overall, the serodiagnosis of toxoplasmosis in animals and humans is hindered by a lack of test standardisation in most countries, which complicates comparison of results across different studies. Although some investigations have shown satisfactory inter-test concordance, MAT is considered the reference serological assay due to its non-species-specific nature, which facilitates comparative analysis across various animal species (KLUN et al. 2006).

To date, only one previous study has investigated the epidemiology of toxoplasmosis in pigs in Croatia, using ELISA to detect *T. gondii* IgG antibodies in serum samples; however, it involved a very small number of animals. The present study is the first large-scale, representative investigation in Croatia to use the MAT for detecting *T. gondii* IgG antibodies in serum samples and to provide a comprehensive risk assessment of *T. gondii* infection in pigs.

2. LITERATURE REVIEW

2.1. A historical overview of *Toxoplasma gondii*

It has been more than 110 years since *T. gondii* was identified and initially described by Nicolle and Manceaux (1908 in Tunis) in the gundi, a North African rodent, and in a rabbit in Brazil by Splendore (1908) (DUBEY 2009). The name *Toxoplasma gondii*, introduced by Nicolle and Manceaux, was based on the shape and morphology of the infectious stage: (Greek) *toxon*: bow or arc, *plasma*: creature. The suffix *gondii* comes from the original host, the gundi (*Ctenodactylus gundi*).

The pathogenic potential of *T. gondii* was first recognised in the 1920s and 1930s, when it was established as a ubiquitous protozoan parasite and an important zoonotic and veterinary pathogen. Early investigations found that in congenitally infected children, the disease presented with the classic triad of symptoms: hydrocephalus, encephalitis, and retinochoroiditis. In veterinary medicine, abortions in sheep due to acute toxoplasmosis were recognised (DUBEY 2022a).

In the 1940s, broad-spectrum sulphonamide antibiotics were found to have anti-*Toxoplasma* activity. Subsequently, combination therapy with sulphonamides and the antiparasitic drug pyrimethamine was introduced and remains the gold standard treatment for toxoplasmosis in humans (DUBEY 2008).

In the 1950s, ocular toxoplasmosis was described in detail and was attributed to postnatally acquired *T. gondii*, in contrast to the previously most commonly recognised mode of transmission – congenital (WEISS and DUBEY 2009).

From the 1960s to the early 1990s, studies on pregnant women and neonates revealed several key findings in congenital toxoplasmosis. Fetal damage was most severe when maternal infection occurred in the first two trimesters, transmission rates depended on the gestational timing of infection, and women who were seropositive before pregnancy did not transmit the parasite to the fetus. Additionally, the macrolide antibiotic spiramycin reduced congenital transmission and proved effective in treating congenitally infected children (WEISS and DUBEY 2009). Since then, spiramycin has been used prophylactically in early pregnancy to reduce mother-to-fetus transmission when fetal infection has not been confirmed.

In the 1980s, *T. gondii* emerged as a one of the major causes of death in humans with acquired immunodeficiency syndrome (AIDS), confirming the theory that reactivation of chronic infection is related to immunocompromised states (WEISS and DUBEY 2009; DUBEY 2022a).

Over the years, many modes of transmission were recognised: congenital, carnivorism, and faecal-oral. In the 1970s, with the discovery of the full life cycle and developmental stages in the feline intestine, the definitive (cats) and intermediate hosts were defined and transmission modes better understood (DUBEY 2009).

The diagnosis and investigation of *T. gondii* relied primarily on experimental infections in mice, known as bioassays, until major advances occurred with the development of the first serological test, the Sabin-Feldman dye test (DT), in 1948, as it is both sensitive and specific, with no evidence of false results (SABIN and FELDMAN 1948; DUBEY 2008). This test was based on identifying specific anti-*T. gondii* antibodies in serum that altered the staining characteristics of tachyzoites allowing the level of specific antibody to be quantified, thus becoming the gold standard. It formed the basis of large-scale studies that revealed a high proportion of humans and domestic animals carrying antibodies to *T. gondii* (FERGUSON 2009; INNES 2010).

Over time, numerous serological tests have been developed for diagnosing toxoplasmosis in humans and animals. In the late 1960s, the detection of immunoglobulin M (IgM) antibodies using ELISA and IFAT in neonatal cord blood became valuable for diagnosing congenital toxoplasmosis, as IgM antibodies, unlike IgG, do not cross the placenta (DUBEY 2008). The direct agglutination test (DAT), developed in the 1960s and later improved in the 1980s as the modified agglutination test (MAT), significantly advanced the diagnosis of toxoplasmosis in both humans and animals, as the MAT does not require species-specific conjugates (DUBEY 2008).

Another major breakthrough in the diagnosis of toxoplasmosis occurred in the late 1980s, when the first *T. gondi*i gene target (B1 gene) was identified for polymerase chain reaction (PCR), enabling direct detection of the parasite's deoxyribonucleic acid (DNA) in tissue samples (DUBEY 2008; INNES 2010).

In the 1990s, identification of specific isoenzymes and comparison of restriction fragment length polymorphisms enabled the distinction and comparison of various strains (types I, II, III, and atypical). Gene mapping was achieved in 2005, facilitating the study of the pathogenic

potential of different genotypes. Mapping of *T. gondii* genes is aiding the search for improved antigens for diagnosis and protection (DUBEY 2008).

Since 2000, it has been possible to separate proteins from the whole parasite or from specific subsets, such as excreted-secreted proteins, using electrophoresis, and to identify individual proteins by mass spectrometry. This approach has greatly facilitated the identification of new proteins, while proteomic data on the parasite also help to fill gaps in genomic data and improve gene identification (FERGUSON 2009).

These advancements in molecular biology, protein separation, and proteomics have collectively supported the development of many different strategies over the last four decades. The first vaccine, developed in the 1980s, was a live attenuated vaccine based on the tachyzoites of the cyst-less strain of T. gondii and was specifically designed to prevent congenital toxoplasmosis in sheep (DUBEY 2008). This vaccine remains the only commericially available option. Despite extensive efforts by the scientific community to develop new and improved vaccines for humans, other intermediate hosts, and felids, an effective vaccine for clinical use is still lacking (MÉVÉLEC et al. 2020; CHU and QUAN 2021).

2.2. Etiology and epidemiology

2.2.1. Taxonomy

Toxoplasma gondii is an obligate intracellular coccidian parasite, with felids serving as the final host and warm-blooded animals, including humans, as intermediate hosts. *T. gondii* is the only species within the genus *Toxoplasma*. Coccidia represent an important group of animal parasites, with the oocyst stage being crucial for their development (DUBEY 2022a). *T. gondii* has a complex life cycle with three infective stages: (1) tachyzoites, (2) bradyzoites and (3) oocysts. A detailed taxonomic classification of the genus *Toxoplasma* can be found in Figure 1.

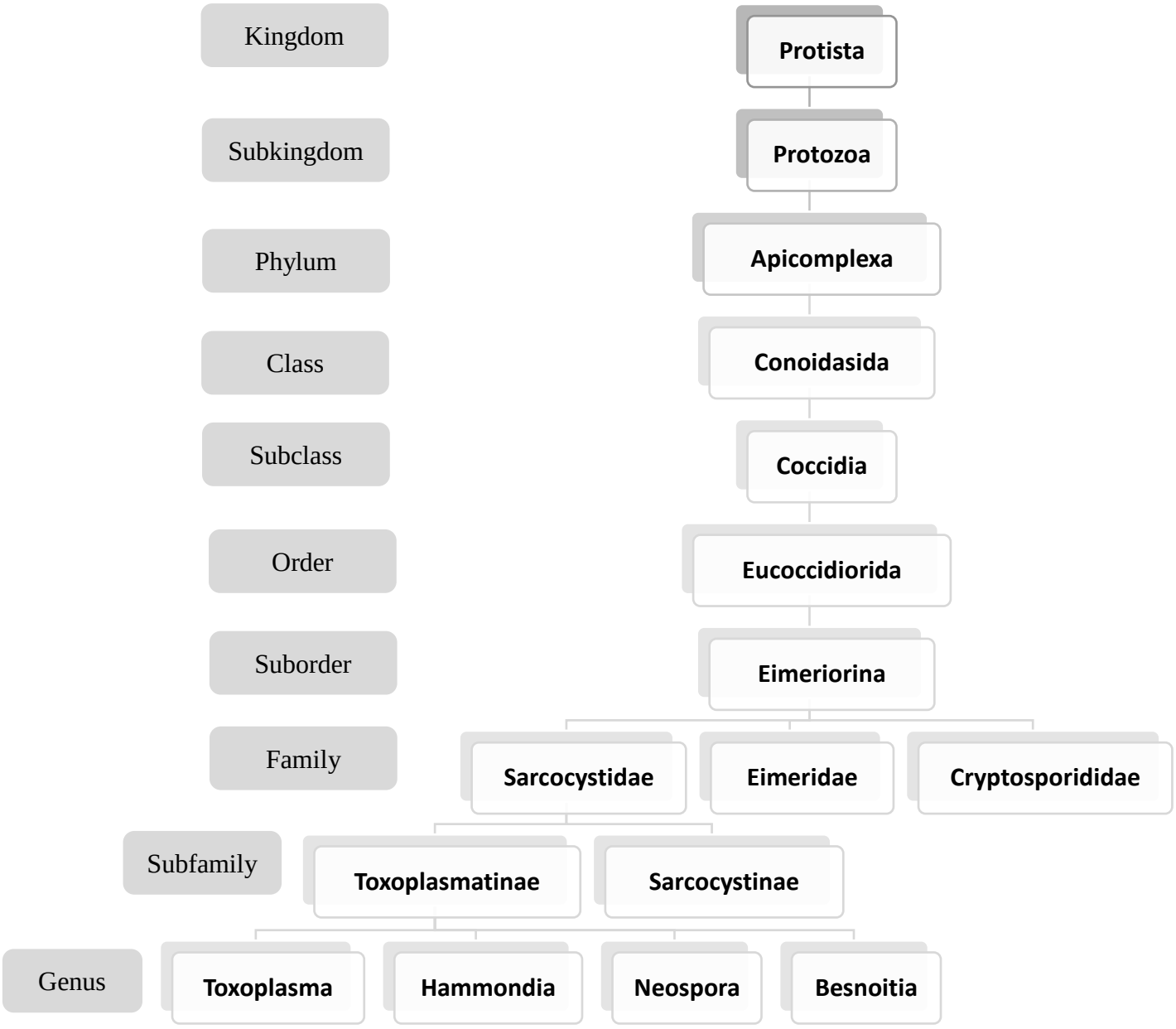

Figure 1. Taxonomic classification of the genus *Toxoplasma* (CURRENT et al. 1990)

2.2.2. Morphology and pathogenesis

During its life cycle, *T. gondii* occurs in three developmental stages: tachyzoite, bradyzoite and oocyst. The three infective stages (tachyzoite, bradyzoite and sporozoite within the sporulated oocyst) have a similar structure, an elongated shape and a typical apical complex in the anterior region containing various organelles (Figure 2). The apical complex, which consists of the cylindrical conoid and secretory organelles (micronemes and rophtries), is essential for the penetration of the parasite into the host cell (ATTIAS et al. 2020).

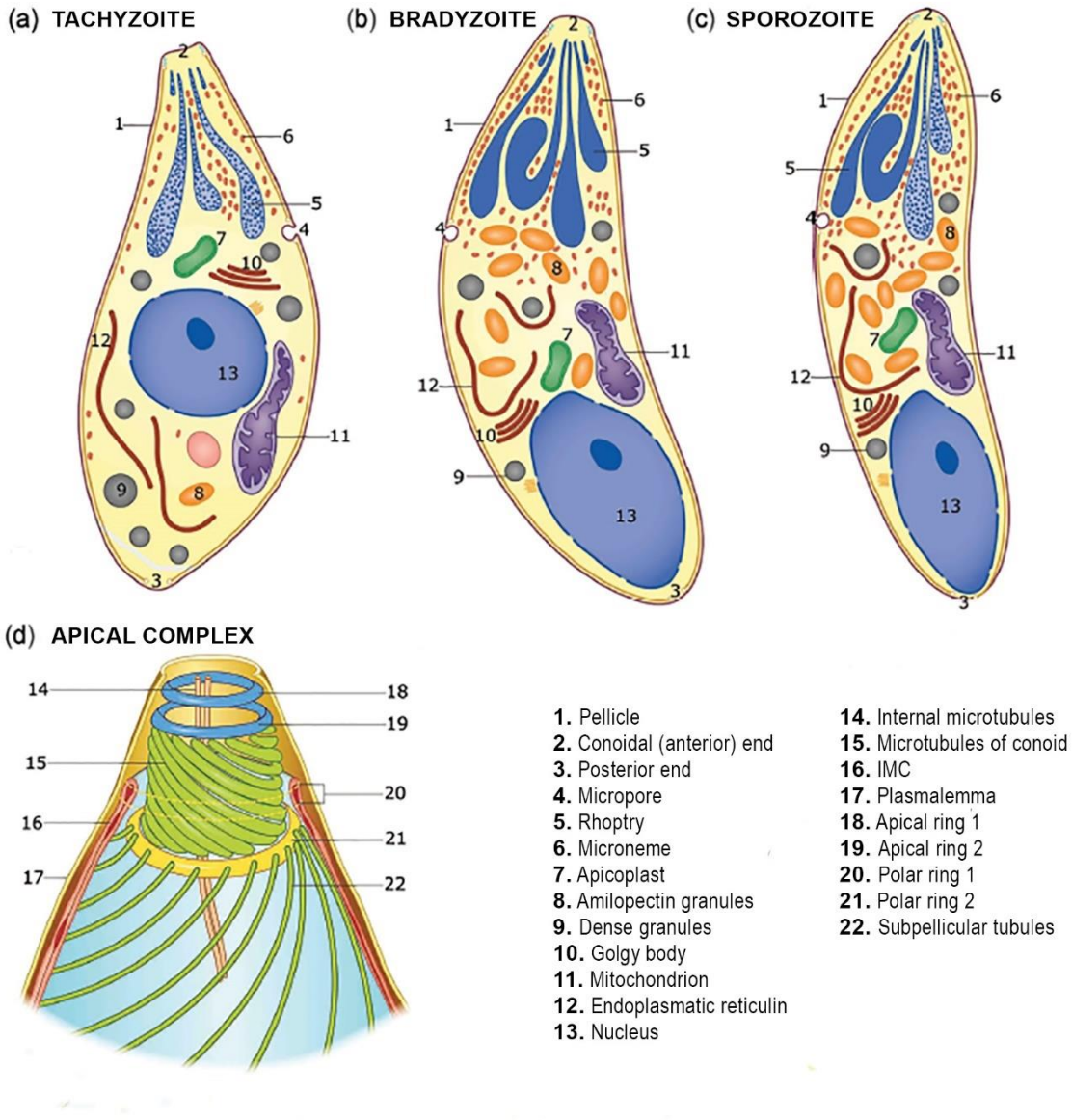

Figure 2. Schematic drawings of *T. gondii* stages: (a) tachyzoite, (b) bradyzoite, (c) sporozite and (d) apical complex (DUBEY 2022a)

Tachyzoites are rapidly multiplying forms typically found in acute infections, capable of proliferating in all nucleated cells of the intermediate host and in the non-intestinal epithelial cells of the final host. Tachyzoite is crescent in shape, measures 2 x 6 μm, and contains a centrally located nucleus with a pointed anterior end and a rounded posterior end. It has a complex structure consisting of several organelles and inclusion bodies. The rhoptries are convoluted, the tachyzoites have only a few micronemes, the dense granules are more numerous than in the bradyzoites, and the amylopectin granules are few, small, or even absent (Figure 2a) (ATTIAS et al. 2020). Tachyzoites can enter a host cell by phagocytosis or by active penetration of the cell membrane, which involves a specific mechanism based on the activity of the actin-myosin motor embedded in the inner membrane complex of the parasite's cytoskeleton (DUBEY 2022b). After entering the host cell, the parasite becomes egg-shaped and is surrounded by a parasitophorous vacuole, formed by both the host cell and the parasite. As the membrane of the parasitophorous vacuole lacks intramembranous components and host cell membrane markers, it cannot fuse with other membranes, thus preventing fusion with lysosomes and potential parasite degradation. In the vacuole, the tachyzoite begins its asexual reproduction by endodyogeny, a special form of reproduction in which two daughter cells are generated from one tachyzoite. Infected host cells expand and eventually burst when they are no longer able to support the growth of tachyzoites, releasing them to infect new cells. The speed of infection and growth depends on the *T. gondii* strain and the host cell (DUBEY et al. 1998).

After several rounds of replication, the tachyzoites transform into the slowly dividing form, bradyzoites, which form tissue cysts and are characteristic of a chronic infection. Bradyzoites are crescent-shaped 5–8.5 × 1–3 μm in size, and exhibit a pronounced tropism for long-lived and highly differentiated cells that are less accessible to the the immune system's defence mechanisms, such as neurons and muscle cells (DUBEY 2022b). They are most commonly found in the central nervous system, eyes, skeletal muscle, and heart. However, they are also found, though less frequently, in visceral organs such as the lungs, liver, and kidneys (DUBEY et al. 1998). The tissue cysts expand as the bradyzoites divide by endodyogeny. The size of each cyst depends on its age, parasite strain, and type of host cell. Their sizes range from about 5 μm in diameter and containing few bradyzoites, to older cysts that can grow up to 60 μm and contain thousands of bradyzoites (DUBEY 2022b) (Figure 3). Under certain conditions, for example, immunosuppression, the tissue cyst may rupture, allowing bradyzoites to transform back into tachyzoites, leading to reactivation of a latent infection (repeating the cycle).

Bradyzoites differ slightly morphologically from tachyzoites. They are more slender, the nucleus is located at the posterior end, rhoptries are electron dense, they have more micronemes, dense granules are less numerous, while amylopectin granules are more numerous and larger (Figure 2b) (ATTIAS et al. 2020).

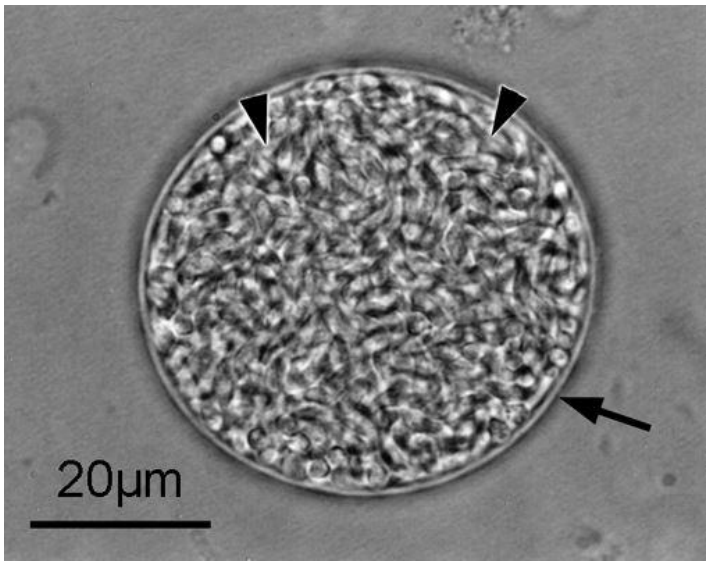

Figure 3. Unstained tissue cyst of *T. gondii* with a cyst wall (arrow) enclosing hundreds of bradyzoites (arrowheads) (DUBEY et al. 1998)

Sporozoites are structurally more similar to bradyzoites, measuring 2 x 6–8 µm. They share a slender, elongated shape, a posteriorly located nucleus, electron-dense rhoptries, and numerous, relatively large amylopectin granules (Figure 2c) (SPEER et al. 1998). However, sporozoites have fewer micronemes and more dense granules than bradyzoites, as well as a substantial number of lipid bodies, which are absent in bradyzoites (DUBEY et al. 1998). Sporozoites are found within the sporocysts of sporulated oocysts. The sporulated oocyst is round to ellipsoidal and measures 11 × 13 µm in diameter. Each sporulated oocyst contains two ellipsoidal sporocysts, each measuring 6 × 8 µm, and each sporocyst contains four sporozoites (Figure 4b) (SPEER et al. 1998). Unsporulated oocysts are produced by the parasite's sexual cycle, which occurs exclusively in the epithelial cells of the cat's small intestine. Oocysts are subspherical to spherical, measuring 10 x 12 µm in diameter, and consist of an oocyst wall with two colourless layers and a sporont that almost fills the oocyst (Figure 4a) (SPEER et al. 1998).

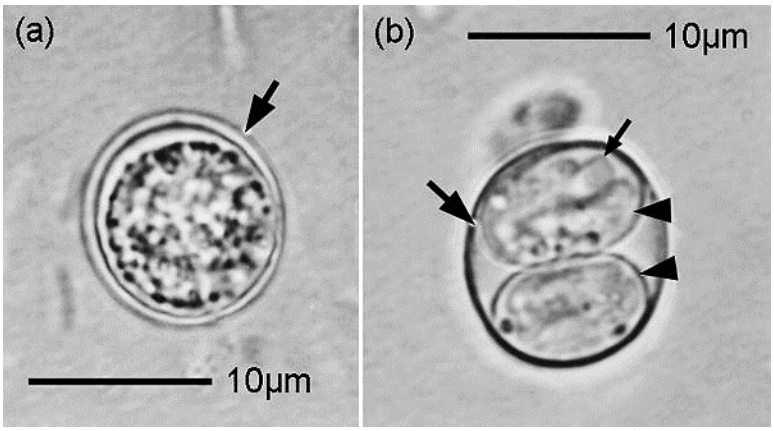

Figure 4. *T. gondii* oocysts: (a) nonsporulated oocysts containing oocyst wall (arrow) and sporont (inside the oocyst), (b) sporulated oocyst with two sporocysts (arrowheads) each containing four sporozoites (arrows) (DUBEY 2022b)

2.3. Life cycle

The parasite *T. gondii* has an indirect life cycle involving a definitive host and intermediate hosts (Figure 5). Domestic and wild felids are the only definitive hosts for *T. gondii*, where both sexual and asexual reproduction occur, encompassing the enteroepithelial and extraintestinal phases of the cycle. In intermediate hosts, reproduction is exclusively asexual and limited to the extraintestinal phase (DUBEY 2022b).

Following infection via tissue cysts in raw meat or infected rodents and birds, the enteroepithelial phase begins in the epithelium of the cat's small intestine. This phase involves a series of asexual divisions known as schizogony, followed by sexual reproduction (gametogony), which ultimately produces oocysts. During oocyst formation, microgametes (male gametes) penetrate the macrogamete (female gamete) via flagella to form a zygote. After fertilisation, a protective wall forms around the zygote; the infected epithelial cell then ruptures, releasing the oocyst into the intestinal lumen. The prepatent period lasts 3–10 days after ingestion of tissue cysts (DUBEY 2001). Oocysts are shed into the environment through feline faeces in an unsporulated form. They measure 10 × 12 µm, are spherical, and consist of a zygote enclosed by an oocyst wall. In cats infected with *T. gondii*, oocyst shedding is brief, lasting up to 13 days, during which oocysts can be detected in faeces by coprological parasitological examination (DUBEY 2001). Although the exact number of oocysts excreted in the faeces of naturally infected cats remains unknown, experimental studies in cats have documented substantial quantities (DUBEY 1995; CORNELISSEN et al. 2014; ZULPO et al. 2018).

However, there are no precise data on the total number of oocysts that a single cat can shed following a primary infection. DUBEY (2022b), in his book, suggests that a cat may excrete more than 500 million oocysts in one day. Notably, once infected, a cat develops immunity to reinfection, making subsequent oocyst shedding rare and rendering coprological parasitological testing highly unreliable (DUBEY 1995). Once excreted into the environment, oocysts mature and sporulate, which takes 1–5 days and results in the formation of two sporocysts within each oocyst, each containing four sporozoites (DUBEY et al. 1998). At this stage, the now-sporulated oocyst becomes infective. If ingested by another animal or human, the parasite's developmental cycle continues in the intermediate host, initiating the extraintestinal phase. Under the influence of digestive juices, sporozoites are released from the oocyst, penetrate the intestinal wall, enter the bloodstream, and invade any nucleated cell. Within these cells, sporozoites transform into rapidly multiplying tachyzoites, which continue to disseminate by invading new cells and dividing via endodyogeny within vacuoles. The rapid proliferation of tachyzoites defines the acute phase of toxoplasmosis. During this stage, the parasite can cross the placenta and infect the foetus, resulting in congenital toxoplasmosis. Notably, transplacental transmission occurs only during primary infection in intermediate hosts (DUBEY 2022a). As the host's immune response develops over time, tachyzoites replication slows down, and the parasites differentiate into slowly dividing bradyzoites. At this point, the infection becomes chronic. Bradyzoites target various tissues but show pronounced neurotropism and myotropism, most commonly forming tissue cysts in neural and muscular tissues (DUBEY 2022b). These tissue cysts are generally believed to persist in a latent state within most hosts for life, but there are no definitive data on this (TENTER et al. 2000; DUBEY 2022b). The three major routes of infection for intermediate and definitive hosts are: (1) ingestion of undercooked meat containing tissue cysts, (2) consumption of food or water contaminated with sporulated oocysts from cat faeces, and (3) transplacental transmission (Figure 5).

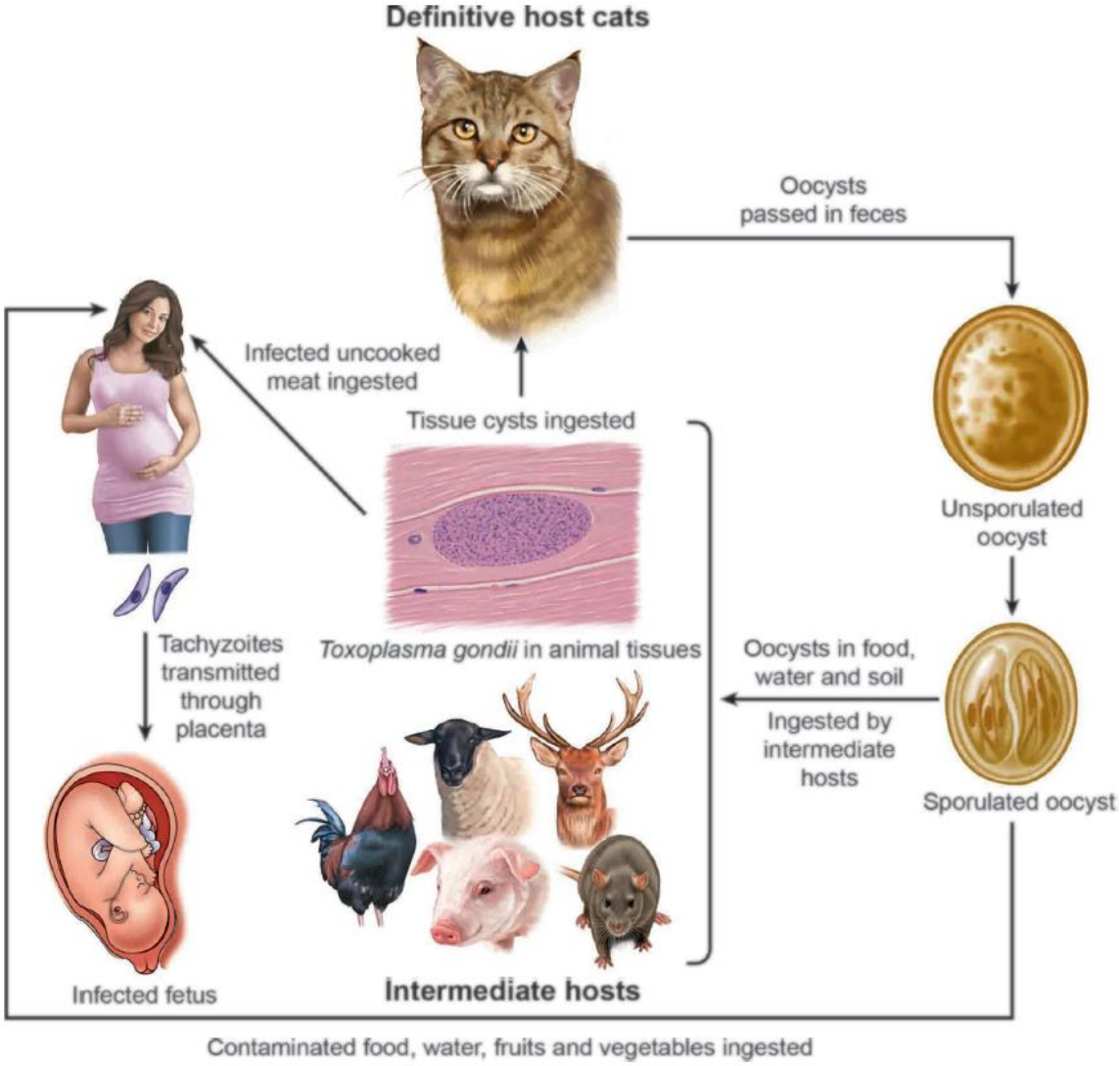

Figure 5. Life cycle of *T. gondii* (DUBEY 2022b)

2.4. Tenacity

The viability and infectivity of oocysts and tissue cysts subjected to different physical and chemical treatments have been extensively studied experimentally, with infectivity of *T. gondii* typically assessed by bioassay in mice and cats.

2.4.1. Oocyst

Among the developmental stages of *T. gondii*, the sporulated oocysts are undoubtedly the most resistant. The increased resistance of sporulated oocysts compared to non-sporulated oocysts is probably due to the development of sporocysts during the sporulation process and to changes in the structure of the oocyst wall. The wall of a sporulated oocyst consists of three layers and serves as a protective barrier against various physical and chemical influences (DUBEY et al. 1998). However, much is still unknown about the sporulation and survival of oocysts under natural conditions.

Experimental studies have shown that sporulation can occur in some cases at temperatures as low as 4°C, while temperatures of 50°C prevent sporulation within 10 minutes (DUBEY et al. 1970a; LINDSAY et al. 2002). Experiments by FRENKEL and DUBEY (1973) showed that non-sporulated oocysts are more sensitive than sporulated oocysts and that freezing is not an effective method for killing sporulated oocysts. In their experiment, non-sporulated oocysts survived in an aqueous suspension of cat faeces for 1-7 days at -6°C and -21°C before being transferred to room temperature to permit sporulation, while sporulated oocysts remained infectious after 28 days at -20°C. Similar observations were made by KUTICIC and WIKERHAUSER (1996), who found that oocysts suspended in water at -20°C were inactivated after three weeks. DUBEY (1998) carried out a further study which showed that sporulated oocysts are more sensitive to lower than to higher temperatures. After 106 days of exposure at -5°C and -10°C, sporulated oocysts remained infective in an aqueous medium. At 35°C they survived for 32 days, but at 55°C they were killed within 2 minutes. Temperature fluctuations appear to have little effect on the survival of oocysts in soil, and oocysts can remain infectious even after severe winters, as shown by two studies. Research carried out in Texas showed that sporulated oocysts could survive in uncovered cat faeces at temperatures ranging from 6°C to 36°C for 46 days, and in covered cat faeces for 334 days, with extreme temperatures ranging from -6°C to +39°C (YILMAZ and HOPKINS 1972). Another experiment in Kansas showed

that oocysts in cat faeces buried shallowly in the soil (3–9 cm) survived temperatures of -20°C to 35°C for up to 18 months (FRENKEL et al. 1975).

T. gondii oocysts have also shown considerable resistance to detergents and disinfectants such as sodium hypochlorite, ozone, and other chemicals used in the food and water industries (DUBEY et al. 1970b; KUTICIC and WIKERHAUSER 1996; WAINWRIGHT et al. 2007). Consequently, fresh products (fruits and vegetables) have recently been recognised as an underestimated source of infection for humans (SHAPIRO et al. 2019). Many of these products are pre-washed and pre-packaged by producers and are sold in stores as 'ready-to-eat' (RTE). One study found that sporulated oocysts on raspberries stored at 4°C could survive for 8 weeks (KNIEL et al. 2002). In more recent studies, oocysts have been found in various vegetables, including RTE salads (CARADONNA et al. 2017; SHAPIRO et al. 2019; SLANY et al. 2019; CALERO-BERNAL et al. 2025). LACOMBE et al. (2017) investigated the sensitivity of oocysts on blueberries to gamma radiation and concluded that it could potentially be used routinely to inactivate oocysts on fruit and vegetables. Ultraviolet radiation also has a deleterious effect on oocysts, although the effects depend on the dose (WAINWRIGHT et al. 2007; DUMÈTRE et al. 2008;).

Interestingly, *T. gondii* oocysts can sporulate and remain viable in salt water for several months, as demonstrated in an experimental study (LINDSAY et al. 2003). This prolonged survival and infectious ability may contribute to the infection of cetaceans and other marine mammals (DUBEY et al. 2020b).

2.4.2. Tissue cyst

Ingestion of viable *T. gondii* tissue cysts in the edible tissues and organs of mammals and birds is one of the main route of infection for both humans and animals. Numerous studies have explored the inactivation of tissue cysts in organs using various physical methods, including thermal treatment (heating, cooking, freezing), irradiation, high pressure processing and curing. A comprehensive review of these studies was presented by MIRZA ALIZADEH et al. (2018). Early reasearch on the effects of freezing on the inactivation of tissue cysts are worth mentioning. JACOBS et al. (1960) found that tissue cysts in mouse brains frozen at -15°C for 24 and 48 hours lost their infectivity. Similarly, DUBEY(1974) reported that tissue cysts in mouse brains stored at -9°C or -20°C for three hours to 7 days were no longer infectious. Tissue cysts in the muscles and brains of experimentally infected pigs also did not survive freezing at -7°C and -12°C for four days (KUTIČIĆ 1992). However, DUBEY and FRENKEL (1973)

observed in their experiment that some tissue cysts remained viable for up to 16 days at -16°C, suggesting that certain *T. gondii* isolates may have greater resistance to low temperatures. To reduce the number of viable tissue cysts, it is generally recommended to store meat at −20°C for at least three days (MIRZA ALIZADEH et al. 2018).

Conventional heat treatment such as cooking and baking, effectively eliminates *T. gondii* tissue cysts in foods of animal origin. DUBEY et al. (1970b) demonstrated that tissue cysts were destroyed after exposure to 55°C for 30 minutes. Similarly, KUTIČIĆ and WIKERHAUSER (1994) found that tissue cysts in mouse brains cooked at 58°C for 15 to 30 minutes lost their infectivity. Other studies, summerized by MIRZA ALIZADEH et al. (2018) confirm that cooking meat and meat products at temperatures between 60°C and 70°C is sufficient to inactivate *T. gondii* tissue cysts.

High hydrostatic pressure has also been shown to be effective in inactivating tissue cysts of *T. gondii*, as well as other foodborne parasites, under laboratory conditions. Studies suggest that a pressure of 340–400 MPa applied for one minute can inactivate *T. gondii* in food. However, this process can negatively affect the colour and texture of food, making it currently unsuitable for widespread use in the food industry (MIRZA ALIZADEH et al. 2018).

Ionising irradiation with gamma rays and X-rays has been investigated in some studies as a method for inactivating tissue cysts of *T. gondii* and proven to be successful in most cases (DUBEY et al. 1986; KUTIČIĆ et al. 1989; CHANG-CUN et al. 1993; DUBEY and THAYER 1994). Notably, WIKERHAUSER et al. (1988) observed that the effective dose of gamma irradiation depends on the specific *T. gondii* isolate, as shown in studies with mouse brains.

Several studies indicate that *T. gondii* tissue cysts do not survive the curing process, with inactivation depending on factors such as maturation time, storage temperature, and salt concentration (DUBEY 1997; HILL et al. 2004, 2006). A recent study found that a low NaCl concentration (1.3%) in dry-cured pork sausage inactivated tissue cysts within the first four hours of fermentation (FREDERICKS et al. 2019). Similarly, HILL et al. (2018) reported that bradyzoites in pork sausage were inactivated during the curing process by a salt solution with a concentration of 1.3% or higher within the first six hours of fermentation. These results indicate that bradyzoites cannot survive the fermentation process, even at lower salt concentrations than those commonly used in curing process. Other authors have demonstrated the susceptibility of tissue cysts to salt concentrations of 2% and above (DUBEY 1997; HILL et al. 2004; DÁMEK et al. 2023). DUBEY (1997) investigated the effects of storing rodent

brains containing *T. gondii* tissue cysts in various NaCl solutions at different temperatures and found that increasing the salt concentration and temperature reduces the survival time of tissue cysts. HILL et al. (2004) demonstrated the susceptibility of tissue. cysts in mouse brains and pork loins to a 2% NaCl solution. Similarly, DÁMEK et al. (2023) showed that processing with NaCl content of 2% and above in traditional French dry sausage and processed pork inactivates tissue cysts.

2.5. Diagnosis

The diagnosis of toxoplasmosis in humans and animals lacks standardisation in most European countries, particularly regarding the type of tissue examined and the diagnostic methods used. Bioassays in laboratory animals have traditionally been considered the gold standard, as they directly confirm the presence and infectivity of *T. gondii*. However, these assays are expensive, time-consuming, and impractical for processing large numbers of samples. Currently, serological tests are the most commonly used method for diagnosing toxoplasmosis in both humans and animals.

Rapid diagnosis of *T. gondii* infection is particularly important in humans, as primary infections can occur in pregnant women, where accurate and timely testing is essential to enable early treatment and prevent congenital transmission to the fetus. In animals, serological tests are used to diagnose *T. gondii*-related abortions in small ruminants and to assess the presence of the parasite in production animals, as well as the risk of human infection from consumption of raw or undercooked meat. Various serological tests have been developed to meet this need, including Sabin-Feldman dye test (DT), indirect fluorescent antibody test (IFAT), enzyme-linked immunosorbent assay (ELISA), enzyme-linked fluorescence assay (ELFA), chemiluminiscent immunoassay (CLIA), indirect haemagglutination test (IHA), modified agglutination test (MAT), Western blot (WB), and others, each with its own advantages and limitations (LIU et al. 2015).

The DT, developed by Sabin and Feldman over seven decades ago, was long considered the gold standard for the detection of anti- *T. gondii* specific antibodies in humans (SABIN and FELDMAN 1948; REITER-OWONA et al. 1999). The DT uses live *T. gondii* tachyszoites for an agglutination reaction, which limits its availability due to the high cost and technical expertise required for antigen production. As a result, only specialised reference laboratories can perform the analysis (REITER-OWONA et al. 1999; LIU et al. 2015). This major drawback

has rendered the DT largely redundant, so that it is rarely used today. In addition, the test has proved to be unreliable for the diagnosis of toxoplasmosis in cattle (DUBEY et al. 1985).

A recent meta-analysis found that ELISA is the most commonly used test in humans and animals (HUERTAS-LÓPEZ et al. 2023). Various ELISA formats have been developed for the detection of anti-*T. gondii* antibodies or antigens, including indirect ELISA, sandwich ELISA, and dot-ELISA. The indirect ELISA is the most commonly used type for the diagnosis of toxoplasmosis in many domestic animals, probably due to the availability of species-specific secondary antibodies (LIYANAGE et al. 2021). In conventional indirect ELISA, a microtitre plate is coated with *T. gondii* antigens and serum samples are applied. Anti-*T. gondii* antibodies bind to the coated antigens and are detected with secondary, enzymatically bound antibodies. After washing out unbound reagents, a substrate is added that produces a colour reaction indicating the presence of antibodies (YBAÑEZ et al. 2020) (Figure 6). This method is mostly used to detect anti-*T. gondii* IgG or IgM antibodies in humans and animals and shows high agreement with DT, IFAT and MAT (KLUN et al. 2007; GLOR et al. 2013; DESHMUKH et al. 2021; HEBBAR et al. 2022; LÓPEZ-UREÑA et al. 2023; KIM et al. 2024). ELISA is easily implemented and allows rapid processing of large sample sizes, making it ideal for high-throughput testing. However, standardisation remains a challenge due to varying protocols, reagents, and diagnostic accuracy across different tests. Numerous ELISA kits are available for diagnosing toxoplasmosis in domestic animals, some of which can be used to test both meat juice and serum. This feature makes ELISA particularly valuable for epidemiological studies, especially those involving meat intended for human consumption. However, a comparative study of serological tests for toxoplasmosis in pigs showed differences in performance between ELISA kits, highlighting the need for harmonised serological methods to ensure consistent and reliable results (LÓPEZ-UREÑA et al. 2023). Additionally, the limited availability of species-specific secondary antibodies for other animals, such as wildlife, restricts the applicability of the test mainly to domestic animals.

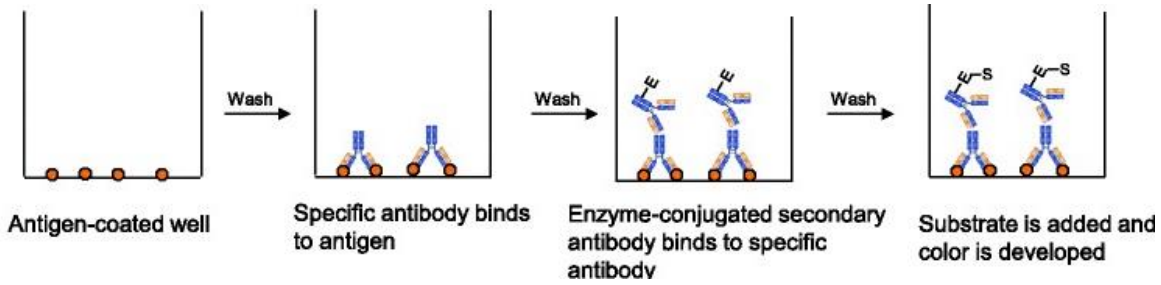

Figure 6. Schematic diagram of indirect enzyme-linked immunosorbent assay (ELISA)

(LIU et al. 2015)

Besides ELISA, IFAT and the MAT are often used for the diagnosis of toxoplasmosis in animals (HUERTAS-LÓPEZ et al. 2023). IFAT involves incubating serum samples with inactivated *T. gondii* tachyzoites, then adding fluorescently labelled antibodies, which are observed under a fluorescence microscope. This test is commonly used to detect IgG and IgM anti-*T. gondii* antibodies in humans and animals (BACHAN et al. 2018; SROKA et al. 2018; HEBBAR et al. 2022; SHAMS et al. 2024; VOYIATZAKI et al. 2024). Although the test is relatively simple and inexpensive, it has some limitations. It requires a fluorescence microscope, and the results depend on visual interpretation, which can lead to variation. In addition, fluorescently labelled antibodies are species-specific and suitable conjugates may be difficult to obtain for some animal species (LIU et al. 2015).

The MAT is one of the most commonly used tests for toxoplasmosis research in animals. This agglutination test was standardised in the 1980s by DESMONTS and REMINGTON (1980) and DUBEY et al. (1985), and does not require conjugated secondary antibodies. It uses formalin-fixed *T. gondii* tachyzoites (antigen), which are produced *in vivo* in only a few specialised laboratories. The diluted sera are applied to U-shaped microtitre plates, followed by fixed *T. gondii* tachyzoites. Positive samples form a thin mat of agglutination, while negative samples form a compact pellet at the bottom of the well. The MAT is versatile, can be applied to all animal species, and allows for comparable results across species (KLUN et al. 2006). Besides serum, it can also test muscle juice, as haemolysis does not affect the results (VILLENA et al. 2012; DJOKIC et al. 2016a). Its simplicity makes it convenient for laboratory diagnosis and epidemiological studies, including testing large numbers of samples. However, the test detects only IgG antibodies and can give false negative results in the early stages of acute infection (DUBEY 2022a). To date, the MAT has been used in serological studies on

toxoplasmosis in cattle, pigs, sheep, chickens, dogs, cats, zoo animals, wild animals, and humans (DUBEY 2022a). The lack of species-specific conjugates makes it particularly valuable for diagnosing toxoplasmosis in wild and zoo animals (FERNÁNDEZ-AGUILAR et al. 2013; TIDY et al. 2017; CANO- TERRIZA et al. 2020; ALMERÍA et al. 2021).

In addition, the MAT has been used to assess the risk of human infection with *T. gondii* through the consumption of meat from domestic animals in France and Serbia and from wild boar meat in France and Poland (RICHOMME et al. 2010; KLUN et al. 2011; DJOKIC et al. 2016a; KURUCA et al. 2017; ROQUEPLO et al. 2017; BLAGA et al. 2019; KORNACKA et al. 2020).

2.6. Toxoplasmosis: a global public health concern

In addition to its previously mentioned significance as a food- and waterborne zoonosis, toxoplasmosis poses a major public health challenge due to its worldwide distribution. It is estimated to infect approximately one-third of the world's population (ROSTAMI et al. 2020). Humans can become infected with *T. gondii* through three main routes: (1) consumption of inadequately heat-treated meat containing tissue cysts with bradyzoites; (2) consumption of food (e.g. fruit and vegetables) and water contaminated with sporulated oocysts; and (3) transplacental transmission of tachyzoites from mother to foetus (TENTER et al. 2000; ALMERIA and DUBEY 2021).

Humans are less likely to become infected by consuming raw milk containing tachyzoites or sporulated oocysts. For example, DUBEY et al. (2014) demonstrated transmammary transmission of tachyzoites during the acute phase of infection in experimentally infected goats, suggesting that raw milk and fresh cheese could be potential sources of infection for humans. Although *T. gondii* DNA has been detected in milk from goats, sheep, cows, donkeys and camels in various studies, there is no conclusive evidence of transmammary tachyzoite transmission, and contamination with oocysts in those studies cannot be excluded (MANCIANTI et al. 2014; ROCHA et al. 2015; SROKA et al. 2017; IACOBUCCI et al. 2019; DELJAVAN et al. 2022; ÇULBASAN et al. 2023; DAHMANE et al. 2025). Additionally, it is well established that tachyzoites are highly sensitive to gastric juice and do not survive exposure to gastric pH (DUBEY et al. 1998).

To date, documented cases of acute toxoplasmosis in humans linked to raw milk consumption have primarily involved goat milk. Evidence from reported outbreaks were often

described as circumstantial, relying on epidemiological associations rather than definitive isolation of viable parasite directly from the implicated milk (DUBEY et al. 2014).

Infection can also occur through transfusion of blood donations containing tachyzoites, organ transplants with tissue cysts, or reactivation of a chronic infection in transplant recipients (FOROUTAN-RAD et al. 2016; ROBERT-GANGNEUX et al. 2018). These routes of transmission are rare, as widespread screening of blood and organ donors for specific IgG and IgM anti-*T. gondii* antibodies is usually performed, which is mandatory for organ exchange in many European countries (ROBERT-GANGNEUX et al. 2018). In Croatia, testing of organ donors has been mandatory since Croatia joined the Eurotransplant programme in 2006 (MIHALJEVIĆ et al. 2013; EUROPEAN COMMITTEE ON ORGAN TRANSPLANTATION 2022).

Outbreaks of toxoplasmosis in humans are rarely noticed or documented, probably due to the non-specific nature of the symptoms (EFSA 2018). Most documented water- and foodborne outbreaks have been reported from Brazil and the USA, with DUBEY (2022a) providing detailed overview in his book. Waterborne outbreaks are usually associated swith municipal water reservoirs contaminated with sporulated oocysts, while foodborne outbreaks are primarily associated with the consumption of undercooked venison, beef or lamb (ALMERIA and DUBEY 2021; DUBEY 2022a).

As previously mentioned, European legislation and EFSA have acknowledged toxoplasmosis as an important zoonosis that needs to be monitored in production animals through meat inspections. In Croatia, the examination of pork for trichinellosis and cysticercosis is obligatory (ANONYMOUS 2019a). However, such mandatory testing does not extend to other meatborne parasitic zoonoses.

2.7. Toxoplasmosis in humans

2.7.1. Clinical manifestation

Toxoplasmosis can be classified into acquired and congenital forms. In immunocompetent individuals, acquired toxoplasmosis is latent in approximately 90% of cases (MCCALL et al. 2022). When symptoms do occur, they are usually non-specific and the disease is generally self-limiting. Acute infection often presents with symptoms similar to those of a viral infection, including general fatigue, headache, excessive sweating, myalgia, joint pain, mild fever, and occasionally a maculopapular rash. Suspicion of toxoplasmosis arises when patients develop generalised lymphadenopathy, particularly involving the cervical and occipital lymph nodes, which may persist for several months and usually resolves spontaneously. In immunocompetent individuals, severe symptoms such as myocarditis, polymyositis, hepatitis, pneumonitis, or encephalitis are rare (MONTOYA and LIESENFELD 2004; SOBANSKI et al. 2013). In immunocompromised individuals, however, toxoplasmosis can be life-threatening (MCCALL et al. 2022). It is most commonly seen in AIDS patients, where it often results from the reactivation of a chronic infection and typically manifests as encephalitis characterised by seizures, incoordination, mental confusion, and sensory abnormalities (MONTOYA and LIESENFELD 2004). Although fatal toxoplasmosis is most commonly observed in immunocompromised patients, it can occasionally occur in individuals without obvious immunodeficiency (LAYTON et al. 2023).

Ocular toxoplasmosis, or toxoplasmic necrotising chorioretinitis, is a specific clinical form of the disease that may develop as a late manifestation of a congenital infection or as a consequence of a postnatally acquired acute infection. Symptoms include visual disturbances, eye pain, and in severe cases, complete loss of vision (MONTOYA and LIESENFELD 2004).

Congenital toxoplasmosis occurs following acute infection in a pregnant woman, resulting in transplacental transmission of *T. gondii* tachyzoites to the fetus, often without clinical symptoms in the mother. This form of infection only occurs in women who have not previously been exposed to the parasite and become infected for the first time during pregnancy. The risk of congenital infection is lowest in the first trimester and increases as the pregnancy progresses (DUBEY et al. 2021). It is noteworthy that 60-70% of babies born to infected mothers escape infection (MONTOYA and LIESENFELD 2004). The severity of the clinical presentation depends primarily on the trimester in which the mother becomes infected. Infections in early pregnancy are particularly dangerous and often lead to spontaneous abortion or stillbirth, while

later infections can lead to mild to severe damage to the central nervous system and eyes, various developmental disorders, or even death of the newborn. Severe forms of congenital toxoplasmosis often manifest as chorioretinitis, blindness, epilepsy, mental retardation, encephalitis, or hydrocephalus (DUBEY et al. 2021). Infants who were exposed to *T. gondii in utero* during the later stages of pregnancy may develop congenital toxoplasmosis years after birth, usually presenting as ocular toxoplasmosis of varying severity.

2.7.2. Seroprevalence of *T. gondii* in the world and Europe

Serological tests provide valuable insight into the prevalence of *T. gondii* infections within populations. Given the severity of congenital toxoplasmosis, it is not surprising that most studies have focused on pregnant women. A recent systematic review and meta-analysis estimated the global seroprevalence (IgG antibody) of *T. gondii* in pregnant women to be 32.9% (BIGNA et al. 2020). The highest seroprevalence was observed in Ethiopia (64%), Gabon (57%) and Brazil (54%), while the lowest was reported in Mexico (7%), South Korea (2%) and Canada (0.2%). This study also looked at seroprevalence in the different World Health Organization (WHO) regions and found that the highest rates were in the Americas (45.2%) and the Eastern Mediterranean (39.7%), and the lowest in Western Pacific (11.2%). In Europe the seroprevalence was estimated at 30%, which is consistent with the results of another meta-analysis conducted by ROSTAMI et al. (2020). Among European countries, the highest pooled seroprevalence among pregnant women was estimated in Hungary (64.8%), Belgium (53.5%) and Albania (48.6%). Significant regional and country-specific differences are attributed to variations in climate, dietary habits and lifestyle. Higher seroprevalence is more commonly observed in regions with mild and humid climates that favour sporulation and oocyst viability, which facilitates transmission and increases the infection rates. In terms of lifestyle, the seroprevalence of *T. gondii* is estimated to be lower in high-income countries, as supported by the review of ROSTAMI et al. (2020) who evaluated the lowest seroprevalence rates in Norway (10.8%), the UK (12.3%) and Sweden (16.8%).

Beyond pregnant women, several studies have investigated the seroprevalence of *T. gondii* in blood and organ donors, as well as immunocompromised individuals. A recent study conducted in Greece among HIV-positive patients reported a seroprevalence of 31.6%, which is similar to the global average (VOYIATZAKI et al. 2024). An earlier meta-analysis by WANG et al. (2017) provided a global overview of seroprevalence in immunocompromised populations, including HIV/AIDS patients, cancer patients and transplant recipients, and

showed higher odds of *T. gondii* infection in these groups. A global review of toxoplasmosis in solid organ transplant recipients assessed the low pooled seroprevalence rates for IgG (9.8%) and IgM (6.4%) anti-*T. gondii* antibodies. However, the authors emphasised the need for increased awareness and prevention, as primary infection or reactivation of a latent infection in such individuals is life-threatening (KHURANA and BATRA 2016; MAMIZADEH et al. 2025). FOROUTAN-RAD et al. (2016) estimated in their meta-analysis that 33% of blood donors worldwide are infected with *T. gondii*, a figure consistent with global seroprevalence. The highest rates were observed in Africa (46%) and the lowest in Asia (29%). Interestingly, at the country level, Brazil (75%) and Ethiopia (73%) were estimated to have the highest seroprevalence, reflecting the seroprevalence in pregnant women reported in the meta-analysis conducted by BIGNA et al. (2020). In Europe, studies on blood donors reported the highest seroprevalence in Romania (45.9%), followed by Portugal (38.1%), Bosnia and Herzegovina (30.6%), Turkey (25.6%) and Serbia (20.5%). It is important to note that these studies used different serological methods to detect IgG anti-*T. gondii* antibodies, which may limit direct comparisons.

2.7.3. Seroprevalence of *T. gondii* in Croatia

A recent review and meta-analysis estimated the pooled seroprevalence of latent toxoplasmosis in pregnant women in Croatia to be 28.7% (ROSTAMI et al. 2020). However, there are eleven studies on the seroprevalence of *T. gondii* infection in humans in Croatia (Table 1). Investigations in the past 20 years have mostly used ELISA and advanced ELISA-based tests such as CLIA, ELFA, and IgG avidity tests, which provide high specificity and sensitivity, enhancing diagnostic accuracy (VILLARD et al. 2016).

Table 1. Studies on seroprevalence of *T. gondii* in humans in Croatia

Study area	Population group	No. examined	Ig[1] class	Positive (%)	Test	Remarks	Reference
Zagreb	Non-pregnant women 18-40 years of age	200	IgG	53	IFAT[2]	Age association with seroprevalence. Higher titres in younger women	DERKOS-MIKULIĆ, 1972
	Parturients 18-40 years of age	200		49			
	Neonates	200		49			
Kutina	Pregnant women	342	IgG	70.2	IFAT	Rural area, domestic animal contact assoc. Raw meat consumption assoc. with higher titres. Acute infection in 13% pregnant women	KONJEVIĆ, 1978
	Control group: Women Men	181 96		73.5 72.4			
	Newborns	18	IgM	5.5			
Zagreb	Female and male patients from 13-59 years of age with begnign lympadenopathy	167	IgM IgG	70 70	DT[3]	Subjects suspected for toxoplasmosis by cytology of lymph nodes tested	JEREN et al. 1991
Splitsko-dalmatinska County	Healthy female subjects 2-84 years of age	*1109 (398 pregnant)	IgG	38.1	ELISA[4]	*31.4% pregnant women positive	PUNDA-POLIĆ et al. 2000
	Neonates	104		30.8			
Splitsko-dalmatinska County	Male and female subjects 8-84 years of age	1464 (355 males *1109 females	IgG	36.4 (30.9, 38.1,	ELISA	*31.4% pregnant women positive	TONKIĆ et al. 2002

		– 398 pregnant women)		31.4)			
Croatia	HIV[5]-patients	166 (43 females and 123 males)	IgA IgM IgG	1.2 1.2 51.8	CLIA[6] IgG avidity test	HIV-patients have 2.7 times greater likelihood of being IgA positive	ĐAKOVIĆ-RODE et al. 2010
	Blood donors	219 (46 females and 173 males)		0.5 0.9 52.5			
Croatia	Pregnant and non-pregnant women 16-45 years of age	502	IgM IgG	2.4 29.1	ELFA[7] IgG avidity test	Age and rural area association with seroprevalence. 0.5% acute infection	VILIBIĆ-ČAVLEK et al. 2011
Nationwide	Organ donors	642	IgM IgG	0.9 70.9	ELFA		MIHALJEVIĆ et al. 2013
Zadarska County	Female and male patients	2 156	IgM IgG	1.4 24	ELFA IgG avidity test	1.4% acute infection (4.4% males and 0.6% females)	PERICA et al. 2016
Croatia	Male schizophrenic patients	210	IgG	52.4	ELFA	TRS association with seroprevalence	VLATKOVIC et al. 2018
	TRS[8]-group / Non TRS-group	100 / 110		70 / 36.4			
Nationwide	Childbearing-aged and pregnant women 16-45 years of age	2791	IgM IgG	1.9 25	ELFA IgG avidity test	Age, settlement assoc. 1.2% acute/recent infection	SVIBEN et al. 2025

[1]Ig – immunoglobulin; [2]IFAT - indirect fluorescent antibody test; [3]DT – dye test; [4]ELISA – enzyme linked immunsorbent assay; [5]HIV – human immunodeficiency virus; [6]CLIA - chemiluminiscent immunoassay; [7]ELFA - enzyme linked fluorescent assay; [8]TRS - treatment-resistant schizophrenia; * the same group of female participants.

2.7.4. Risk factors associated with *T. gondii* infection

Consumption of raw or undercooked meat is consistently identified as a risk factor in epidemiological studies of *T. gondii* seroprevalence in humans (COOK 2000; TENTER et al. 2000). An older European multicentre study conducted on pregnant women, including selected cities in Belgium, Denmark, Italy, Norway, Switzerland, and the UK, identified raw meat consumption, contact with soil, and travel outside Europe and North America as strong risk factors for acquiring *T. gondii* infection (COOK 2000). The consumption of different types of raw meat is identified as a risk factor in various countries, probably due to differences in consumer eating habits or different prevalences of infection in meat-producing animals among countries. A recent systematic review and meta-analysis showed that, besides consumption of undercooked meat, consumption of unwashed vegetables, raw milk, and shellfish significantly increases the odds of acquiring toxoplasmosis. In this study, almost all meat categories were identified as risk factors: pork, poultry, beef, processed meat, lamb, and game meat (THEBAULT et al. 2021).

Professional groups such as abattoir workers, butchers, and hunters may also become infected during evisceration and handling of meat (TENTER et al. 2000). Contamination of soil and water with sporulated oocysts, as well as eating unwashed fresh products (vegetables and fruits), is also identified in another review as a risk factor (UREÑA et al. 2022). Contact with soil is shown to be associated with *T. gondii* infection, probably via gardening or farming, in a seroepidemiological study conducted in pregnant women in Belgrade (Serbia) and in a cross-sectional study conducted among blood donors (STOPIĆ et al. 2022; MARKOVIĆ-DENIĆ et al. 2023). Recently, two studiess carried out in pregnant women and blood donors in Romania also identified low educational level as an important risk factor for seroconversion (OLARIU et al. 2020; LUPU et al. 2022).

2.7.5. Control and prevention

Toxoplasmosis poses a significant challenge for public health surveillance, particularly in detecting acute infections in pregnant women and immunocompromised individuals. Primary prevention of toxoplasmosis focuses on educating the public about the risks of consuming raw or undercooked meat and promoting hygiene practices such as handwashing and thorough washing of fresh products (fruits and vegetables). Secondary prevention, especially for congenital toxoplasmosis, relies heavily on serological testing for *T. gondii*, which detects

specific IgG and IgM antibodies to assess infection status in pregnant women throughout pregnancy. According to the One Health Zoonoses Report for 2023, only ten countries have active surveillance programmes for congenital toxoplasmosis. Six countries, France, Austria, Belgium, Slovakia, Slovenia and Greece, require mandatory screening for pregnant women, while voluntary screening is offered in Bulgaria, the Czech Republic, Germany and Hungary (EFSA and ECDC 2024). Between 2018 and 2022, a decline in congenital toxoplasmosis cases was observed in Germany, France and Poland. In France, a continuous downward trend has been observed over the last three decades, which is due to an effective surveillance programme that has been in place since 1992. This programme facilitates early detection and treatment of infections and provides accessible guidelines for the prevention of toxoplasmosis during pregnancy (SAWERS et al. 2022). It includes compulsory serological screening in the first trimester, followed by monthly testing of seronegative women until delivery. Owing to this robust surveillance system, France reported 74.6% of all congenital toxoplasmosis cases in the EU in 2022 (an average of 128 cases per year from 2018 to 2022), despite the overall decline (EFSA and ECDC 2024). The declining seroprevalence of toxoplasmosis in French pregnant women over the last 50 years is partly attributed to changing dietary habits and improved hygiene practices (PICONE et al. 2020).

A similar downward trend in seroprevalence among pregnant women has been observed in Serbia and Italy, likely due to increased awareness of *T. gondii* infection, improved socioeconomic conditions and changes in dietary habits (FANIGLIULO et al. 2020; STOPIĆ et al. 2022; MARKOVIĆ-DENIĆ et al. 2023). In Croatia there is no national screening programme for *T. gondii* during pregnancy, although tests are sometimes offered as part of antenatal care. When offered, these may include toxoplasmosis, other infections, *Rubella*, *Cytomegalovirus* and *Herpes simplex virus* (TORCH) panel or *T. gondii* IgM and IgG avidity antibody tests to detect recent infections, with costs usually covered by primary health insurance.

2.8. Toxoplasmosis in animals

2.8.1. Clinical manifestation

Similar to humans, toxoplasmosis in animals is usually asymptomatic. Sheep and goats are the most vulnerable species, as they are susceptible to congenital toxoplasmosis. The severity of symptoms depends on the stage of gestation at which the infection occurs, with earlier infections leading to more severe clinical outcomes, similar to those observed in humans. In sheep, toxoplasmosis is a leading cause of stillbirths and abortions in New Zealand, the United Kingdom, Norway, and the USA, resulting in significant economic losses (DUBEY et al 2020c). Goats appear to be more susceptible to *T. gondii* than sheep. Cases of acute toxoplasmosis in adult goats have been reported and were fatal. Congenital infections in goats can lead to mummification, maceration, abortions, stillbirths, or the birth of weak kids that die shortly after birth (DUBEY et al. 2020d).

Clinical manifestations of toxoplasmosis have also been described in other domestic animals, including dogs, cats, pigs, cattle, and chickens. Dogs and cats rarely show clinical signs of toxoplasmosis, which are often associated with immunosuppressive therapy. Symptoms are varied and range from general signs such as fever and dyspnoea to more specific neurological, respiratory, ocular, and cutaneous manifestations. In cats, congenital infections are the most severe and can lead to hepatitis, pneumonia, and encephalitis in kittens. Fatal cases of acute toxoplasmosis have been reported in adult cats (CALERO-BERNAL and GENNARI 2019). Pigs rarely show clinical signs of toxoplasmosis such as fever, dyspnoea, skin cyanosis, pneumonia, or lymphadenopathy, and the disease is considered a rare cause of reproductive losses, including stillbirths and abortions (DUBEY et al. 2020a). Cattle are generally resistant to clinical toxoplasmosis, with congenital infections and subsequent abortions occurring only in rare cases (DUBEY 2022a).

Research over the last decade has shown that *T. gondii* is also a common parasite in wildlife. In certain wild canids, such as wolves and coyotes, no clinical cases have been reported. In contrast, numerous clinical cases have been documented in other wild carnivores, such as foxes and mustelids. Fatal toxoplasmosis, including congenital infections, has been reported in minks, other mustelids and civets (DUBEY et al. 2021b). Fatal cases have also been documented in European and Iberian hares (JOKELAINEN et al. 2011; FERNÁNDEZ-AGUILAR et al. 2013; TSOKANA et al. 2020). In marine mammals such as sea otters,

dolphins, sea lions, seals, and whales, toxoplasmosis is associated with increased morbidity and mortality (DUBEY et al. 2020b).

Clinical toxoplasmosis has been described in numerous animals in captivity, some of which have succumbed to the disease. This susceptibility is likely due to evolutionarily novel exposure, as these species have evolved largely separately from the parasite. Wild felids in captivity, particularly Sand cats and Palla's cats, are considered very susceptible and there are reports of fatalities (DUBEY et al. 2020e). Australian marsupials have long been known to be susceptible to toxoplasmosis, with numerous reports of fatal outcomes worldwide (DUBEY 2022c; GONG et al. 2025). New World non-human primates, such as howler monkeys, squirrel monkeys, ansd ring-tailed lemurs, are highly susceptible to *T. gondii* infection compared to Old World non-human primates, and there are documented cases of clinical toxoplasmosis, including fatalities and congenital infections. Clinical toxoplasmosis has also been reported in various avian species in zoos (DUBEY 2022c).

2.8.2. Seroprevalence of *T. gondii* in the world and Europe

T. gondii has been found in almost all species of domestic and wild mammals and birds using various diagnostic methods. Numerous studies across the world have investigated the seroprevalence of toxoplasmosis in domestic animals, using a variety of serological tests. Some of the highest seroprevalence rates were found in sheep (98.4%) in Egypt using ELISA on a small sample of 62 animals, and in pigs (96.6%) in Mexico using ELISA on 60 blood samples collected at the slaughterline (GHONEIM et al. 2010; HERNÁNDEZ-CORTAZAR et al. 2016). On the other hand, the lowest seroprevalence rates were detected in pigs (1.4%) in Mexico using the MAT, and in cattle (1.9%) from China using the MAT (DONG et al. 2018; CUBAS-ATIENZAR et al. 2019). In Europe, high seroprevalence was detected in sheep (98.9%) in Turkey using the DT and in goats (73.3%) in Serbia using the MAT, while low seroprevalence was found in pigs (2.1%) in Italy using the MAT and in horses (1.7%) in Greece using ELISA (KOUAM et al. 2010; ÇIÇEK et al. 2011; DJOKIĆ et al. 2014; PAPINI et al. 2017).

Numerous serological studies have also been carried out on wild animals in the last two decades. For example, some of the highest seroprevalence rates were found in Egyptian mongooses (85.3%) in Portugal using the MAT, in feral cats (84.7%) in the Balearic Islands, Spain using the MAT, and in foxes (84.7%) in Germany using WB (MILLÁN et al. 2009; HERRMANN et al. 2012; WAAP et al. 2016). High seroprevalence was also observed in

American minks in Spain (78.8%) and the USA (77%) using the MAT, and in coyotes (72.7%) in the USA using the MAT (AHLERS et al. 2015; GERHOLD et al. 2017; RIBAS et al. 2018). Among the lowest seroprevalence rates in wildlife were those in mouflons (1.3%) in Spain and rabbits (2.8%) in Portugal, both assessed using the MAT (WAAP et al. 2016; ALMERÍA et al. 2021).

In most previously cited studies, IgG anti-*T. gondii* antibodies were detected in animal serum samples. Many recent studies have also examined alternative matrices, such as meat juice, to determine the seroprevalence of *T. gondii*. These studies focus primarily on domestic and game animals, particularly those whose meat is intended for human consumption, to highlight the importance of toxoplasmosis seroprevalence in these species and the potential risk of human infection. For example, PLAZA et al. (2020) used ELISA and the MAT to detect IgG anti-*T. gondii* antibodies in meat juice from retail beef, chicken, lamb, pork, and venison.

Similarly, HAMID et al. (2020) tested meat juices from retail beef, goat meat, and lamb in Malaysia, along with diaphragm samples collected at slaughterhouses, using ELISA. Several other studies have also used ELISA on meat juice samples for IgG detection: diaphragm and heart from goats and sheep in Italy, diaphragm from pigs in Switzerland, and heart from wild boars in Italy (GAZZONIS et al. 2020; KELBERT et al. 2021; SINI et al. 2024). Meat juice is proposed as an alternative when blood sampling is difficult or impractical, which is often the case for animals in the food supply chain, particularly pigs. However, the results of serological tests using meat juice appear to be less reliable for detecting low antibody levels, as the specific antibody concentrations are lower in meat juice compared to serum (WALLANDER et al. 2015). Therefore, serum remains the preferred matrix to obtain more accurate seroprevalence data and provide more reliable results in seroepidemiological studies.

2.8.3. Seroprevalence of *T. gondii* in Croatia

Several serological studies using different serological tests have been carried out on domestic and wild animals in Croatia. In a serological study using the MAT, 14% of goats on two farms in Varaždinska County tested positive (RAJKOVIĆ-JANJE et al. 1993). A year later, RAJKOVIĆ-JANJE et al. (1994) found a seroprevalence of 11.6% among sheep and 9.4% in lambs using MAT. Another study in central Croatia reported a seroprevalence of 48.4% in sheep on ten farms using ELISA (MARINCULIĆ et al. 1997). In Vukovarsko-srijemska County, 86 pig sera were tested with a commercial ELISA, revealing a seroprevalence of 62% (BARIĆ 2012). KIŠ et al. (2021) analysed the muscle juice of 92 domestic pigs and 92 wild

boars from Karlovačka County using ELISA and found a prevalence of 38% in domestic pigs and 55.4% in wild boars. A recent study on cardiac fluid from 103 wild boars in four hunting areas in Croatia reported a seroprevalence of 54.4% using MAT (GRBAVAC et al. 2025).

2.8.4. Seroprevalence of *T. gondii* and risk factors in pigs

According to a systematic review and meta-analysis of *T. gondii* seroprevalence in pigs, the estimated global seroprevalence is approximately 19% (FOROUTAN et al. 2019). A subsequent review by DUBEY et al. (2020a) noted that most serological studies in pigs relied on convenience blood samples collected at slaughter lines, with prevalence rates varying widely due to differences in the pig populations examined, such as fattening pigs versus sows, indoor versus outdoor farming systems, as well as the use of different serological tests.

Over the past decades, a substantial body of research in Europe has focused on the seroprevalence of toxoplasmosis in pigs destined for human consumption, covering various age groups such as piglets, fattening pigs, and breeding animals. These European studies report prevalence rates ranging from as low as 2.1% in intensively reared fattening pigs in Italy using the MAT to as high as 60% in sows on organic farms in Denmark using ELISA (PAPINI et al. 2017; OLSEN et al. 2020). In the Italian study, the low seroprevalence was attributed to the animals' confinement in closed farming systems; however, potential routes of infection for pigs in such environments remain speculative, as the study did not attempt to determine the risks associated with the occurrence of seropositive animals. In Denmark, rearing pigs on organic farms increases the odds of *T. gondii* infection 16-fold compared to conventional systems, while sows face a 22-fold higher risk of exposure than fattening pigs. Outdoor access and age in livestock are among the most frequently identified risk factors for acquiring *T. gondii* infection (STELZER et al. 2019). Many other epidemiological studies on pigs have shown that those raised outdoors or in indoor systems with access to outdoor areas have a substantially higher risk of *T. gondii* infection than pigs confined strictly indoors, particularly on large intensive farms. This pattern has been consistently observed in several European countries, including Poland, the Netherlands, France, Denmark, Latvia, and England (DEKSNE and KIRJUŠINA 2013; DJOKIC et al. 2016a; LIMON et al. 2017; SWANENBURG et al. 2019; OLSEN et al. 2020; SROKA et al. 2020; EPPINK et al. 2022). For example, in a study conducted in the Netherlands over four years by SWANENBURG et al. (2019) involving an exceptionally large sample size (226 340 pigs), the lowest seroprevalence of 1.4% using ELISA was detected among conventionally reared pigs, while the highest rate was 53% recorded in pigs on organic

farms. In the same study, the authors found that the overall risk of infection was 2.63 times higher for organically raised pigs than for those reared conventionally. In addition to housing type, it is well established that seroprevalence increases with age in most animal species, including pigs (OPSTEEGH et al. 2016; OLSEN et al. 2019). Age has been identified as a risk factor in several studies conducted in European countries such as Spain, France, Poland, Denmark, and Serbia, which examined risk factors across different pig age categories (GARCÍA-BOCANEGRA et al. 2010; DJOKIC et al. 2016b; OLSEN et al. 2020; SROKA et al. 2020; BETIĆ et al. 2022). This age-related trend undoubtedly contributes to the consistently higher seroprevalence reported in sows compared to fattening pigs in many serological surveys conducted in Europe and worldwide (DUBEY et al. 2020a; CASTILLO-CUENCA et al. 2021; AUGUSTYNIAK et al. 2025). European serological studies conducted in Italy, France, Spain, Denmark, and Estonia that included risk analysis have confirmed that sows are more likely to be seropositive than fattening pigs (DJOKIC et al. 2016a; KOFOED et al. 2017; SANTORO et al. 2017; GAZZONIS et al. 2018; PIPIA et al. 2018; CASTILLO-CUENCA et al. 2020; OLSEN et al. 2020).

In addition to important factors such as age, housing type, and pig production category, many authors have analysed and identified associations with other factors in seropositive pigs, including farm size, sex, presence of cats, poor hygiene and animal husbandry conditions, farm type, lower biosecurity level, absence of disinfection foot dips and Danish entry, type of feed storage, lack of rodent control, mode of rodent control, and type of drinking water (KLUN et al. 2006; GARCÍA-BOCANEGRA et al. 2010; VERONESI et al. 2011; DJOKIC et al. 2016a; HERRERO et al. 2016; LIMON et al. 2017; SANTORO et al. 2017; GAZZONIS et al. 2018; BLAGA et al. 2019; CASTILLO-CUENCA et al. 2020; SROKA et al. 2020; BETIĆ et al. 2022; EPPINK et al. 2022; CHEPYATICH et al. 2023).

2.8.5. Control and prevention

Prevention of toxoplasmosis in livestock production should focus on identifying and eliminating risk factors at the farm level. While the primary source of infection in domestic animals is ingestion of feed and water contaminated with sporulated oocysts excreted in cat faeces, pigs kept extensively could also become infected by accidentally ingesting rodent or other small animal carcasses containing tissue cysts. Appropriate biosecurity measures should therefore be implemented to minimise contact with oocysts. These include restricting access of cats (including free-roaming cats) to farms, ensuring proper storage of feed to prevent

contamination, applying measures to prevent water contamination with oocysts, and implementing effective rodent control (ALMERIA and DUBEY 2021). Interestingly, an older study showed that vaccinating cats with an experimental vaccine against *T. gondii* in the vicinity of the farm reduces the infection rate in pigs (MATEUS-PINILLA et al. 1999) In small ruminants, toxoplasmosis is particularly important as a cause of abortion, which has led researchers to focus on developing an effective vaccine as a preventive measure. Although a vaccine (Toxovax™, MSD Animal Health) is currently available and licensed for use in sheep in New Zealand, the United Kingdom, Ireland, and France, it provides only partial protection to ewes, while still helping to reduce reproductive losses (INNES et al. 2019). The objective of immunoprophylaxis should be to control congenital, acute, and chronic infections in small ruminants, thereby improving meat safety for human consumption and reducing environmental contamination with oocysts (HASAN and NISHIKAWA 2022). One control measure for congenital toxoplasmosis in small ruminants should be testing sheep and goats in all reported cases of abortion. However, in Croatia, current legislation on the prevention, control, and monitoring of animal diseases no longer requires ruling out toxoplasmosis as a cause of abortion in domestic animals (ANONYMOUS 2023).

As mentioned in the introduction, establishing routine testing for toxoplasmosis in domestic animals at the slaughter line could serve as an effective surveillance system – a measure not currently implemented anywhere and still only a suggestion. This is particularly relevant, as numerous studies based on blood samples collected at the slaughter line have demonstrated high seroprevalence in pigs, sheep, goats, cattle, and horses (LOPES et al. 2013; PENA et al. 2018; GAZZONIS et al. 2020; SROKA et al. 2020; BETIĆ et al. 2022; CANO-TERRIZA et al. 2023; CONDOLEO et al. 2023).

2.9. Seroprevalence of *T. gondii* in pigs in Croatia – a pilot study

We conducted a pilot study on a small sample of pigs from Croatia to evaluate the seroprevalence of *T. gondii* and to calculate the sample size required for a large-scale study. The pilot study performed at the laboratory of the Department of Parasitology and Parasitic Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb, used a commercial indirect ELISA (PrioCHECK^{TM} Porcine Toxoplasma Ab Kit, ThermoFisher Scientific, Germany) for detection of IgG anti-*T. gondii* antibodies in 92 fattening pig sera and 92 sow sera. ELISA was chosen because the MAT was not available at the time. The ELISA test is commercially available, with sensitivity and specificity according to the manufacturer of 98% and 99.6%, respectively. In the test kit, the antigen (protein extract from cell culture-derived tachyzoites) is bound to the walls of a polystyrene microtitration plate (microplate) containing 96 (12 x 8) wells. The PrioCHECK™ Porcine Toxoplasma Ab Kit follows a four-step protocol: sample preparation, sample incubation, conjugate incubation and detection. Initially, the sera are diluted at a ratio of 1/9 on a dummy plate. Then, 20 µl of the diluted samples are transferred from the dummy plate to the wells of the test plate and further diluted at a 1/4 ratio. After incubation at room temperature and washing of the wells, the previously prepared specific conjugate, an anti-IgG antibody labelled with enzyme peroxidase (POD), is added and binds to IgG antibodies previously captured on antigens during a second incubation. The plate is then washed to remove unbound conjugate. Next, the substrate/chromogen is added, initiating the enzymatic reaction in positive samples: the POD enzyme catalyses the oxidation of the substrate, resulting in blue colour development. After a short incubation at room temperature, the reaction is stopped by adding stop solution. If specific IgG antibodies against *T. gondii* are present in the serum tested, the labelled conjugated anti-IgG antibodies will bind to them and a yellow colour will appear after adding the stop solution (Figure 7).

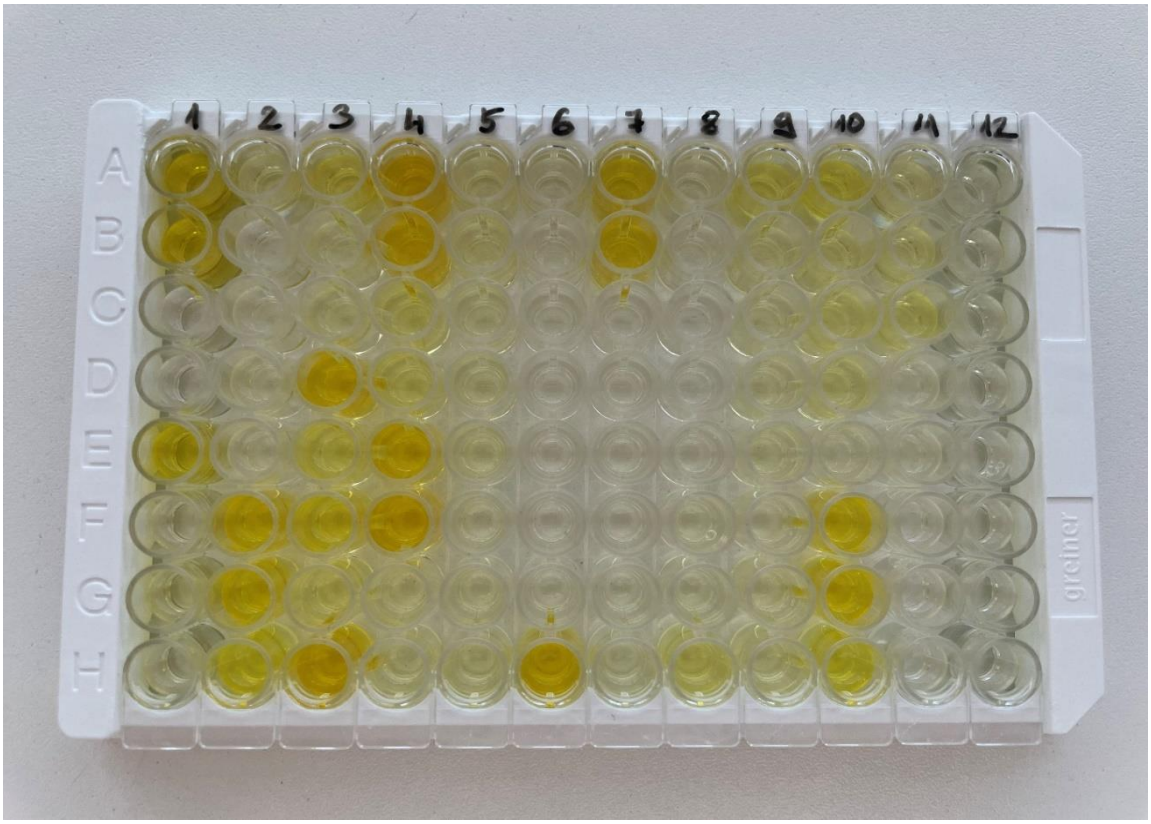

Figure 7. Microtiter plate after adding stop solution to the serum samples of sows. Yellow colour indicates the presence of specific IgG antibodies against *T. gondii* in tested samples. A1 and B1: positive controls; C1 and D1: negative controls.

The optical density (OD) of the resulting colour is measured using a spectrophotometer at a wavelength of 450 nm. The results are obtained by dividing the difference between the mean OD value of the sample and the negative control (NC) by the difference between the mean OD value of the positive control (PC) and negative control. This gives the mean percentage of positivity (PP) according to the calculation shown.

$$\mathbf{PP} = \left[\frac{\mathbf{OD_{450\ Sample} - OD_{450\ NC}}}{\mathbf{OD_{450\ PC} - OD_{450\ NC}}}\right] \mathbf{x\ 100}$$

If the results obtained are equal to or above the cut-off value of 20 PP, the sample is considered positive for antibodies against *T. gondii*. If the results are below the cut-off value of 20 PP, the sample is considered negative for antibodies against *T. gondii*. According to a pilot study conducted prior to the large-scale study, the results showed that seroprevalence in fattening pigs was 5.4% (5/92) and in sows 26.1% (24/92). These results were used to design the large-scale study described later in this manuscript.

3. HYPOTHESIS AND OBJECTIVES

Given the public health significance of toxoplasmosis and the important role of undercooked pork as a source of human infection, this study assessed *T. gondii* exposure in Croatian pigs by analysing serum samples for IgG antibodies using the MAT. An epidemiological questionnaire was also used to identify risk factors associated with higher infection rates and to evaluate farm biosecurity measures in relation to seropositivity. This is the first large-scale investigation of *T. gondii* seroprevalence in Croatian pigs using the MAT. The research aims to clarify the biosecurity risk factors influencing *T.gondii* seroprevalence in pigs and to contribute to the standardisation of the MAT for diagnostic use. The results will improve understanding of toxoplasmosis epidemiology in Croatian pig farms, support the development of surveillance systems and risk-reduction strategies in livestock production, and provide valuable insights into the public health risk of *T. gondii* transmission to humans through pork consumption.

Research hypotheses

It is hypothesised that pigs from farms with lower biosecurity categories will have higher exposure to *T. gondii*. It is also hypothesised that the seroprevalence of *T. gondii* will be higher in sows than in fattening pigs.

Objectives of this study:

1. Determine the *T. gondii* seroprevalence in pigs slaughtered in Croatia
2. Determine the differences in seroprevalence of *T. gondii* between fattening pigs and sows in the studied population
3. Identify risk factors for seropositivity in relation to the biosecurity category of the farm

4. MATERIALS AND METHODS

4.1. Samples and sampling strategy

In this study, serum samples from fattening pigs and sows collected in 2021 and 2022 were analysed for the presence of IgG antibodies against *T. gondii*. Blood samples from fattening pigs and sows were collected at the slaughter line during exsanguination, while some sow serum samples were obtained from the archives of the Croatian Veterinary Institute after testing for various infectious diseases under the Agreement on the Protection of Animal Health. The use of archived sera for this study was authorised by the Directorate of Veterinary and Food Safety of the Croatian Ministry of Agriculture and the Director of the Croatian Veterinary Institute.

Results from previously conducted pilot study were used for sample size calculation. The publicly available software package Epitools – Epidemiological Calculators https://epitools.ausvet.com.au) was used to determine the sample size and true prevalence in groups of fattening pigs and sows. A minimum of 340 animals was required to determine a true prevalence of 5% in fattening pigs (n>10,000) with a precision of 5%, assuming a MAT specificity of 90% and sensitivity of 83% (DUBEY et al. 1995). A minimum of 535 animals was required to determine a true prevalence of 20% in sows (n>10,000) with an accuracy of 5% and the same specificity and sensitivity.

According to the Ministry of Agriculture, the pig population on Croatian farms and across geographical regions fluctuates significantly on an annual basis, which is why the sampling strategy was determined to be random.

4.2. Sampling, labeling and storage of samples

At the slaughter line, blood samples from fattening pigs and sows were collected by authorised veterinarians with the consent of the slaughterhouse owner (Figure 8). For serological examination, 10 ml of blood was collected in tubes with a clot activator during exsanguination by puncture. Labelled tubes containing blood were stored at +4°C and delivered to the laboratory of the Department of Parasitology and Parasitic Diseases with Clinic, Faculty of Veterinary Medicine, University of Zagreb within four days of sample collection. In the Department laboratory, the sera were separated immediately after delivery by centrifugation at 700 g force for 5 minutes.

Archived sow serum samples were aliquoted into 1 ml volumes in microcentrifuge tubes after testing for different infectious diseases at the Croatian Veterinary Institute, labelled, and stored at - 20°C until delivery to the Department laboratory.

Each serum sample from fattening pigs and sows was aliquoted into 500 µl volumes for serological examination and stored at - 20°C. All samples were sent by plane in a portable refrigerator provided by the biopharmaceutical courier service to the UMR BIPAR (Unité Mixte de Recherche, Biologie moléculaire et Immunologie des PARasites), Laboratoire de Santé Animale, L'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (Anses), Maisons-Alfort, France.

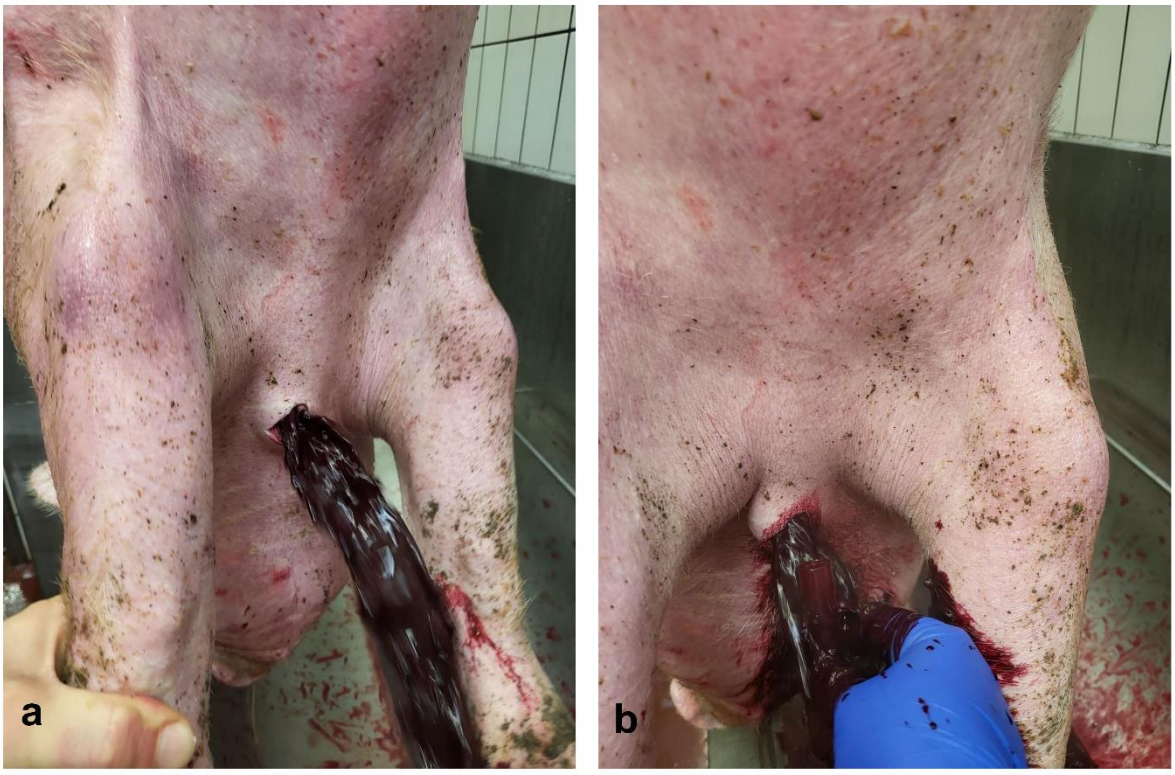

Figure 8. Blood sample collection at the slaughter line: (a) a cut in the central part of the neck (throat area) made by sharp knife, (b) collection of blood in 10 ml tube

4.3. Serological diagnosis

The MAT is used to detect IgG antibodies against *T. gondii* in pig sera at the UMR BIPAR in Maisons Alfort, France, as described by Desmonts and Remington (DESMONTS and REMINGTON 1980). The antigen for this research was provided by the Centre National de Référence de la Toxoplasmose in Reims, France. Before performing the test, the reagents were prepared according to the number of samples to be tested.

Reagent preparation:

1) Dithiothreitol (DTT) 1/50
    - DTT (1M) was diluted in Phosphate-Buffered Saline (PBS) in 1/50 ratio
2) Antigen mixture: formalin fixed tachyzoites ($2x10^8$ parasites/ml) 1/17
    - Antigen was diluted in Bovine Albumin Buffer Solution (BABS) in 1/17 ratio

Procedure:

To conduct the test, a 1/3 dilution of the serum and the positive and negative controls was initially prepared in PBS on a polystyrene microtitration plate, also known as a dummy plate, containing 96 (12 x 8) U-bottom wells (Figure 9). First, 50 µl of PBS was added to each well of the dummy plate using a multichannel pipette. Controls and serum samples were briefly vortexed and centrifuged before being placed into the wells. Then, 25 µl of the controls was added to well A1 (positive) and well B1 (negative) and mixed by pipetting up and down three times. 25 µl of each serum sample was added to the remaining wells of the dummy plate, starting with well E1, and mixed by pipetting up and down three times.

After preparing and labelling the test plate, 25 µl of the previously prepared DTT (1/50) was added to each well using a multichannel pipette (Figure 10). Then, 25 µl of the diluted controls was transferred from the test plate to wells A1-D1 of the test plate, ensuring duplicate positive and negative controls. Next, 25 µl of the serum samples was also transferred from the first row of the dummy plate to the first row of the test plate, starting with well E1. Serial dilution were made for both the controls and samples down to the fourth row using a multichannel pipette. From the final dilution row, 25 µl was removed from each well and discarded. In this way, two-fold serial dilutions were made, ranging from 1/6 to 1/48 (Figure 10).

Then, 25 µl of the previously prepared antigen mixture (1/17) was carefully added to each well using a multichannel pipette, ensuring that the tips did not touch the sample. Each test plate was covered with sealing tape and incubated at room temperature for 18-24 hours. Positive

serum samples were further analysed at higher dilutions (1/6 to 1/12 288) to establish the endpoint titre. The antibody titre was determined by the last dilution of the serum in which a positive reaction was observed by forming white agglutinates covering more than 50% of the well surface, visible under a magnifying glass (Figure 11). To avoid bias, each plate was analysed blind by two assessors. All samples reactive at ≥1/6 were considered positive as previously published by DJOKIC et al. (2016b).

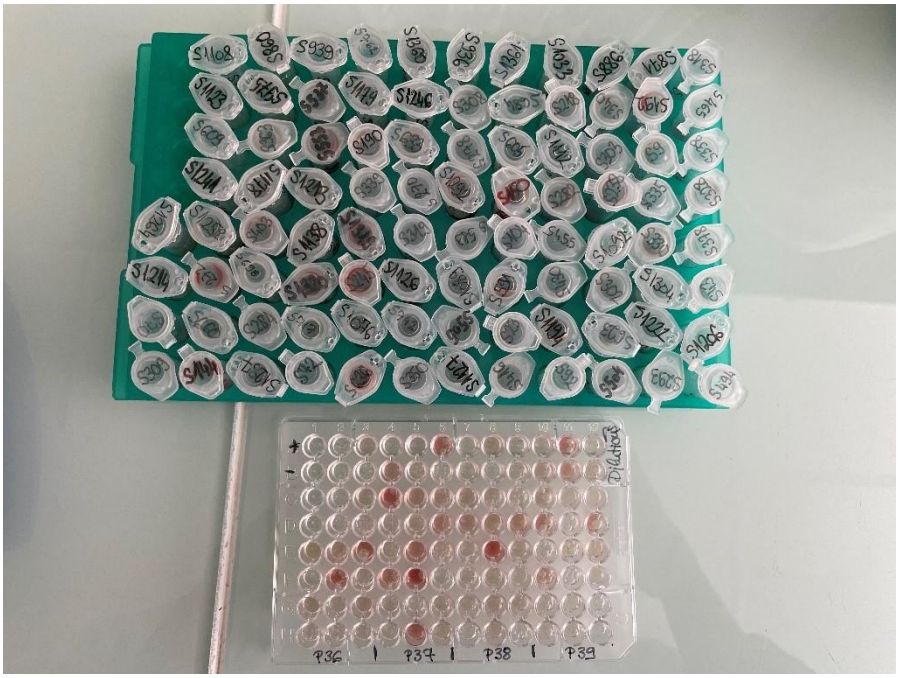

Figure 9. Initial 1/3 dilution of serum samples, positive and negative controls on a microtitration 96-well U-bottom dummy plate

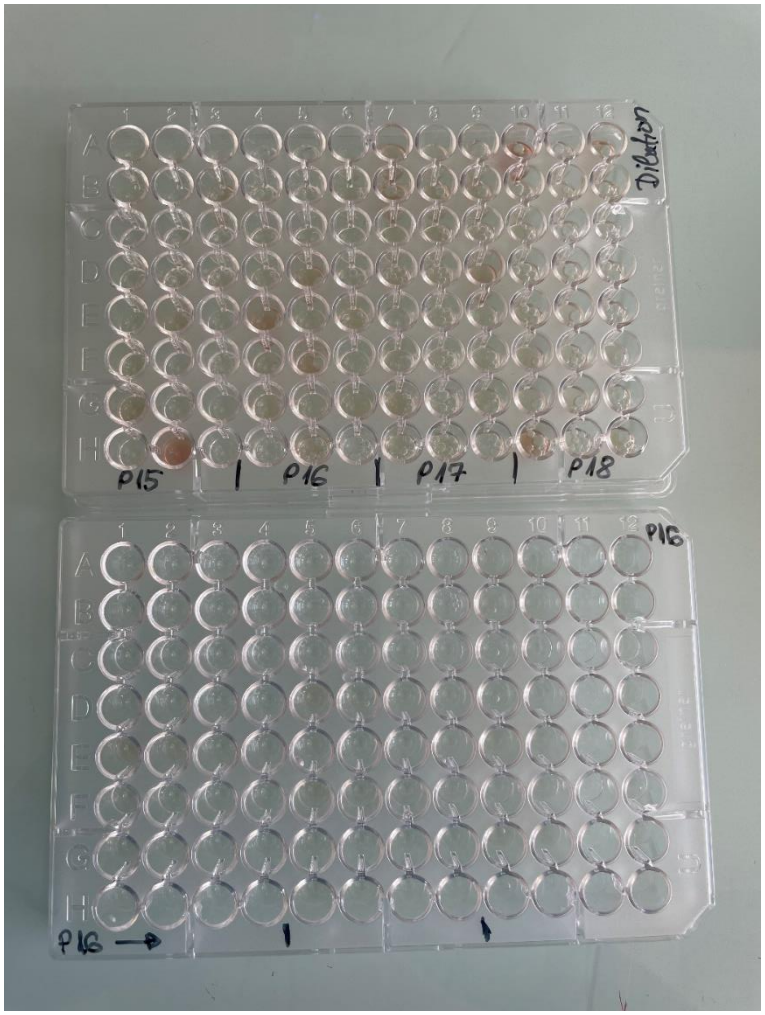

Figure 10. Initial 1/3 dilution of serum samples, positive and negative controls on a microtitration 96-well U-bottom dummy plate (upper plate). Four two-fold serial dilutions (from 1/6 to 1/48) of serum samples on a microtitration 96-well U-bottom test plate (lower plate)

Figure 11. Reading MAT results: In rows B and E, samples are positive and agglutinates are visible. In rows C and D, samples are negative; non-agglutinated antigen has settled at the bottom of the well, forming a sedimentation button.

4.4. Data on farms and pigs

Farm data from the database of the Veterinary and Food Safety Administration, Ministry of Agriculture, within the VetIs system, a comprehensive database containing all information on farms and activities related to different infectious disease surveillance and farm categorisation, were used in this study. The farm data included the unique farm identification number, and the address, comprising county, city, settlement, street, and house number, as recorded in VetIs during initial registration. Data on pig category (fattening pig or sow), sex and age category were obtained from pig travel certificates issued for transport to the slaughterhouse or from referral forms attached to serum samples sent to the Croatian Veterinary Institute.

4.4.1. Biosecurity categorisation

In this study, data on the biosecurity categorisation of pig farms were used, as provided by the Veterinary and Food Safety Administration, Ministry of Agriculture, for the purposes of this research. The dataset included information on the biosecurity category assigned to each farm, and the results from the biosafety questionnaire as of 16 March 2022. Although the questionnaire was originally designed to assess farm biosecurity in relation to African swine

fever (ASF), several questions were identified as useful for evaluating potential risk factors for the occurrence of toxoplasmosis on pig farms. Therefore, eight questions were selected from the original questionnaire for this purpose. The selected questions and their possible answers (Yes/No) are presented in Appendix 1, and refer to farm size, presence of pets, protection against entry of other animals, housing type, footwear disinfection, presence of a foot dip, feed production, and feed storage.

The categorisation process began in 2019 to review biosecurity measures and collect data on farms as part of the national measures for preventing the introduction and spread of the ASF virus. These measures were established in the Order on measures for the prevention of the occurrence and early detection of the introduction of ASF virus in the Republic of Croatia (ANONYMOUS 2019b). According to the regulation, every farm that keeps pigs must be categorised in terms of biosecurity. The categorisation was carried out by an authorised veterinarian who, during the farm inspection, completed a biosecurity questionnaire consisting of 95 questions. Based on the responses, the "Categorisation" application within VetIs automatically calculated and assigned the farm's biosecurity category. Each farm was classified into one of five categories:

- Category "0" - smallholdings that keep one fattening pig for their own needs
- Category "1" - non-eligible farms that do not meet the required conditions

Farms in this category fail to meet key biosecurity criteria. Exclusion criteria, such as feeding pigs with offal, keeping unmarked pigs or pigs without proper documentation, or keeping pigs outdoors without authorisation from the veterinary inspectorate, automatically place a farm in this category. Failure to comply with a specified number of other question groups is also taken into account. Depending on the responses, farm owners receive written recommendations for improving biosecurity measures.

- Category "2" –farms that partially meet the biosecurity requirements

Owners receive guidance and recommendations for improvement.

- Category "3" –farms that fully comply with all biosecurity requirements
- Category "4" –farms that keep pigs outdoors and are approved by the veterinary inspectorate

These farms keep pigs outdoors for most of the year. They consist of fenced pastures with suitable shelters and typically raise traditional pig breeds (Figure 12). Outdoor pig keeping is only permitted with official approval from the veterinary inspectorate.

Figure 12. Traditional farming of the autochthonous pig breed (Black Slavonian pig); biosecurity category 4.

4.5. Risk factors

To select potential risk factors relevant to the epidemiology of toxoplasmosis, chosen questions and available results from the biosafety questionnaire (Appendix 1) were used, along with other data on pigs and farms. The following variables were considered as potential risk factors for *T. gondii* infection in pigs: pig category (fattening pig or sow), age category (<12 months, ≥12 months), sex (male or female), total number of pigs on the farm (≤20, 21-100, >100), biosecurity category (0, 1, 2, 3, 4), presence of pets on the farm (yes/no), protection against entry of other animals (yes/no), indoor housing without outdoor access (yes/no), outdoor housing (yes/no), footwear disinfection upon entry into the facility (yes/no), presence of a foot dip for staff at the farm entrance (yes/no), feed production on the farm (yes/no), and proper feed storage and pest protection (yes/no).

A simulation table was created to determine the number of samples required per risk factor group for reliable detection of 10% or 20% differences in seroprevalence (Appendix 2). The table was created using STATA 13.1 (StataCorp. 2016. Stata Statistical Software: Release 13.1, College Station, Texas, USA).

4.6. Statistical analysis and risk factor assessment

To investigate the seroprevalence of *T. gondii* in pigs and to identify the main risk and protective factors associated with infection, a series of complementary statistical approaches was applied. First, the overall prevalence was estimated. Prevalence values were also calculated for each potential risk factor category, with corresponding 95% confidence intervals. In the second step, descriptive statistics were performed to explore the distributions of seropositive and seronegative animals across the categories of each potential risk factor. For each category, the frequencies of positive and negative samples were reported, along with the total number of animals in each category and the percentage of positive samples. The association between each potential individual factor and seroprevalence was then tested using Pearson's chi-square test. Associations were considered statistically significant at $p < 0.05$.

Univariate logistic regression models were developed to evaluate the independent effects of individual factors on seropositivity. For each variable, the odds ratio (OR) with a 95% confidence interval (95% CI) was calculated to indicate the strength, direction, and precision of the association with infection. A variable was considered statistically significant at $p < 0.05$ and when the 95% CI for the OR did not include 1.

All variables that were statistically significant in the univariate logistic regression analyses were included as candidates in the multivariate logistic regression model. A multivariate logistic regression model was constructed using a stepwise variable selection approach. Variables were iteratively entered or removed based on their statistical contribution to the model fit, and only those that remained statistically significant after mutual adjustment were retained in the final model. This comprehensive model enabled identification of the most important predictors of *T. gondii* seroprevalence while accounting for potential confounding effects of other variables.

Finally, statistical analysis of antibody titres was performed. Antibody titres were expressed as reciprocal serum dilutions (1/6 to 1/12 288) and summarised as frequency distributions. Statistical comparisons between groups were performed using a t-test on log-transformed titre

values, and the geometric mean (GM) ratio with 95% CI was calculated to assess the magnitude and significance of the difference.

All statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA).

5. RESULTS

5.1. Results of serological screening for *T. gondii* in pigs in Croatia

During the study period in 2021 and 2022, a total of 1693 serum samples were collected from pigs. Due to incomplete data on the origin of the animals and the biosafetey questionnaire, 1621 samples were included in the analysis, of which 319 tested positive and 1302 were negative. Based on the percentage of positive samples, the overall seroprevalence was estimated at 19.68% (95% CI 17.77%–21.70%).

County-level and biosecurity category distributions were evaluated descriptively. Table 2 presents the seroprevalence of *T. gondii* by county.

Table 2. Seroprevalence of *T. gondii* by county, with frequencies of positive and negative samples, Pearson's chi-square p-values, and 95% confidence interval

County	Serology result		Total (1621)	Pearson Chi-Square p-value	Seroprevalence (%)	95% CI (%)
	Positive (319)	Negative (1302)				
Bjelovarsko-bilogorska	3	20	23		13.04	1.39–30.20
Brodsko-posavska	3	53	56		5.36	0.56-13.06
Grad Zagreb	10	10	20		50.00	27.64-72.36
Istarska	0	23	23		-	-
Karlovačka	3	11	14		21.43	2.33-46.86
Koprivničko-križevačka	3	64	67		4.48	0.47-10.98
Krapinsko-zagorska	52	91	143	<.0001	36.36	28.48-44.53
Međimurska	1	54	55		1.82	0.02-7.53
Osječko-baranjska	102	343	445		22.92	19.06-26.97
Požeško-slavonska	11	14	25		44.00	24.47-64.26
Sisačko-moslavačka	38	77	115		33.04	24.50-42.03
Varaždinska	5	29	34		14.71	3.88-28.83

Virovitičko-podravska	5	45	50		10.00	2.51-20.06
Vukovarsko-srijemska	25	264	289		8.65	5.54-12.20
Zagrebačka	55	190	245		22.45	17.30-27.93
Šibensko-kninska	3	14	17		17.65	1.90-39.60

Table 2 shows that, when stratified by county, there are statistically significant differences in seroprevalence. The highest seroprevalence is observed in the Grad Zagreb (50%), Požeško-Slavonska (44%) and Krapinsko-zagorska County (36.36%), while among the lowest is recorded in Međimurska County (1.82%), and no positive pigs are detected in Istarska County. The table also shows substantial variation in sample sizes between counties. Larger sample sizes provide narrower confidence intervals and thus more precise estimates, whereas smaller datasets are associated with wider intervals and greater uncertainty. Counties with large sample sizes (>200 samples) include Osječko-baranjska, Vukovarsko-srijemska, and Zagrebačka County. Medium-sized samples (100–199) are obtained in Krapinsko-zagorska and Sisačko-moslavačka County. Counties with smaller sample sizes (30–99) include Brodsko-posavska, Koprivničko-križevačka, Međimurska, Varaždinska, and Virovitičko-podravska. Counties with very small sample sizes (<30) are Grad Zagreb, Požeško-slavonska, Karlovačka, Šibensko-kninska, Bjelovarsko-bilogorska, and Istarska County.

Geographically, the distribution of *T. gondii* seroprevalence by county is presented on the map of Croatia in Figure 13 and in the mosaic plot, which also illustrates the distribution of seropositive and seronegative pigs in relation to sample size (Figure 14).

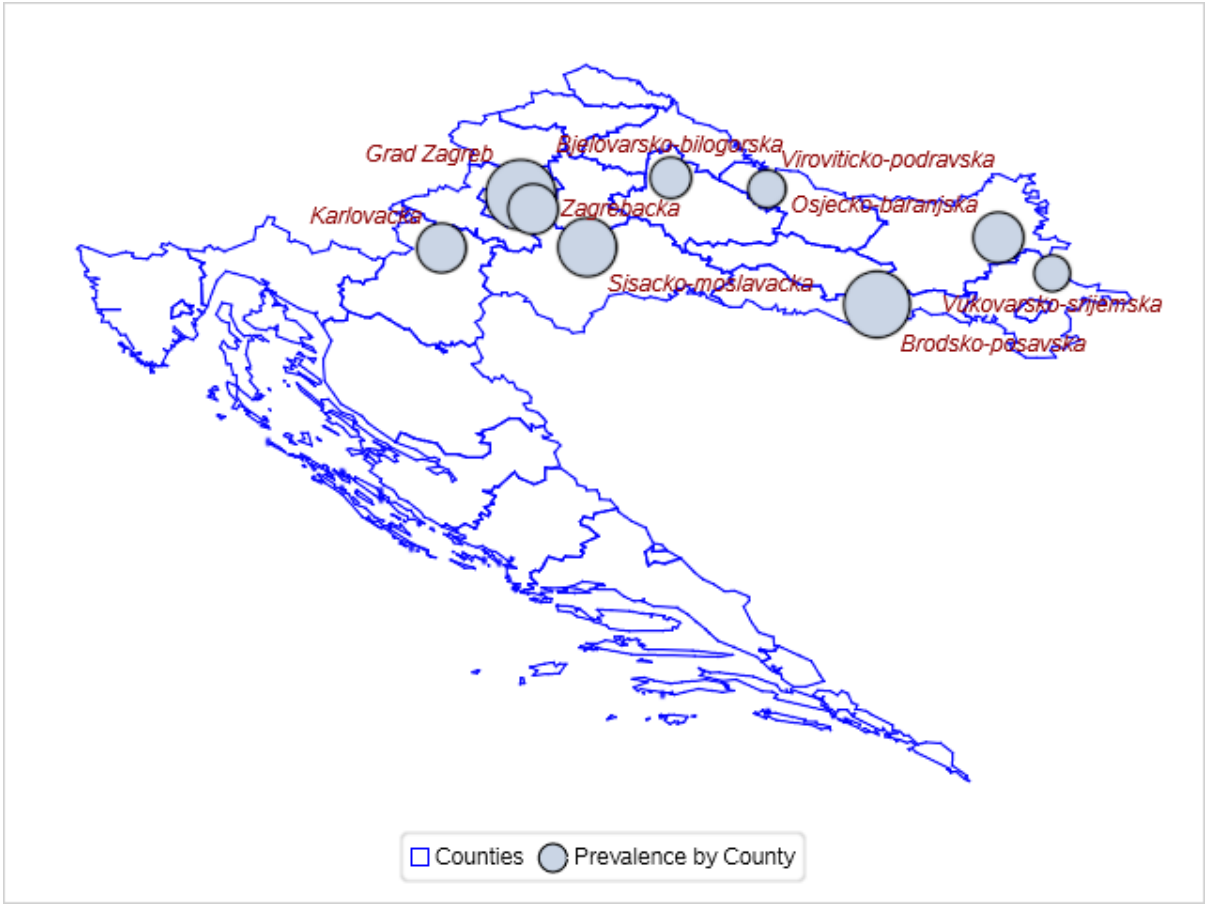

Figure 13. Geographical distribution of *T. gondii* seroprevalence by county in Croatia (SAS/GRAPH, SAS 9.4)

In the mosaic plot (Figure 14), serology results are displayed on the X-axis, with column widths proportional to the number of positive (lower blue area) and negative (upper red area) samples in each county. The county variable is displayed on the Y-axis, and bar heights are proportional to the number of positive and negative samples within each county.

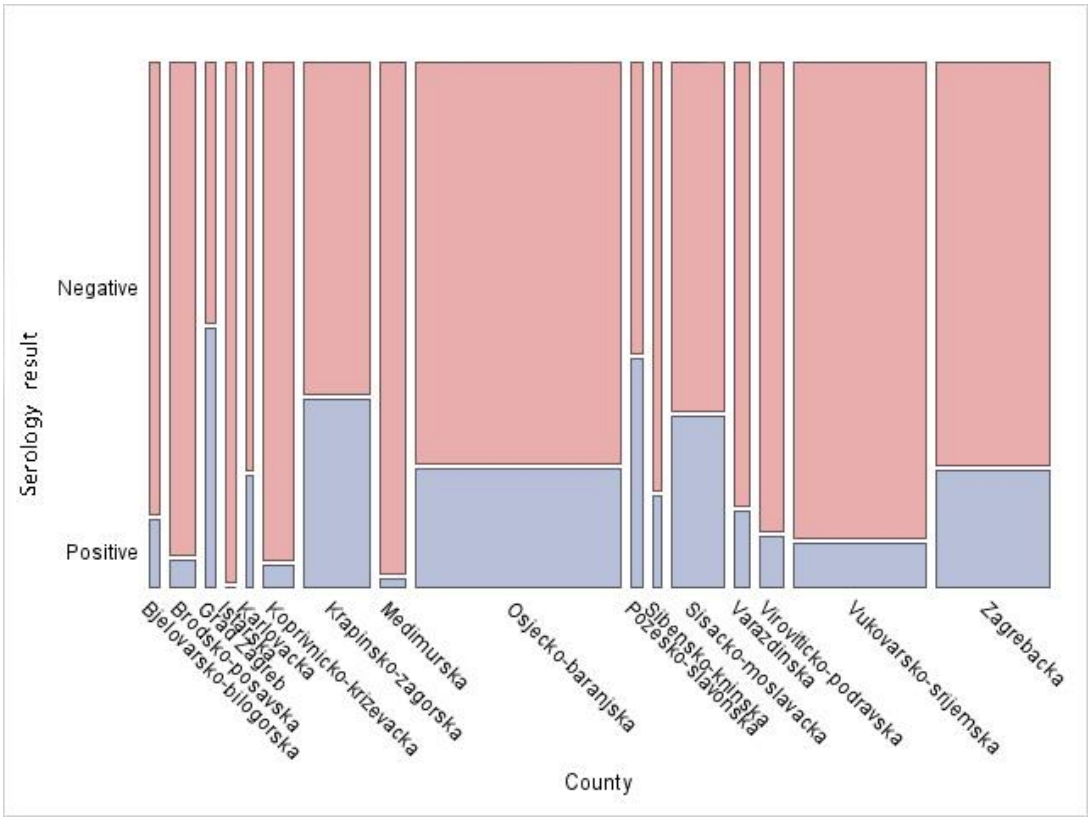

Figure 14. Mosaic plot of *T. gondii* seroprevalence in pigs by county in Croatia

Both the map and the mosaic plot show that there are geographical variations in *T. gondii* seroprevalence across Croatian counties, while the mosaic plot also illustrates unequal sample sizes between counties.

5.2. Risk factor analysis and association of risk factors with seropositivity

The aim of this analysis was to identify which farm-level factors are associated with seropositivity for *T. gondii* in pigs. The following independent variables were evaluated for each of the 1621 pigs sampled: pig category (fattening pig or sow), age category (<12 months, ≥12 months), sex (male or female), total number of pigs on the farm (≤20, 21-100, >100), biosecurity category (0, 1, 2, 3, 4), presence of pets on the farm (yes/no), protection against entry of other animals (yes/no), indoor housing without outdoor access (yes/no), outdoor housing (yes/no), footwear disinfection upon entry into the facility (yes/no), presence of a foot

dip for staff at the farm entrance (yes/no), feed production on the farm (yes/no), and proper feed storage and pest protection (yes/no). All potential risk factors showed a strong association with seropositivity (with most p-values < 0.0001). Tables 3 and 4 summarise, for each potential risk factor, the frequencies of seropositive and seronegative pigs, p-values from chi-square tests of association between seropositivity and each risk factor, as well as the prevalence estimates and their corresponding 95% confidence intervals.

Table 3. Seroprevalence of *T. gondii* in pigs across different potential risk factors, with frequencies of positive and negative samples, Pearson's chi-square p-values, and 95% confidence intervals

Variable		Serology result		Total (1621)	Pearson Chi-Square p-value	Seroprevalence (%)	95% CI[1] (%)
		Positive (319)	Negative (1302)				
Pig category	Fattening pigs	119	856	975	<0.0001	12.21	10.18-14.34
	Sows	200	446	646		30.96	27.40-34.60
Age category	<12 months	56	715	771	<0.0001	7.26	5.48-9.21
	≥12 months	263	587	850		30.94	27.84-34.11
Sex	Female	260	913	1173	<0.0001	22.17	19.80-24.60
	Male	59	389	448		13.17	10.11-16.48
Total number of pigs	≤20	150	259	409	<0.0001	36.67	32.00-41.45
	21–100	51	143	194		26.29	20.17-32.78
	>100	118	900	1018		11.59	9.66-13.64
Pets on the farm	No	147	864	1011	<0.0001	14.54	12.40-16.79
	Yes	172	438	610		28.20	24.64-31.86

Protection against entry of other animals	No	51	103	154	<0.0001	33.12	25.72-40.85
	Yes	268	1199	1467		18.27	16.31-20.29
Indoor housing without outdoor access	No	123	270	393	<0.0001	31.30	26.73-36.01
	Yes	196	1032	1228		15.96	13.93-18.07
Outdoor housing	No	247	1162	1409	<0.0001	17.53	15.56-19.57
	Yes	72	140	212		33.96	27.60-40.55
Footwear disinfection upon entry into the facility	No	85	136	221	<0.0001	38.46	32.03-45.05
	Yes	234	1166	1400		16.71	14.78-18.72
Foot dip for staff at the farm entrance	No	134	244	378	<0.0001	35.45	30.63-40.39
	Yes	185	1058	1243		14.88	12.93-16.92
Feed production on farm	No	34	424	458	<0.0001	7.42	5.11-10.02
	Yes	285	878	1163		24.51	22.05-27.03
Proper feed storage	No	19	28	47	0.0003	40.43	26.38-55.09
	Yes	300	1274	1574		19.06	17.13-21.04

[1]CI – Confidence interval

Table 3 shows statistically significant differences in seroprevalence across all variable categories presented. Sows have a higher seroprevalence (30.96%, 95% CI 27.40-34.60%) compared with fattening pigs (12.21%, 95% CI 10.18-14.34%). Likewise, pigs older than 12

months exhibit a higher seroprevalence (30.94%, 95% CI 27.84-34.11%) compared with younger pigs (7.26%, 95% CI 5.48-9.21%), while females are more frequently seropositive than males (22.17%, 95% CI 19.80-24.60% vs 13.17%, 95% CI 10.11-16.48%). Preventive measures on farms show similar trends: farms lacking a foot dip for staff or footwear disinfection upon entry into the facility have higher seroprevalence of 35.45% (95% CI 30.63-40.39%) and 38.46% (95% CI 32.03-45.05%), compared with 14.88% (95% CI 12.93-16.92%) and 16.71% (95% CI 14.78-18.72%) where these measures are in place. Housing type also shows differences in seroprevalence, with pigs housed outdoors or indoors with outdoor access, having nearly twice the seroprevalence (33.96% and 31.30%) compared to pigs housed indoors (17.53% and 15.96%). Similarly, farms with pets show nearly twice the seroprevalence (28.20%, 95% CI 24.64-31.86%) compared to farms without pets (14.5%, 95% CI 12.40-16.79%). Variables related to feed management show that farms with on-farm feed production have over threefold higher seroprevalence (24.51%, 95% CI 22.05-27.03%) compared with farms that do not produce their own feed (7.42%, 95% CI 5.11-10.02%). Farms that store feed properly, ensuring protection from pests, exhibit half the seroprevalence (19.06%, 95% CI 17.13-21.04%) compared to farms lacking such practices (40.43%, 95% CI 26.38-55.09%). Finally, herd size shows an inverse effect, with small herds (≤20 pigs) reaching a seroprevalence of 36.67% (95% CI 32.00-41.45%), medium herds (21-100 pigs) 26.29% (95% CI 20.17-32.78%), and large herds (>100 pigs) only 11.59% (95% CI 9.66-13.64%).

The Table 4 presents the distribution of seroprevalence by biosecurity categories.

Table 4. Seroprevalence of *T. gondii* by biosecurity category, with frequencies of positive and negative samples, Pearson's chi-square p-values, and 95% confidence interval

Biosecurity category	Serology result		Total (1621)	Pearson Chi-Square p-value	Seroprevalence (%)	95% CI (%)
	Positive (319)	Negative (1302)				
1	38	79	117	<.0001	32.48	24.05-41.36
2	141	496	637		22.14	18.94-25.46
3	79	604	683		11.57	9.22-14.09
4	61	123	184		33.15	26.38-40.21

As presented in Table 4, herds in biosecurity categories 1 and 4 show a high seroprevalence of 32.48% (95% CI 24.05-41.36%) and 33.15% (95% CI 26.38–40.21%), whereas categories 2 and 3 have lower seroprevalence rates of 22.14% (95% CI 18.94-25.46%) and 11.57% (95% CI 9.22-14.09%), respectively.

The mosaic plot in Figure 15 illustrates the relative sample sizes for each farm biosecurity category and the corresponding serological results. In the mosaic plot, serology results are displayed on the X-axis, with row widths proportional to the number of positive (lower blue area) and negative samples (upper red area) in each biosecurity category. The farm biosecurity category is shown on the Y-axis, with bar heights proportional to the number of positive and negative samples in each category.

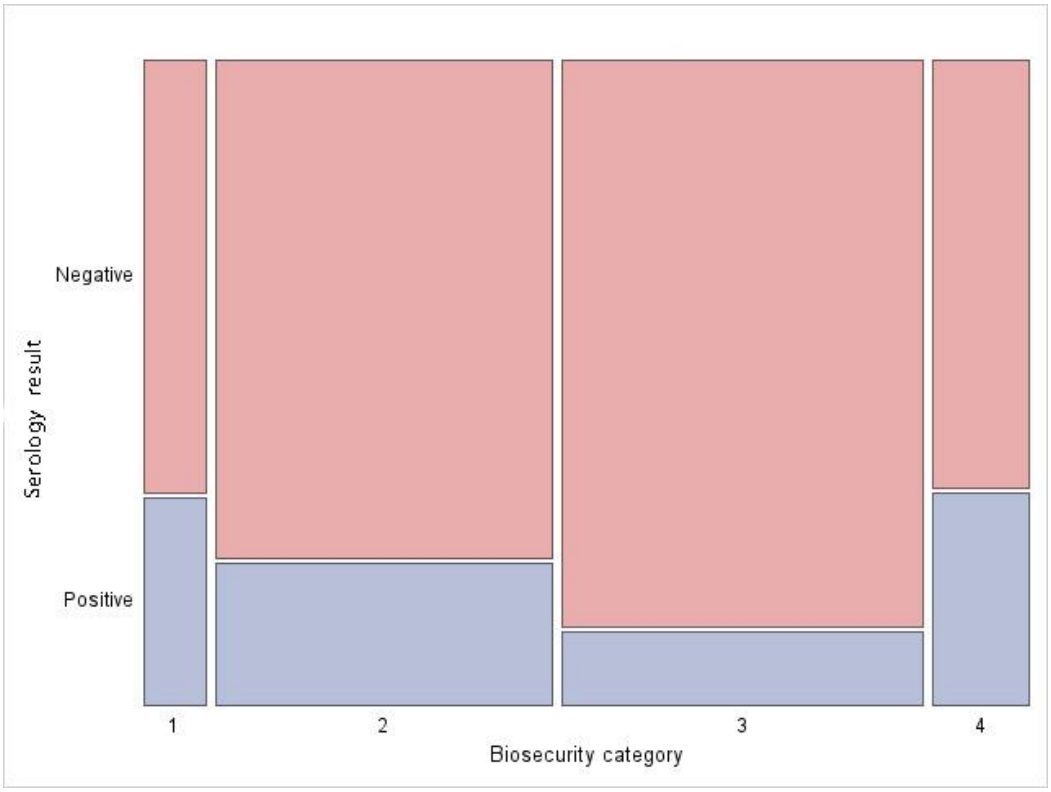

Figure 15. Mosaic plot of *T. gondii* seroprevalence in pigs by farm biosecurity categories in Croatia

The mosaic plot shows variations in *T. gondii* seroprevalence and sample sizes across the biosecurity categories. Categories 2 and 3 contribute the largest numbers of pigs, while categories 1 and 4 contain fewer samples. The plot highlights differences in seroprevalence between categories, with the lowest proportion of positives in category 3 and the highest in

categories 1 and 4 (around one-third of the samples), suggesting a U-shaped distribution of seroprevalence across the ordered biosecurity categories of the farms.

5.2.1. Results of univariate logistic regression

Univariate logistic regression analysis revealed that all investigated risk factors were significantly associated with *T. gondii* seropositivity in pigs ($p<0.05$ and 95% confidence interval for the OR that does not include 1). The ORs, confidence intervals (95% CI), and p-values for all parameters included in the univariate analysis are presented in the Table 5.

Table 5. Univariate logistic regression results: odds ratio estimates with 95% confidence intervals for risk factors of *T. gondii* seroprevalence in pigs

Variable	Effect	OR[1]	95% CI[2]	p-value[3]
Pig category	Sows vs Fattening pigs	3.23	2.50-4.16	<0.0001
Age category	≥12 months vs <12 months	5.72	4.20-7.79	<0.0001
Sex	Female vs Male	1.88	1.38 -2.55	<0.0001
Total number of pigs	≤20 vs >100	4.42	3.34-5.83	<0.0001
	21–100 vs >100	2.72	1.87-3.95	<0.0001
Pets on the farm	Yes vs No	2.31	1.80-2.96	<0.0001
Protection against entry of other animals	Yes vs No	0.45	0.31-0.65	<0.0001
Indoor housing without outdoor access	Yes vs No	0.42	0.32-0.54	<0.0001
Outdoor housing	Yes vs No	2.42	1.76-3.32	<0.0001
Footwear disinfection upon entry into the facility	Yes vs No	0.32	0.24-0.44	<0.0001
Foot dip for staff at the farm entrance	Yes vs No	0.32	0.24-0.41	<0.0001
Feed production on farm	Yes vs No	4.05	2.78-5.88	<0.0001
Proper feed storage	Yes vs No	0.35	0.19-0.63	0.0005

[1]Odds ratio-OR; [2]Confidence interval-CI; [3]Statistically significant if $p < 0.05$

According to Table 5, a statistically significant difference is found for all variables between seropositive and seronegative pigs. The pig category variable shows that sows have more than

three times the odds of infection compared with fattening pigs (OR = 3.23, 95% CI: 2.50–4.16, $p < 0.0001$). Similarly, sex shows that females have nearly double the odds of seropositivity compared with males (OR = 1.88, 95% CI: 1.38–2.55, $p < 0.0001$). Age is a particularly strong predictor, as pigs older than 12 months have almost a sixfold higher risk of being seropositive compared with younger animals (OR = 5.72, 95% CI: 4.20–7.79, $p < 0.0001$). Herd size is inversely associated with risk: compared with large herds (>100 pigs), small herds (≤20 pigs) have 4.42 times greater odds of seropositivity (OR = 4.42, 95% CI: 3.34–5.83, $p < 0.0001$), while medium herds (21–100 pigs) have 2.72 times greater odds compared with large herds (OR = 2.72, 95% CI: 1.87–3.95, $p < 0.0001$). As shown in Table 4, protection against the entry of other animals is negatively associated with seropositivity, indicating that the odds of seropositivity in pigs on farms implementing this measure are more than 50% lower than in those without it (OR = 0.45, 95% CI: 0.31–0.65, $p < 0.0001$). Similarly, the presence of footwear disinfection upon entry (OR = 0.32, 95% CI: 0.24–0.44, $p < 0.0001$) and a foot dip for staff at the farm entrance (OR = 0.32, 95% CI: 0.24–0.41, $p < 0.0001$) are both strongly protective, with pigs from farms implementing these measures having almost 70% lower odds of seropositivity. Likewise, indoor housing without outdoor access reduces the odds of seropositivity by more than half (OR = 0.42, 95% CI: 0.32–0.54, $p < 0.0001$). Risk increases on farms with outdoor housing (OR = 2.42, 95% CI: 1.76–3.32, $p < 0.0001$), with pigs in that group having almost two and a half times greater odds of being seropositive. The risk also significantly increases with the presence of pets on the farm (OR = 2.31, 95% CI: 1.80–2.96, $p < 0.0001$), where pigs have 2.3 times greater odds of seropositivity compared with those on farms without pets. The presence of on-farm feed production is positively associated with risk, with pigs on such farms having fourfold greater odds of being positive for toxoplasmosis compared with those on farms without on-farm feed production (OR = 4.05, 95% CI: 2.78-5.88, $p < 0.0001$), while proper feed storage shows a significant protective effect, with pigs from such farms having more than 60% lower odds of seropositivity (OR = 0.35, 95% CI: 0.19–0.63, $p = 0.0005$).

5.2.2. Results of the risk factor analysis using a multivariate logistic regression model

In this study, all independent variables from the previous univariate analysis were included in the multivariate logistic regression model using a stepwise selection approach, to more thoroughly examine the statistical associations and the complexity of the relationships between the mentioned variables and seropositive pigs, as well as identify the main risk factors for

T. gondii seropositivity. Individual variables were excluded from the model through stepwise elimination at each step when they did not show a statistically significant association. Several independent variables that showed strong associations with seropositivity in the univariate analyses (e.g., pig category, presence of pets on the farm, foot dip for staff, and footwear disinfection) did not remain in the final model due to their strong association with variables already included in the model.

The final model showed that age, herd size, housing type, and feed production on farm emerged as consistent independent risk factors, retaining statistical significance after mutual adjustment (Table 6, Figure 16, and Table 7).

Table 6. Odds ratio estimates and 95% Wald confidence intervals from the final multivariate logistic regression model assessing risk factors of *T. gondii* seroprevalence in pigs

Variable		**OR**[1]	**95% CI**[2]
Age category	≥ 12 months vs <12 months	2.32	1.59- 3.39
Farm size	≤ 20 vs 21–100	1.88	1.27 - 2.78
	≤ 20 vs >100	4.25	2.77 - 6.52
	21–100 vs >100	2.26	1.39 - 3.65
Indoor housing without outdoor access	Yes vs No	0.55	0.37 - 0.80
Outdoor housing	Yes vs No	2.09	1.24 - 3.54
Feed production on farm	Yes vs No	1.63	1.06 - 2.51

[1]Odds ratio-OR; [2]Confidence interval-CI

In Table 6, age remains a strong determinant, with pigs older than 12 months having more than twice the odds of seropositivity compared with younger animals (OR = 2.32, 95% CI: 1.59–3.39). Herd size retains its strong inverse association. Small herds (≤20 pigs) have over a fourfold higher risk compared with large herds (>100 pigs) (OR = 4.25, 95% CI: 2.77–6.52), and nearly double the odds compared with medium-sized herds (21-100 pigs) (OR = 1.88, 95% CI: 1.27–2.78). Medium herds also show increased risk

compared with large herds (OR = 2.26, 95% CI: 1.39–3.65). As shown in Table 6, housing type demonstrates contrasting effects. Indoor housing without outdoor access is protective (OR= 0.55, 95% CI: 0.37–0.80), while outdoor housing more than doubles the odds of infection (OR = 2.09, 95% CI: 1.24–3.54). Table 6 also shows that pigs from farms with on-farm feed production have 1.63 times higher odds of seropositivity (OR = 1.63, 95% CI: 1.06–2.51).

OR estimates from the final model assessing predictors of *T. gondii* seroprevalence in pigs are shown in a forest plot in Figure 16. A forest plot visualises the results from a multivariate logistic regression model, displaying the OR as a measure of association between each predictor and the odds of a pig testing seropositive for *T. gondii*. Among the predictors in Figure 16, indoor housing shows decreased odds (OR < 1) of seropositivity, while the others show increased odds (OR > 1). On the plot, the predictor farm size (≤20 vs >100) is highlighted as the strongest driver.

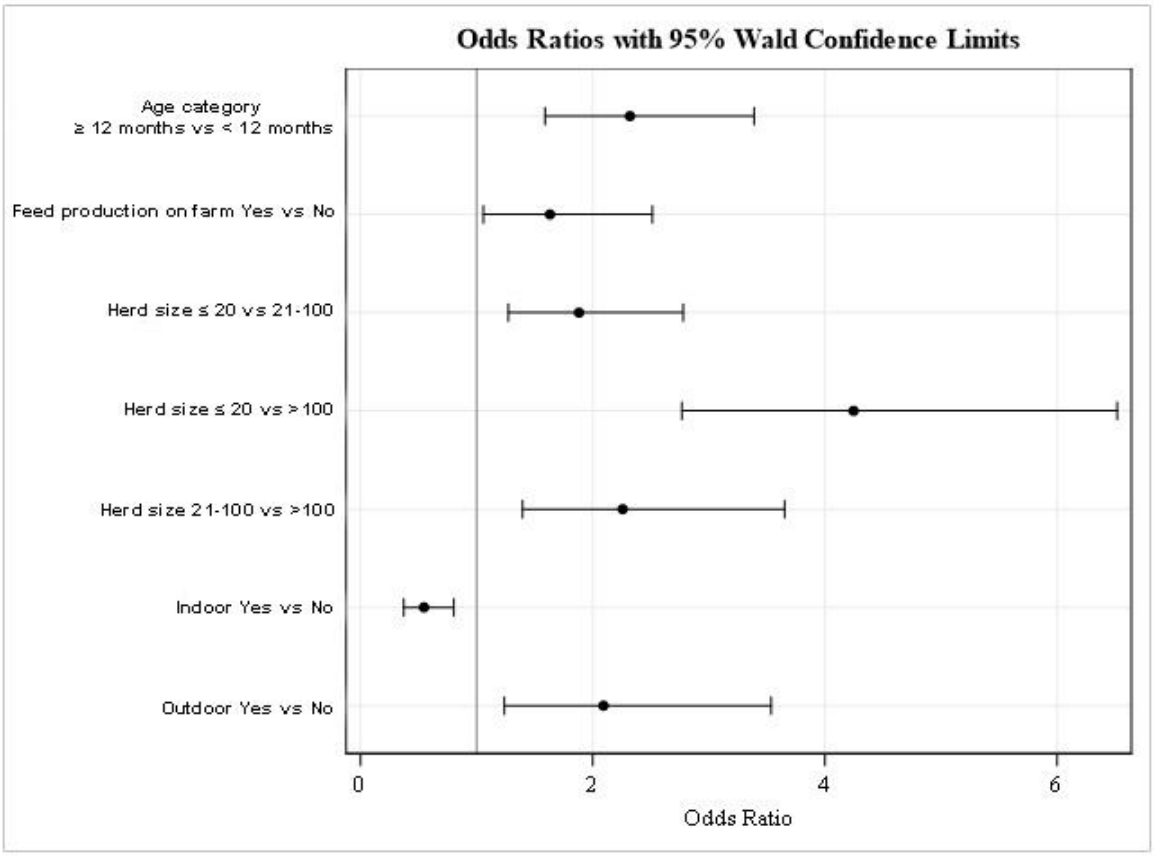

Figure 16. Forest plot of odds ratio estimates with 95% Wald confidence intervals from the final multivariate logistic regression model assessing risk factors of *T. gondii* seroprevalence in pigs

Table 7 presents the strength of the association between the risk factors included in the final model and seropositivity in pigs.

Table 7. Type III Analysis of effects for the final multivariate logistic regression model assessing risk factors of *T. gondii* seroprevalence in pigs

Variable	DF[1]	Wald Chi-Square	p-value
Age category	1	19.00	<0.0001
Feed production on farm	1	4.92	0.0266
Indoor housing	1	9.42	0.0021
Outdoor housing	1	7.61	0.0058
Herd size	2	45.16	<0.0001

[1]DF- Degrees of freedom

From Table 7, it is evident that all variables are statistically significanty associated with seropositivity in pigs (p<0.05). Each variable contributes independently to variations in *T. gondii* seroprevalence in pigs, even after adjusting for the others. The very high Wald values for herd size (45.16) and age category (19.00), together with low p-values (<0.0001) indicate the strongest associations with seropositivity among all variables included in the final model.

5.3. Results of the statistical analysis of antibody titres

The distribution of IgG anti-*T. gondii* antibodies among seropositive animals by pig category (fattening pigs and sows) and overall is shown in Table 8.

Table 8. Distribution of *T. gondii* antibody titres among seropositive pigs in Croatia by pig category (fattening pigs and sows) and overall

Pig category	**Titre**										
	1/6	**1/12**	**1/24**	**1/48**	**1/96**	**1/192**	**1/384**	**1/768**	**1/1536**	**1/12288**	**Total**
Fattening pigs	26	21	24	23	8	8	6	3	0	0	119
Sows	30	32	37	16	25	24	18	9	5	4	200
Total	56	53	61	39	33	32	24	12	5	4	319

This distribution shows that most infected pigs had titres ranging from 1/6 to 1/192, while only a few exhibited titres of 1/1 536 and 1/12 288, which were only found in sows.

The overall distribution of antibody titres among seropositive pigs (n = 319), expressed as percentages, is presented in a Figure 17.

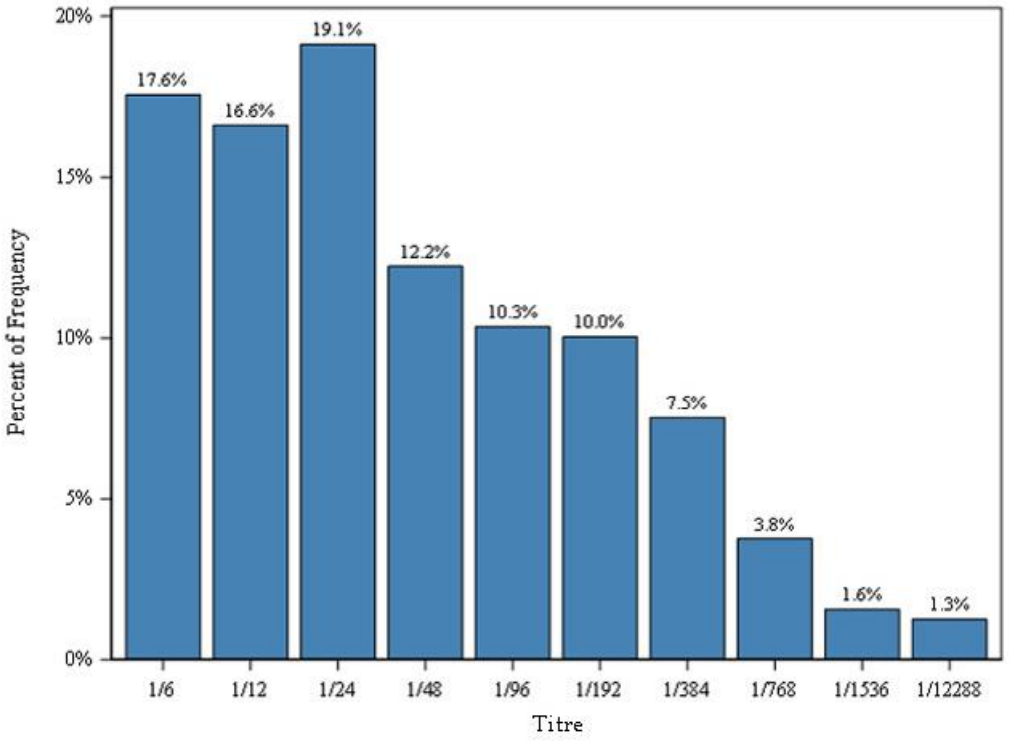

Figure 17. Percentage distribution of *T. gondii* antibody titres in seropositive pigs

From the graph in Figure 17, the overall titre distribution among seropositive pigs displays a right-skewed pattern, with most animals exhibiting low to moderate titres. The most frequent titre is 1/24 (19.12%), followed by 1/6 (17.55%) and 1/12 (16.61%). These three titres together account for over half of all positive samples (53.29%). Intermediate titres are less common, including 1/48 (12.23%), 1/96 (10.34%), and 1/192 (10.03%), whereas high titres (≥1/384) comprise less than 15% of positive samples (1/384 = 7.52%, 1/768 = 3.76%, 1/1536 = 1.57%, and 1/12288 = 1.25%).

Results of the comparison of titres between the pig categories (fattening pigs and sows) are shown in Table 9.

Table 9. Comparison of antibody titres between fattening pigs and sows, expressed as geometric means, coefficients of variation, and geometric mean ratios with 95% confidence intervals

Pig category	Method	GM[1]	95% CI[2] GM[1]	CV[3]	95% CI[2] CV[3]
Fattening pigs		28.42	22.44 - 35.99	2.11	1.67 - 2.87
Sows		53.07	41.70 - 67.55	4.35	3.31 - 6.21
GM[1] Ratio (1/2)	Satterthwaite	0.53	0.38 - 0.75		

[1]GM- Geometric mean; [2]CI – Confidence interval; [3]CV-Coefficient of variation

There is a statistically significant difference in antibody levels between the two pig categories. Sows have significantly higher antibody titres than fattening pigs, with GM of 53.07 and 28.42, respectively. The GM ratio (fattening pigs/sows) is 0.53, with a 95% confidence interval of 0.38 to 0.75 (which does not include 1), further confirming the statistical significance of the difference between groups. This ratio indicates that the GM titre in fattening pigs is approximately 53.5% of that in sows, or, inversely, that sows have approximately 1.87 times higher antibody titres than fattening pigs. Table 9 also shows that the coefficient of variation (CV) is higher among sows (CV = 4.35) compared to fattening pigs (CV = 2.11), suggesting greater variability in antibody response within the sow population.

6. DISCUSSION

In this study, 1621 serum samples collected from slaughtered pigs across Croatia were examined, revealing that 19.68% (139/1621; 95% CI: 17.77–21.70%) tested positive for IgG antibodies against *T. gondii*. Among these, samples from 646 sows showed a notably higher seropositivity of 30.96% (200/646; 95% CI 27.40-34.60%), approximately two and a half times the rate observed in the 975 samples from fattening pigs, where seroprevalence was 12.21% (119/975; 95% CI 10.18-14.34%). These findings differ markedly from those reported by BARIĆ (2012), whose analysis of only 53 serum samples from pigs in Vukovarsko-srijemska County found a very high seroprevalence of 61.6% using a commercial ELISA assay. Such stark contrasts likely result from the limited scope of Barić's work, which was sourced exclusively from small rural holdings. These holdings featured housing with outdoor access and poor biosecurity measures that allowed cats to enter freely and potentially contaminate feed, water and bedding with oocysts. Therefore, these conditions have most probably influenced the high seropositivity rate, as factors such as management systems and housing types that allow access to cats are known to affect seroprevalence of *T. gondii* in pigs (STELZER et al. 2019; DUBEY et al. 2020a). This was probably coupled with the use of a different serological method and a lack of clarity on whether samples were from sows or fattening pigs, as seroprevalence in sows is reported to be consistently higher than in fattening pigs in many studies (CASTILLO-CUENCA et al. 2021; AUGUSTYNIAK et al. 2025). A similar discrepancy is observed in the seroprevalence of 12.2% for fattening pigs detected in this study compared to the 38% recently reported by KIŠ et al. (2021) in 92 diaphragm meat juice samples from Karlovačka County, which also used a commercial ELISA. Factors such as modest sample sizes, methodological differences, and the use of a different sample matrix, probably account for this.

A review of the literature shows that seroprevalence studies in pigs have been conducted in many countries worldwide. For instance, a recent review by DUBEY et al. (2020a) compiles a comprehensive list of relevant investigations carried out between 2009 and 2020. Most of these studies, including the most recent, use convenience sampling strategies, typically involving the collection of pig blood samples at the slaughter line during exsanguination. Seroprevalence of IgG antibodies to *T. gondii* in these investigations varies considerably, particularly across different pig housing systems (indoor versus outdoor) and pig categories (fattening pigs versus sows). However, comparing the seroprevalence results from these studies—either among

themselves or with our own findings—remains challenging, mainly due to the diverse serological methods and cut-off values used. This variability poses a significant obstacle for researchers seeking to compare and discuss their results. Similarly, FOROUTAN et al. (2019), in their systematic review and meta-analysis of *T. gondii* seroprevalence in pigs, were unable to undertake a fully standardised global comparison due to the absence of a uniform diagnostic test. Their meta-analysis estimated a global seroprevalence in pigs of approximately 19%, which aligns closely with the 19.68% observed in the present study. Overall, the seroprevalence detected in our study falls within the ranges reported for several major pig-rearing nations. For example, pooled estimates indicate 19–30% in China, 15–36% in the USA, 18–48% in Mexico, and 14–27% in Brazil (FOROUTAN et al. 2019).

Among all surveys, the most accurate comparisons involve studies employing the same diagnostic test (MAT), cut-off value, and pig category (fattening pigs versus sows). A literature review, most clearly illustrated by DUBEY et al. (2020a) in their comprehensive synthesis, identified approximately 20 investigations that assessed pig sera for *T. gondii* IgG antibodies using the MAT across diverse global regions and countries, including Brazil, China, Mexico, the Democratic Republic of São Tomé and Príncipe, Ethiopia, Estonia, the United Kingdom, Poland, Portugal, Spain, France, Italy, and Serbia. However, not all of these studies examined both pig categories and they used cut-off values that differ from those used in the present study.

A recent investigation by BETIĆ et al. (2022) conducted in neighbouring Serbia reported an overall seroprevalence of 16.5% among 825 pigs, yielding results somewhat comparable to our findings. In their study, seroprevalence was higher among sows (43.6%) than in our cohort (30.96%), whereas the rate among fattening pigs (15.1%) was relatively similar to ours (12.21%). Another substantial study in southwestern Spain, encompassing 2970 pigs, detected antibodies in 16.6% of samples (GARCÍA-BOCANEGRA et al. 2010). Within that cohort, 9.7% of fattening pigs and 24.2% of sows were seropositive for *T. gondii*, representing lower rates than those observed here. Notably, both this and the aforementioned Serbian study used a cut-off value of 1/25. We selected 1/6 as the cut-off value, based on our previously published work and because MAT, by using formalin fixed whole parasite as antigen, allows detection of low antibody concentrations in serum, which can be seen in chronically infected pigs (DJOKIC et al. 2016b; GRBAVAC et al. 2025, personal communication with V. Djokić). With this cut-off, the overall seroprevalence was 19.68%, with most animals having IgG titres ranging from 1/6 to 1/24 (53.3%; Figure 17). The MAT cut-off value remains a matter of debate, as some other studies on *T. gondii* seroprevalence have chosen a cut-off of 1/25 without further

explanation (GARCÍA-BOCANEGRA et al. 2010; BETIĆ et al. 2022). One study using the same protocol as ours was conducted by DJOKIC et al. (2016b) in France on 3595 pigs. That study included serum samples from fattening pigs, weaned piglets, and suckling piglets, resulting in an overall seroprevalence of 6.9%, which is substantially lower than the 12.21% detected among fattening pigs in our research. The lower seroprevalence rates observed in their study may be due to the inclusion of piglets and the fact that all animals were from closed facilities. In contrast, our study includes pigs from closed facilities as well as from facilities with outdoor access and fully outdoor housing. Another study conducted in France on sows, fattening pigs, and piglets from both outdoor and intensive farms, used the same protocol (DJOKIC et al. 2016a). This study found seroprevalence rates of 3.3% among fattening pigs and 10.5% among sows, again, lower than our results. This discrepancy could result from their use of cardiac fluid as the sample matrix, which underscores the need for caution when making direct comparisons. Additionally, differences in biosecurity practices on intensive farms between France and Croatia may also contribute to these variations.

Other European studies have also reported lower seroprevalence rates in pigs than the 19.68% observed in our study. Examples include Poland using the DAT (11.9%), southwestern Spain using the MAT (8.9%), the United Kingdom using ELISA (7.5%), Estonia using the MAT (5.8%), the Netherlands using ELISA (1.4 - 2.8%), northern Italy using ELISA (3.8%) and the MAT (2.1%), and Finland using ELISA (0.6%) (LIMON et al. 2017; PAPINI et al. 2017; SANTORO et al. 2017; GAZZONIS et al. 2018; PABLOS-TANARRO et al. 2018; FELIN et al. 2019; SWANENBURG et al. 2019; SROKA et al. 2020). Most of these studies focused on fattening pigs raised in similar or different farming systems. The lower seroprevalence rates compared to our results can largely be attributed to differences in sampling targets (primarily fattening pigs) and the serological tests used. Higher seroprevalence rates were observed in southwestern Spain using ELISA (24.1%), Sardinia using ELISA (51.7%), the Czech Republic using ELISA (21%), and Romania using the IFAT (23.1%) (PAȘTIU et al. 2013; SLANY et al. 2016; PIPIA et al. 2018; CASTILLO-CUENCA et al. 2020). These higher rates can be explained by differences in pig husbandry practices, as all these studies included pigs that were mostly raised extensively.

In our study, a pronounced geographical variation in *T. gondii* seroprevalence across Croatian counties was observed (Table 2, Figure 13), a pattern consistent with findings from some studies in other European countries (GARCÍA-BOCANEGRA et al. 2010; BETIĆ et al. 2022; AUGUSTYNIAK et al. 2025).

A study in Spain found that farms in regions with high temperatures and medium relative humidity had significantly higher seroprevalence (GARCÍA-BOCANEGRA et al. 2010). Another example from Serbia explained higher infection risk in certain regions by differences in farm sizes and biosecurity measures, which are generally much stricter on larger, highly industrialised farms (BETIĆ et al. 2022). A similar trend has also been reported outside Europe. Researchers in China have attributed regional differences in seroprevalence to variations in climatic conditions between provinces (LIU et al. 2025). These findings underscore the need for caution when comparing and explaining regional differences, as climatic conditions alone may not be sufficient. Beyond climate, such variations could be influenced by confounding factors, such as farm management practices, proximity to surrounding villages, presence of cats, other farm characteristics, and unequal sample sizes, as observed in another study (AUGUSTYNIAK et al. 2025).

In the present work, most samples were collected from northern and north-eastern Croatia, where the majority of pig farms are located, and which share similar climatic conditions. Regarding potential differences in farm management and other characteristics, we could not reliably link them to regions, likely due to substantial variation in sample sizes across counties (Figure 14). Therefore, regional differences in seroprevalence must be interpreted with caution, and no definitive conclusions can be drawn from these findings, given the large disparities in sample sizes per county. Ultimately, counties were not considered a potential risk factor, as sample size variability affected the analysis, and county-level distinctions in this study are considered irrelevant for the epidemiology and risk factors of toxoplasmosis in pigs in Croatia.

When examining the data on the percentage of seropositive pigs across biosecurity categories, higher proportions were observed in categories 1 and 4 (32.48% and 33.15%, respectively) compared to categories 2 and 3 (22.14% and 11.57%, respectively) (Table 4). This pattern is likely due to the low biosecurity levels and poor housing conditions in category 1, which increase exposure risks, while in category 4, outdoor housing allows cats, the definitive hosts of *T. gondii*, easy access to pigs, potentially contaminating their environment. Additionally, pigs in this category are at risk of infection through ingestion of rodent carcasses or other small animals containing tissue cysts.

In this study, biosecurity category was evaluated only descriptively and excluded from logistic regression analyses because it is a composite indicator derived from several underlying predictors and is therefore correlated with them across biosecurity levels, while also accounting for the different sample sizes in each group. Notably, no blood samples were collected from pigs from small holdings in biosecurity category 0, which keep one pig for their own needs (Figure 15). Obtaining these samples was highly unlikely because the sampling method was based on random convenience sampling, and such pigs represented only a small proportion (5.5% of the total number of pigs produced in 2021) (ACINGER-ROGIĆ 2022). These pigs are usually slaughtered traditionally in the owners' backyards, with notification to the authorised veterinary organisation. With the arrival of ASF in Croatia (16 June 2023), such slaughters have been prohibited in certain zones where the disease has appeared, which was after our sampling was completed. According to the categorisation results as of 31 December 2021, the majority of farms in Croatia belonged to biosecurity category 1 (31.1%) and category 2 (52.1%), while the largest number of pigs was kept on farms in biosecurity categories 2 and 3 (>1.1 million, approximately 88% of the total pig population) (ACINGER-ROGIĆ 2022). Accordingly, in our study, most samples were collected from pigs originating from farms with biosecurity categories 2 and 3 (Figure 15). Surprisingly, seroprevalence reached 11.57% (79/117; 95% CI: 9.22-14.09%) in category 3, which primarily includes large intensive farms expected to fully comply with biosecurity standards. This outcome could arise from several factors. First, our sample included both seropositive fattening pigs and sows, with these production categories often housed differently – sows on smaller and medium-sized farms frequently have outdoor access. This leads to a second point: farm size. Even if assigned the highest biosecurity category, smaller farms may not implement measures as rigorously as larger ones. Additionally, the type of production on larger farms could play a role, as finishing farms have shown lower biosecurity quality compared to farrow-to-finish farms (KLUN et al. 2011; BETIĆ et al. 2022).

To better understand the situation in our case, the characteristics of farms with seropositive pigs warrant further investigation. Notably, we observed that some farms in biosecurity category 3 house pigs in enclosed facilities with outdoor access points. Of the 79 animals seropositive for *T. gondii* in biosecurity category 3, 27 came from farms with outdoor access, which could explain their positivity (Appendix 3). Such arrangements could pose a risk for *T. gondii* infection, as stray cats may access these areas and contaminate them. However, the remaining 52 seropositive animals originated from farms without outdoor access, which was unexpected. Further breakdown of these 52 pigs by production category revealed that 21 were

fattening pigs and 31 were sows. All fattening pigs came from large (>100 animals), intensive farms with closed production systems – namely, farrow-to-finish operations – that, according to the questionnaire, met maximum biosecurity standards. Identifying the weak link in these cases is challenging, but it is most likely attributable to human factors, such as non-adherence to strict biosecurity protocols. Seropositive pigs from such farms indicate the presence of critical points, a common phenomenon, as similar findings have been reported from high-tech industrialised farrow-to-finish farms in other European countries (DJOKIC et al. 2016b; GAZZONIS et al. 2018; OLSEN et al. 2020; BETIĆ et al. 2022; EPPINK et al. 2022).

Visits to some intensive farrow-to-finish farms revealed that, after passing the disinfection barrier at the entrance and changing clothes and footwear, workers move between buildings without repeated disinfection or changing footwear (personal communication with Ž. Acinger-Rogić). On these farms, different production categories are housed in separate buildings, often separated by green areas, where access by free-roaming cats remains possible. Considering this, a critical point could involve movement in the same footwear across the farm, leading to accidental introduction of oocysts into housing areas. Responses to the questionnaire on protective footwear and disinfection barriers do not necessarily reflect proper application of these measures. The same applies to other biosecurity-related questions, as questionnaire answers do not confirm the effectiveness of implementation, a limitation noted by ACINGER-ROGIĆ (2022) in her study on biosecurity levels and Aujeszky's disease occurrence in pigs in Croatia. Moreover, in 2025, ASF was confirmed on one of these intensive farms from which toxoplasmosis-positive pigs originated. This further indicates gaps in biosecurity and may suggest incomplete implementation of measures even in these high-standard facilities. When focusing on the group of seropositive sows housed without outdoor access, most originated from small and medium-sized farms. The reason for their seropositivity could be poorer biosecurity implementation on smaller farms, as demonstrated in prior studies (DJOKIC et al. 2016b; SROKA et al. 2020; BETIĆ et al. 2022).

Finally, it is noteworthy that farms belonging to less highly industrialised pig producers and breeders, which were assigned to biosecurity category 3, allow pigs outdoor access. The biosafetey questionnaire does not fully define when such a farm qualifies for this category, and based on our results, one might question whether it should. ACINGER-ROGIĆ (2022) made a similar observation in her study, using the entire biosafety questionnaire, and recommended revising the categorisation questionnaire to reduce potential bias. Although our research did not

use the full questionnaire but only selected questions, we observed similar limitations related to the phrasing of the questions, their scope, and the provided answer options.

Taking all this into account, pigs seropositive for *T. gondii* may indicate that there are gaps in the implementation of biosecurity measures within intensive production systems classified at the highest biosecurity level. In future, this approach could potentially be applied in practice to assess the overall effectiveness of biosecurity protocol implementation.

This study analysed risk factors for *T. gondii* infection in pigs in Croatia, as well as their interactions, to provide a basis for designing toxoplasmosis surveillance programmes in pig production. Identifying these factors improves understanding of the disease's distribution, enabling better estimation of the likelihood of seropositivity on farms.

To assess the effects of individual factors, variables such as pig category, age, sex, and responses to selected questions from the biosafety questionnaire (Appendix 1) were analysed as independent variables. All 12 investigated risk factors were statistically significant for *T. gondii* infection in pigs, with age, pig category, on-farm feed production, herd size, presence of pets, housing type, and protection against the entry of other animals into pig housing being particularly prominent (Table 5). For instance, univariate analysis showed that pigs older than 12 months had nearly a sixfold higher risk of seropositivity (OR = 5.72, $p < 0.0001$). Pig category was also a strong predictor, with sows having over threefold higher odds of infection compared to fattening pigs (OR = 3.23, $p < 0.0001$). Several other studies have also shown that sows are at greater risk, likely due to their longer lifespan compared to fattening pigs (DJOKIC et al. 2016a; KOFOED et al. 2017; GAZZONIS et al. 2018; PIPIA et al. 2018; CASTILLO-CUENCA et al. 2021). Fattening pigs are commonly slaughtered at approximately nine months old, while sows are usually slaughtered at a much older age (three to five years), resulting in prolonged exposure to the parasite. However, all these associations should be interpreted cautiously, as they do not account for potential confounding factors and are further evaluated in a multivariate model. It is particularly interesting that pig category was not significant in the final model. This variable was likely not retained in the multivariate model due to overlap with the age category, which did enter the final model. Although pig category remains biologically important, its effect in the final model is partially confounded by age category. This could be explained by the strong association between these two variables, in other words, most pigs older than 12 months were sows, while the younger ones were fattening pigs.

The final multivariate model showed that the main risk factors for *T. gondii* seroprevalence in pigs in Croatia are age, herd size, housing type, and on-farm feed production (Table 6). Among these, herd size and age showed the strongest association with seropositivity (Figure 16, Table 7).

Age is a well-established and important factor influencing *T. gondii* seroprevalence in animals, as most infections occur postnatally (DUBEY 2022a). A recent review of risk factors for toxoplasmosis in farm animals highlighted that age is frequently identified as a significant predictor in studies involving pigs, sheep, goats, and chickens (STELZER et al. 2019). Essentially, an animal's age is proportional to its cumulative exposure time to the parasite, given that *T. gondii* is a ubiquitous parasite capable of infecting any warm-blooded host. In this review, the authors underscore the importance of age as a risk factor and suggest that it should always be included in risk analyses for studies involving multiple age categories. They also note that age can act as a confounder or effect modifier in statistical data analysis. A similar effect of the age variable was observed in the present study; as mentioned earlier, age appears to influence the pig category variable in the multivariate analysis of risk factors. An animal's age often depends on the production type. For instance, animals used in the meat industry typically belong to lower age categories compared with those used for breeding and dairy production.

In the present study, age was positively associated with seroprevalence, and the final model showed that pigs older than 12 months had more than twice the odds of seropositivity compared with younger animals (OR = 2.32, 95% CI: 1.59–3.39). Age was identified as a risk factor in several other studies conducted in European countries such as Spain, France, Poland, Denmark, and Serbia, which explored risk factors among different pig age categories (GARCÍA-BOCANEGRA et al. 2010; DJOKIC et al. 2016b; BETIĆ et al. 2022; OLSEN et al. 2020; SROKA et al. 2020). Similar findings were reported in some studies conducted outside Europe, such as in Cuba, Brazil, and India (SOUSA et al. 2014; CASTILLO-CUENCA et al. 2021; MOUDGIL et al. 2024).

Analysis of farm size in relation to seroprevalence revealed a statistically significant increase in smaller herds (Table 6), consistent with findings from other studies (DJOKIC et al. 2016b; HERRERO et al. 2016; LIMON et al. 2017; SROKA et al. 2020). Risk analysis indicated that pigs on small farms had over four times higher odds of infection compared to large farms with more than 100 animals (OR = 4.25, 95% CI: 2.77–6.52), and nearly double the odds compared to medium-sized farms (OR = 1.88, 95% CI: 1.27–2.78). This pattern likely arises because

small farms are typically less confined, often provide outdoor access, and implement fewer stringent biosecurity measures than larger, intensive operations, which usually maintain fully confined systems. In Croatia, most pig farms are small, family-run enterprises with low biosecurity and suboptimal housing conditions, which undoubtedly increase *T. gondii* infection risks (ACINGER-ROGIĆ 2022). Domestic cats, as definitive hosts, can easily access pig housing, feed, bedding, and surrounding farmland, contaminating them with oocysts. These observations align with a broader trend in the literature, where pigs in smaller production groups consistently show higher seropositivity due to suboptimal hygiene and animal husbandry standards (STELZER et al. 2019). For instance, a previously mentioned large-scale study in France confirmed the association between farm size and seropositivity among fattening pigs and piglets in confined systems, linking it to lower technical and hygienic standards on small holdings (DJOKIC et al. 2016b). Similarly, a large-scale epidemiological study in England found higher infection risks on farms with fewer than 200 animals, often involving outdoor pig access and open feed storage (LIMON et al. 2017). Comparable results have also been reported from other European countries, including Serbia, Spain, and Poland, where farm size has been identified as a significant risk factor for *T. gondii* infection in pigs (KLUN et al. 2011; HERRERO et al. 2016; SROKA et al. 2020). A recent study conducted in Kenya identified farm size as a key risk factor, with seroprevalence dropping significantly in herds exceeding 100 animals, attributed to improved management and hygiene practices in such farms (CHEPYATICH et al. 2023). This may indicate a lack of hygiene and animal husbandry practices in small holdings and small farms worldwide.

When examining pig housing systems, a significant association was found with *T. gondii* seroprevalence, particularly in closed systems with outdoor access and fully open housing (Table 6). Other studies have identified farming systems that allow outdoor access and open housing as key risk factors, primarily because cats can access pigs and contaminate the soil and pasture with oocysts (KLUN et al. 2006; GARCÍA-BOCANEGRA et al. 2010; DJOKIC et al. 2016a; LIMON et al. 2017; SWANENBURG et al. 2019; MOUDGIL et al. 2024).

In our study, open housing increased the likelihood of infection by a factor of 2.09 (95% CI: 1.24–3.54), meaning pigs in such systems were more than twice as likely to be seropositive for toxoplasmosis compared to those kept indoors. Pigs from open housing primarily came from larger farms (>100 animals) classified under biosecurity category 4, where animals are managed extensively most of the year. Most of these farms focus on rearing autochthonous breeds, such as Black Slavonian, Turopolje, and Mangalica pigs. Consequently,

higher seroprevalence and infection risk are expected in these traditional production systems, where pigs are raised extensively on pastures and woodlands, foraging for natural food sources such as acorns, beech mast, and wild grains. Feeding in these systems relies almost entirely on pasture and acorns, with supplemental corn or grains required only in lean years, particularly during autumn and winter. This foraging behaviour increases the risk of infection, as pigs root in the soil and may ingest contaminated soil or water containing sporulated *T. gondii* oocysts from feline faeces. Additionally, like wild boars, these pigs can acquire the parasite by consuming infected rodents or other small animals, driven by their natural scavenging and predatory instincts (STELZER et al. 2019). This traditional pig rearing system differs from other outdoor farming types, such as free-range or organic farms, which have become more popular in recent years. These systems generally provide outdoor access alongside indoor housing and use standard pig feed, although organic farms in the EU must comply with specific regulations and ensure feed is free from pesticides and artificial fertilisers (EUROPEAN COMMISSION 2023).

In the present study, seropositive pigs kept outdoors also originated from medium-sized (21-100 animals) and large farms (>100 animals) in lower biosecurity categories (1 and 2). These farms either do not meet or only partially fulfil biosecurity requirements. Although modest wooden or concrete shelters are available, pigs are typically kept outdoors for most of the time. It is evident that various systems of extensive pig rearing exist, with their names differing between countries. Consequently, the terms “organic“ or “free-range“ pigs cannot fully apply to Croatia's autochthonous breeds raised traditionally, or to pigs from low biosecurity farms that keep pigs extensively. For simplicity, we will refer to these groups as outdoor pigs.

Outdoor pigs have been shown to have increased infection risks in Poland, the Netherlands, France, Denmark, and Latvia (DEKSNE and KIRJUŠINA 2013; DJOKIC et al. 2016a; SWANENBURG et al. 2019; OLSEN et al. 2020; SROKA et al. 2020; EPPINK et al. 2022). Outside Europe, pigs kept outdoors in Mexico, India, and Ethiopia also showed higher infection probabilities (ALVARADO-ESQUIVEL et al. 2014; GEBREMEDHIN et al. 2015; MOUDGIL et al. 2024). Furthermore, a relatively high seroprevalence of 33.96% (72/212; 95% CI: 27.60-40.55%) was detected in outdoor pigs in this study. In Europe, seroprevalence rates range from 3.8% to 5.8% by ELISA in the Netherlands, 6.3% by the MAT in France, 24.1% by ELISA in Spain, 51.7% by ELISA in Italy, and 52.6% by ELISA in Poland (DJOKIC et al. 2016a; PIPIA et al. 2018; SWANENBURG et al. 2019; CASTILLO-CUENCA et al. 2020;

PUCHALSKA et al. 2022). On other continents, rates reach 32% by WB in Peru, and 32% by ELISA in Argentina, 39.7% by the DAT in Ethiopia, and 45.3% by the MAT in Mexico (ALVARADO-ESQUIVEL et al. 2014; GEBREMEDHIN et al. 2015; FLORES et al. 2021; KUNIC et al. 2022). High seroprevalence in outdoor pigs, as reported in numerous studies across Europe and worldwide, further underscores the role of housing systems in the epidemiology of toxoplasmosis. The high seroprevalence observed in this study may be explained by the predominance of autochthonous breeds raised traditionally, which likely increases parasite exposure through specific feeding practices such as foraging in natural environments, compared to other extensive rearing systems. Overall, elevated seroprevalence in outdoor pigs, as documented in numerous studies across Europe and worldwide, further emphasises the role of housing systems in the epidemiology of toxoplasmosis in pigs. In contrast, fully indoor housing without outdoor access in this study acted as a protective factor against *T. gondii* infection (OR = 0.55, 95% CI: 0.37–0.80). Pigs in these confined systems had approximately 45% lower odds of infection compared to those with outdoor access. This protective effect is due to reduced environmental contact with oocysts shed by cats in soil, feed, or water, along with intensive biosecurity protocols that further minimise infection risks. Some other studies evaluating infection risk for pigs in confined systems have shown higher chances of infection for pigs with access to the outdoors (SOUSA et al. 2014; LIMON et al. 2017; EPPINK et al. 2022).

The questionnaire included feed-related variables, as ingestion of sporulated *T. gondii* oocysts through contaminated feed is an important infection route for pigs. These questions addressed feed production and storage. Open or less confined feed storage has been identified as a risk factor in some studies, as it allows cat access and contamination with sporulated oocysts (STELZER et al. 2019). Although the variable on feed storage could not be included in the multivariate model in our study due to collinearity with other variables, the construction of the question and its response options warrants attention. The question and answer choices were superficial, focusing only on whether feed was stored properly or not. No details were provided on the type of storage or what constitutes "proper feed storage," such as whether this refers only to silos or to other methods that may protect feed from cats and rodents. Small and medium-sized farms are unlikely to have silos like large intensive operations, yet the questionnaire responses do not allow for such distinctions. The variable retained in the multivariate analysis was on-farm feed production, which was associated with higher odds of seropositivity (OR = 1.63, 95% CI: 1.06–2.51). One possible explanation is that on-farm feed production may

attract cats and rodents, particularly to supplement storage sites, even on larger farms. On small and medium-sized farms, less rigorous biosecurity and technical differences in the production process compared to large intensive farms could additionally contribute to a higher risk of feed contamination. For instance, a large study on conventional pig farms in the Netherlands found that heated feed in the production process was associated with lower seroprevalence (EPPINK et al. 2022). To fully elucidate the role of on-farm feed production, further research is needed to identify potential critical points in these systems, particularly where technological differences may exist. Future studies should also include detailed assessments of feed storage methods, with the development of more specific questions on this aspect.

Antibody titres in this study varied widely, ranging from low levels (1/6 in 19.1% of animals) to very high levels (1/12 288 in 1.3% of animals). More than half of the animals (65.5%) exhibited low titres (≤1/48) (Figure 17). The highest titres were observed in sows, with five animals showing titres of 1/1536 and four reaching 1/12 288 (Table 8). Elevated titres may indicate a potential acute infection, as suggested in an earlier study conducted in Serbia using the MAT by KLUN et al. (2006), who also detected the highest titre of 1/12 288 using a cut-off of 1/25. However, no definitive conclusions can be drawn from high IgG titre alone. A more recent Serbian study, also using the MAT with a cut-off of 1/25, found that over 80% of pigs had low titres (≤ 1/50), with the highest titre of 1/800 recorded in one sow (BETIĆ et al. 2022). The authors also performed bioassay experiments in mice by inoculating them with heart digests from seropositive sows. Although no direct correlation was found between antibody titres and parasite isolation, the majority of viable parasites were isolated from sows with titres ≥ 1/100. Consequently, the authors suggested that such high titres could signal an elevated risk to human consumers of that meat. A similar correlation between antibody titres measured by IFAT, cut-off 1/20, and the presence of viable tissue cysts was reported in a Spanish study (HERRERO et al. 2016). The researchers concluded that titres ≥ 1/80 may serve as an indicator of viable tissue cysts in meat and help identify higher-risk animals in food chain production. This may mean that animals with higher titres detected with the MAT or IFAT could represent a significant source of human toxoplasmosis transmission through the consumption of undercooked or raw pork. In the present study, 34.5% of seropositive pigs had titres ≥ 1/96. However, no further conclusions can be drawn, as a different test and/or cut-off value was used, and serological testing alone does not allow accurate assessment of the true risk of human toxoplasmosis transmission via pork. Confirming this risk would require studies that combine serology with isolation of viable parasites from retail pork. Ultimately, post-slaughter handling

of pig carcasses, including storage in cold chambers and other post-harvest treatments, may significantly reduce or eliminate *T. gondii* tissue cysts (HILL et al. 2006; DUBEY et al. 2020a). Indeed, the prevalence of viable tissue cysts isolated from pork samples obtained from grocery stores is low as shown in a recent study conducted by DUBEY et al. (2020f) in the USA.

Titre analysis revealed a significant difference in antibody titres against *T. gondii* between fattening pigs and sows, with sows exhibiting markedly higher titres overall (Table 9). The results indicate that sows have titres 1.87 times higher (GM = 53.07) than fattening pigs (GM = 28.42). This difference can be difficult to interpret, as elevated IgG titres may indicate a recent acute infection, a chronic infection, or immune reactivation, and the MAT detects only IgG antibodies. Distinguishing acute from chronic infection requires additional tests to detect IgM antibodies and assess IgG avidity. Furthermore, antibody titres can be influenced by multiple factors, including the size of the infective dose and the individual immune responsiveness of the intermediate host, making a definitive explanation for the observed differences challenging (OPSTEEGH et al. 2016). The dynamics of *T. gondii* specific IgG antibodies in pigs remain poorly understood, as in animals generally. Unlike in humans, where *T. gondii* specific IgG antibodies are known to persist lifelong, it remains unclear how long pigs can remain seropositive for *T. gondii* (VILLARD et al. 2016). Experimental studies following infected pigs for 2.5 and 4 months post-infection showed fluctuating IgG antibody levels but persistent seropositivity (LIND et al. 1997; BASSO et al. 2017). A more recent study that explored *T. gondii* antibody dynamics in sows over a year following natural infection showed that not all sows test consistently positive or negative for *T. gondii* (OLSEN et al. 2021). The authors of that study attributed this to the possibility of false positive results, which can occur with ELISA tests due to cross-reactivity with closely related parasites, as previously documented by LIND et al. (1997), and the possibility of false negative results with ELISA, as samples from those sows were positive when tested with MAT and WB.

The analysis of antibody titres in this study also revealed greater heterogeneity in antibody responses among sows (CV=4.35), likely related to their older age and different physiological and production stages. However, there is no evidence supporting stress-induced reactivation of chronic infection in pigs. Instead, an experimental study in chronically infected pregnant sows documented a decline in IgG antibody levels at farrowing (BASSO et al. 2017). Increases in antibody levels during pregnancy have been observed in experimental studies involving cattle and goats, although there are no data for the postpartum period (DE OLIVEIRA et al. 2022; DE OLIVEIRA et al. 2023). In contrast, the more uniform titres observed in fattening pigs

(CV = 2.11) may reflect their shorter lifespan and more consistent production stage. However, definitive conclusions cannot be drawn from these findings.

Taken together, these results highlight the substantial influence of age and production stage on *T. gondii* seropositivity and antibody titre magnitude. Notably, a literature review identified no previous serological studies in pigs that performed titre analysis in a manner comparable to the present work. To conclusively explain the higher titres observed in sows, further research is needed, including longitudinal monitoring of titres in both fattening pigs and sows over an extended period, and the use of multiple serological methods that allow IgM detection and IgG avidity testing to better understand titre dynamics. IgG avidity assessment is crucial for distinguishing past from recent infections (TEIMOURI et al. 2020). However, unlike in humans, no commercial tests are available for pigs (GARNAUD et al. 2020). Only adapted in-house protocols or modified commercial ELISA tests have been used to assess the binding strength of IgG antibodies to an antigen (*T. gondii*) (SUARÉZ-ARANDA et al. 2000; BASSO et al. 2017; ROY et al. 2024).

As noted in the introduction, pork is one of the most common sources of *T. gondii* infection in humans. Given that pork is the most consumed meat in Croatia, this study highlights a potential risk for human infection, particularly when pork is consumed undercooked. Identifying key risk factors for toxoplasmosis in pigs enables targeted interventions to reduce prevalence at both animal and farm levels, thereby enhancing the safety of pork for human consumption. The risk factors identified in this study can be used in planning toxoplasmosis monitoring systems. Additionally, the biosafety questionnaire should be revised to include questions that more thoroughly assess specific risk factors while minimising nonspecific responses to reduce bias. Monitoring, diagnosis, and prevention of this zoonosis are fully aligned with the One Health initiative. Standardising diagnostic methods in animals, such as adopting the MAT test, is advisable, along with conducting similar large-scale studies in Croatia for other livestock species.

7. CONCLUSIONS

- This is the first large-scale study on toxoplasmosis in pigs in Croatia
- The seroprevalence of toxoplasmosis in Croatian pigs is 19.68%, which aligns with global rates observed in pig populations. This suggests that toxoplasmosis may pose a potential risk for human infection
- The higher seroprevalence of toxoplasmosis in sows (30.96%) compared to fattening pigs (12.21%) likely reflects an age-related effect, as sows are exposed to the parasite for longer due to their extended lifespans
- The higher seroprevalence in pigs from farms in biosecurity categories 1 and 4 (32.48% and 33.15%) than those from categories 2 and 3 (22.14% and 11.57%) indicates that housing conditions affect the exposure of pigs to the parasite
- The higher antibody titres against *T. gondii* observed in sows than in fattening pigs remain unexplained by this study
- The key risk factors significantly influencing the prevalence of toxoplasmosis are: age, farm size (herd size), housing type and feed production on farm
- Targeted interventions must be implemented to reduce or eliminate risk factors on farms, thereby lowering seroprevalence and enhancing pork safety for human consumption
- Planning toxoplasmosis monitoring systems should include all farms exposed to identified risk factors
- Questions from the biosafety questionnaire should be revised, featuring questions that more thoroughly examine specific risk factors
- Toxoplasmosis should be addressed from the One Health perspective, adopting the MAT as the diagnostic standard in animals, and conducting further research on other livestock species whose meat is consumed by humans

8. BIBLIOGRAPHY

ACINGER-ROGIĆ, Ž. (2022): Utvrđivanje čimbenika rizika i učinka razine biosigurnosti na prevalenciju infekcije virusom bolesti Aujeszkoga u populaciji domaćih svinja u Hrvatskoj. PhD Thesis. University of Zagreb, Zagreb, Croatia.

AHLERS, A. A., M. A. MITCHELL, J. P. DUBEY, R. L. SCHOOLEY, E. J. HESKE (2015): Risk factors for *Toxoplasma gondii* exposure in semiaquatic mammals in a freshwater ecosystem. J. Wildl. Dis. 51, 488–492.
DOI: 10.7589/2014-03-071.

ALMERÍA, S., D. CANO-TERRIZA, P. PRIETO, J. P. DUBEY, D. JIMÉNEZ-MARTÍN, S. CASTRO-SCHOLTEN, J. PANIAGUA, I. GARCÍA-BOCANEGRA (2021): Seroprevalence and risk factors of *Toxoplasma gondii* infection in wild ungulates that cohabit in a natural park with human–animal interaction in the Mediterranean ecosystem. Zoonoses Public Health 68, 263–270.
DOI: 10.1111/zph.12821.

ALMERIA, S., J. P. DUBEY (2021): Foodborne transmission of *Toxoplasma gondii* infection in the last decade. An overview. Res. Vet. Sci. 135, 371–385.
DOI: 10.1016/j.rvsc.2020.10.019.

ALVARADO-ESQUIVEL, C., D. ROMERO-SALAS, Z. GARCÍA-VÁZQUEZ, M. CRIVELLI-DIAZ, M. BARRIENTOS-MORALES, L. LOPEZ-DE-BUEN, J. P. DUBEY (2014): Seroprevalence and correlates of *Toxoplasma gondii* infection in domestic pigs in Veracruz State, Mexico. Trop. Anim. Health Prod. 46, 705–709.
DOI: 10.1007/s11250-014-0551-3.

ANONYMOUS (2019): Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625.

ANONYMOUS (2019): Naredba o mjerama za sprječavanje pojave i rano otkrivanje unosa virusa afričke svinjske kuge u Republici Hrvatskoj (NN 96/2019).

ANONYMOUS (2023): Naredba o provedbi i financiranju mjera sprječavanja, kontrole i nadziranja bolesti životinja na području Republike Hrvatske (NN 1/23).

ATTIAS, M., D. E. TEIXEIRA, M. BENCHIMOL, R. C. VOMMARO, P. H. CREPALDI, W. DE SOUZA (2020): The life-cycle of *Toxoplasma gondii* reviewed using animations. Parasites and Vectors 13, 588.
DOI: 10.1186/s13071-020-04445-z.

AUGUSTYNIAK, A., A. DORS, R. NIEMYJSKI, M. POMORSKA-MÓL (2025): Serological survey of antibodies against *Toxoplasma gondii* and *Neospora caninum* in pigs from various regions of Poland. BMC Vet. Res. 21, 98.
DOI: 10.1186/s12917-025-04566-6.

BACHAN, M., A. R. DEB, B. R. MAHARANA, N. R. SUDHAKAR, V. SUDAN, B. C. SARAVANAN, A. K. TEWARI (2018): High seroprevalence of *Toxoplasma gondii* in goats in Jharkhand state of India. Vet. Parasitol. Reg. Stud. Reports 12, 61–68.
DOI: 10.1016/j.vprsr.2018.02.004.

BARIĆ, J. (2012): Uloga svinja invadiranih protozoonom *Toxoplasma gondii* u epizootiologiji zoonoze. PhD Thesis. University of Zagreb, Zagreb, Croatia.

BASSO, W., F. GRIMM, M. RUETTEN, V. DJOKIC, R. BLAGA, X. SIDLER, P. DEPLAZES (2017): Experimental *Toxoplasma gondii* infections in pigs: Humoral immune response, estimation of specific IgG avidity and the challenges of reproducing vertical transmission in sows. Vet. Parasitol. 236, 76–85.
DOI: 10.1016/j.vetpar.2017.01.026.

BETIĆ, N., N. KARABASIL, O. DJURKOVIĆ-DJAKOVIĆ, V. ĆIRKOVIĆ, B. BOBIĆ, I. B. LAZIĆ, V. DJORDJEVIĆ, I. KLUN (2022): Seroprevalence, Direct Detection and Risk Factors for *Toxoplasma gondii* Infection in Pigs in Serbia, and Influence of Biosecurity Measures. Microorganisms 10, 1069.
DOI: 10.3390/microorganisms10051069.

BIGNA, J. J., J. N. TOCHIE, D. N. TOUNOUGA, A. O. BEKOLO, N. S. YMELE, E. L. YOUDA, P. S. SIME, J. R. NANSSEU (2020): Global, regional, and country seroprevalence of *Toxoplasma gondii* in pregnant women: a systematic review, modelling and meta-analysis. Sci. Rep. 10.
DOI: 10.1038/s41598-020-69078-9.

BLAGA, R., D. AUBERT, A. THÉBAULT, C. PERRET, R. GEERS, M. THOMAS, A. ALLIOT, V. DJOKIC, N. ORTIS, L. HALOS, B. DURAND, A. MERCIER, I. VILLENA, P. BOIREAU (2019): *Toxoplasma gondii* in beef consumed in France: Regional variation

in seroprevalence and parasite isolation. Parasite 26.
DOI: 10.1051/parasite/2019076.

CALERO-BERNAL, R., M. BETSON, I. SLANA, B. BARTOSOVA, G. MARUCCI, A. POSSENTI, G. ÁLVAREZ-GARCÍA, N. BIER, A. MAYER-SCHOLL, R. P. BERG, U. CHAUDHRY, N. M. LÓPEZ-UREÑA, W. PIOTROWSKA, J. SROKA, G. S. JOHANNESSEN, R. DAVIDSON, F. DÁMEK, R. BLAGA, S. THOUMIRE, B. ZALEWSKÁ, H. C. WAAP, P. JOKELAINEN, M. LALLE (2025): Molecular detection of *Toxoplasma gondii* in ready-to-eat salad mixes: multi-country survey using a validated and harmonised standard operating procedure, Europe, 2021 to 2022. Eurosurveillance 30.
DOI: 10.2807/1560-7917.ES.2025.30.22.2400594.

CALERO-BERNAL, R., S. M. GENNARI (2019): Clinical toxoplasmosis in dogs and cats: An update. Front. Vet. Sci. 6, 54.
DOI: 10.3389/fvets.2019.00054.

CANO-TERRIZA, D., S. ALMERÍA, J. CABALLERO-GÓMEZ, D. JIMÉNEZ-MARTÍN, S. CASTRO-SCHOLTEN, J. P. DUBEY, I. GARCÍA-BOCANEGRA (2020): Exposure to *Toxoplasma gondii* in zoo animals in Spain. Prev. Vet. Med. 176.
DOI: 10.1016/j.prevetmed.2020.104930.

CANO-TERRIZA, D., J. J. FRANCO, E. JOSE-CUNILLERAS, F. BUONO, S. ALMERÍA, V. VENEZIANO, E. ALGUACIL, J. GARCÍA, I. VILLENA, J. P. DUBEY, D. JIMÉNEZ-MARTÍN, I. GARCÍA-BOCANEGRA (2023): Seroepidemiological study of *Toxoplasma gondii* in equids in different European countries. Zoonoses Public Health 70, 276–283.
DOI: 10.1111/zph.13026.

CARADONNA, T., M. MARANGI, F. DEL CHIERICO, N. FERRARI, S. REDDEL, G. BRACAGLIA, G. NORMANNO, L. PUTIGNANI, A. GIANGASPERO (2017): Detection and prevalence of protozoan parasites in ready-to-eat packaged salads on sale in Italy. Food Microbiol. 67, 67–75.
DOI: 10.1016/j.fm.2017.06.006.

CASTILLO-CUENCA, J. C., J. M. DÍAZ-CAO, Á. MARTÍNEZ-MORENO, D. CANO-TERRIZA, S. JIMÉNEZ-RUIZ, S. ALMERÍA, I. GARCÍA-BOCANEGRA (2020): Seroepidemiology of *Toxoplasma gondii* in extensively raised Iberian pigs in Spain. Prev. Vet. Med. 175, 104854.

DOI: 10.1016/j.prevetmed.2019.104854.

CASTILLO-CUENCA, J. C., Á. MARTÍNEZ-MORENO, J. M. DIAZ-CAO, A. ENTRENA-GARCÍA, J. FRAGA, P. C. ARIAS, S. ALMERÍA, I. GARCÍA-BOCANEGRA (2021): Seroprevalence of *Toxoplasma gondii* and associated risk factors in domestic pigs raised from Cuba. Parasitol. Res. 120, 2897–2903.
DOI: 10.1007/s00436-021-07245-1.

CHANG-CUN, S., Y. XING-ZHENG, S. LI-YING, G. XIAO-XIAN, D. JIANG-ZU (1993): The effect of cobalt-60 irradiation on the infectivity of *Toxoplasma gondii*. Int. J. Parasitol. 23, 89–93.
DOI: 10.1016/0020-7519(93)90101-4.

CHEPYATICH, D., D. N. SENTAMU, N. BOR, J. ONONO, P. B. GATHURA, J. M. AKOKO, L. F. THOMAS (2023): Seroprevalence of *Toxoplasma gondii* in Slaughtered Pigs in Kiambu, Kenya. Zoonotic Dis. 3, 301–306.
DOI: 10.3390/zoonoticdis3040024.

CHU, K. B., F. S. QUAN (2021): Advances in *Toxoplasma gondii* vaccines: Current strategies and challenges for vaccine development. Vaccines 9, 413.
DOI: 10.3390/vaccines9050413.

ÇIÇEK, H., C. BABÜR, M. ESER (2011): [Seroprevalence of *Toxoplasma gondii* in Pirlak sheep in the Afyonkarahisar Province of Turkey]. Turkiye Parazitol. Derg. 35, 137–139.
DOI: 10.5152/tpd.2011.34.

CONDOLEO, R., P. ROMBOLÀ, R. PALUMBO, D. SANTORI, S. SERRA, S. TONON, A. BOSCO, E. SEZZI (2023): *Toxoplasma gondii* in sheep: Serological occurrence at slaughterhouse level in Italy and environmental risk factors. Front. Vet. Sci. 10, 1057277.
DOI: 10.3389/fvets.2023.1057277.

COOK, A. J. C. (2000): Sources of toxoplasma infection in pregnant women: European multicentre case-control study Commentary: Congenital toxoplasmosis---further thought for food. BMJ 321, 142–147.
DOI: 10.1136/bmj.321.7254.142.

CORNELISSEN, J. B. W. J., J. W. B. VAN DER GIESSEN, K. TAKUMI, P. F. M. TEUNIS, H. J. WISSELINK (2014): An experimental *Toxoplasma gondii* dose response challenge model to study therapeutic or vaccine efficacy in cats. PLoS One 9, e104740.

DOI: 10.1371/journal.pone.0104740.

CROATIAN BUREAU OF STATISTICS (2021): Basic characteristics of household consumption, 2019. Available online: https://podaci.dzs.hr/en/archives/personal-consumption-and-poverty-indicators/basic-characteristics-of-household-consumption/

CROATIAN BUREAU OF STATISTICS (2025a): Slaughtering of livestock and poultry, 2024. Available online: https://podaci.dzs.hr/2025/en/97396

CROATIAN BUREAU OF STATISTICS (2025b): Livestock production, 2024. Available online: https://podaci.dzs.hr/2025/en/97392

CUBAS-ATIENZAR, A. I., G. HIDE, J. E. SMITH (2019): Mat Seroprevalence Infers Low Rates of *Toxoplasma gondii* in Domestic Pigs from Yucatan, Mexico. J. Parasitol. 105, 738–747.

ÇULBASAN, V., C. GUNGOR, D. A. GUNDOG, K. KOSKEROGLU, N. E. ONMAZ (2023): Molecular surveillance of *Toxoplasma gondii* in raw milk and Artisan cheese of sheep, goat, cow and water buffalo origin. Int. J. Dairy Technol. 76, 948–954.
DOI: 10.1111/1471-0307.12988.

CURRENT, W. L., S. J. UPTON, P. L. LONG (1990): Taxonomy and life cycles. pp. 1–16 in: P.L. Long (ED.), Coccidiosis Man Domest. Anim. Boca Raton: CRC Press.

DAHMANE, A., A. VISMARRA, K. PASSEBOSC-FAURE, N. REGHAISSIA, D. BAROUDI, H. SAMARI, M. SEMERARO, H. YERA, A. E. LAATAMNA (2025): First Molecular Detection of *Toxoplasma gondii* DNA in Blood and Milk of Goats from Algeria. Pathogens 14, 174.
DOI: 10.3390/pathogens14020174.

ĐAKOVIĆ-RODE, O., S. ŽIDOVEC LEPEJ, M. VODNICA MARTUCCI, V. LASICA POLANDA, J. BEGOVAC (2010): Prevalence of antibodies against *Toxoplasma gondii* in patients infected with human immunodeficiency virus in Croatia. Infektološki Glas. 30, 5–10.

DÁMEK, F., B. FREMAUX, D. AUBERT, S. THOUMIRE, M. DELSART, J. L. MARTIN, S. VUILLERMET, M. OPSTEEGH, P. JOKELAINEN, D. LE ROUX, P. BOIREAU, I. VILLENA, R. BLAGA (2023): Inactivation of *Toxoplasma gondii* in dry sausage and processed pork, and quantification of the pathogen in pig tissues prior to production. Food Waterborne Parasitol. 31, e00194.

DOI: 10.1016/j.fawpar.2023.e00194.

DE OLIVEIRA, J. M. B., B. P. e. SILVA, M. RIBEIRO-ANDRADE, W. J. N. PORTO, R. P. B. DE MELO, J. W. P. JUNIOR, A. A. DA FONSECA OLIVEIRA, R. A. MOTA (2022): *Toxoplasma gondii* infection in goats: serological, pathological, and clinical monitoring during gestation. Parasitol. Res. 121, 3147–3153.
DOI: 10.1007/s00436-022-07633-1.

DE OLIVEIRA, U. V., V. C. S. DE MAGALHÃES, S. C. L. COSTA, I. B. ALLAMAN, A. D. MUNHOZ (2023): Fluctuations of antibody serum titers for *Toxoplasma gondii* and *Neospora caninum* in naturally infected crossbred cows during gestation. Rev. Bras. Med. Vet. 45, e003023.
DOI: 10.29374/2527-2179.bjvm003023.

DEKSNE, G., M. KIRJUŠINA (2013): Seroprevalence of *Toxoplasma gondii* in domestic pigs (*Sus scrofa domestica*) and wild boars (*Sus scrofa*) in Latvia. J. Parasitol. 99, 44–47. DOI: 10.1645/GE-3187.1.

DELJAVAN, N., M. H. MOOSAVY, N. HAJIPOUR (2022): Molecular detection of *Toxoplasma gondii* DNA in goats (*Capra hircus*), sheep (*Ovis aries*), and donkey (*Equus asinus*) milk using PCR in East Azerbaijan province, Iran. Res. Vet. Sci. 152, 58–60. DOI: 10.1016/j.rvsc.2022.07.020.

DESHMUKH, A. S., B. K. HEBBAR, P. MITRA, S. SHINDE, S. CHAUDHARI, S. B. BARBUDDHE (2021): Seroprevalence and risk factors of *Toxoplasma gondii* infection among veterinary personnel and abattoir workers in Central India. Parasitol. Int. 84, DOI: 10.1016/j.parint.2021.102402.

DESMONTS, G., J. S. REMINGTON (1980): Direct agglutination test for diagnosis of *Toxoplasma* infection: Method for increasing sensitivity and specificity. J. Clin. Microbiol. 11, 562–568.
DOI: 10.1128/jcm.11.6.562-568.1980.

DJOKIC, V., R. BLAGA, D. AUBERT, B. DURAND, C. PERRET, R. GEERS, T. DUCRY, I. VALLEE, O. DJURKOVIC DJAKOVIC, A. MZABI, I. VILLENA, P. BOIREAU (2016a): *Toxoplasma gondii* infection in pork produced in France. Parasitology 143, 557–567.
DOI: 10.1017/S0031182015001870.

DJOKIC, V., C. FABLET, R. BLAGA, N. ROSE, C. PERRET, O. DJURKOVIC-DJAKOVIC, P. BOIREAU, B. DURAND (2016b): Factors associated with *Toxoplasma gondii* infection in confined farrow-to-finish pig herds in western France: An exploratory study in 60 herds. Parasites and Vectors 9, 466.
DOI: 10.1186/s13071-016-1753-5.

DJOKIĆ, V., I. KLUN, V. MUSELLA, L. RINALDI, G. CRINGOLI, S. SOTIRAKI, O. DJURKOVIĆ-DJAKOVIĆ (2014): Spatial epidemiology of *Toxoplasma gondii* infection in goats in Serbia. Geospat. Health 8, 479–488.
DOI: 10.4081/gh.2014.37.

DJURKOVIĆ-DJAKOVIĆ, O., B. BOBIĆ, A. NIKOLIĆ, I. KLUN, J. DUPOUY-CAMET (2013): Pork as a source of human parasitic infection. Clin. Microbiol. Infect. 19, 586–594.
DOI: 10.1111/1469-0691.12162.

DONG, H., Y. Y. LU, R. J. SU, Y. H. WANG, M. Y. WANG, Y. B. JIANG, Y. R. YANG (2018): Low prevalence of antibodies against *Toxoplasma gondii* in dairy cattle from China's central region. BMC Vet. Res. 14, 315.
DOI: 10.1186/s12917-018-1629-3.

DUBEY, J P (1974): Effect of freezing on the infectivity of *Toxoplasma* cysts to cats. J. Am. Vet. Med. Assoc. 165, 534–536. .

DUBEY, J P (1995): Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. J. Parasitol. 81, 410–415. .

DUBEY, J.P. (1997): Survival of *Toxoplasma gondii* tissue cysts in 0.85-6% NaCl solutions at 4-20 C - PubMed. J. Parasitol. 83, 946–949.

DUBEY, J. P. (1998): *Toxoplasma gondii* Oocyst Survival under Defined Temperatures. J. Parasitol. 84, 862.
DOI: 10.2307/3284606.

DUBEY, J. P. (2001): Oocyst shedding by cats fed isolated bradyzoites and comparison of infectivity of bradyzoites of the VEG strain *Toxoplasma gondii* to cats and mice. J. Parasitol. 87, 215–219.
DOI: 10.1645/0022-3395(2001)087[0215:osbcfi]2.0.co;2.

DUBEY, J. P. (2008): The history of *Toxoplasma gondii* - The first 100 years. J. Eukaryot.

Microbiol. 55, 467–475.
DOI: 10.1111/j.1550-7408.2008.00345.x.

DUBEY, J. P. (2009): History of the discovery of the life cycle of *Toxoplasma gondii*. Int. J. Parasitol. 39, 877–882.
DOI: 10.1016/j.ijpara.2009.01.005.

DUBEY, J. P. (2021): Outbreaks of clinical toxoplasmosis in humans: five decades of personal experience, perspectives and lessons learned. Parasites and Vectors 14.
DOI: 10.1186/s13071-021-04769-4.

DUBEY, J. P. (2022): Clinical toxoplasmosis in zoo animals and its management. Emerg. Anim. Species 2, 100002.
DOI: 10.1016/j.eas.2022.100002.

DUBEY, J. P. (2022a): Toxoplasmosis of animals and humans.Toxoplasmosis Anim. Humans. Boca Raton and Oxon: CRC Press.

DUBEY, J. P. (2022b): Biology of *Toxoplasma gondii*. pp. 7–89 in: Toxoplasmosis Anim. Humans. Boca Raton and Oxon: CRC Press.

DUBEY, J. P., C. K. CERQUEIRA-CÉZAR, F. H. A. MURATA, O. C. H. KWOK, D. HILL, Y. YANG, C. SU (2020a): All about *Toxoplasma gondii* infections in pigs: 2009–2020. Vet. Parasitol. 288.
DOI: 10.1016/j.vetpar.2020.109185.

DUBEY, J. P., C. K. CERQUEIRA-CÉZAR, F. H. A. MURATA, O. C. H. KWOK, Y. R. YANG, C. SU (2020e): All about toxoplasmosis in cats: the last decade. Vet. Parasitol. 283.
DOI: 10.1016/j.vetpar.2020.109145.

DUBEY, J. P., G. DESMONTS, C. MCDONALD, K. W. WALLS (1985): Serologic evaluation of cattle inoculated with *Toxoplasma gondii*: comparison of Sabin-Feldman dye test and other agglutination tests. Am. J. Vet. Res. 46, 1085–1088. .

DUBEY, J. P., D. E. HILL, V. FOURNET, D. HAWKINS-COOPER, C. K. CERQUEIRA-CÉZAR, F. H. A. MURATA, S. K. VERMA, O. C. H. KWOK, S. RANI, J. FREDERICKS, B. ADAMS, J. L. JONES, R. E. WIEGAND, Y. YING, M. GUO, C. SU, A. K. PRADHAN (2020f): Low prevalence of viable *Toxoplasma gondii* in fresh, unfrozen, American pasture-raised pork and lamb from retail meat stores in the United

States. Food Control 109.
DOI: 10.1016/j.foodcont.2019.106961.

DUBEY, J. P., D. S. LINDSAY, C. A. SPEER (1998): Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin. Microbiol. Rev. 11, 267–299.
DOI: 10.1128/cmr.11.2.267.

DUBEY, J. P., N. L. MILLER, J. K. FRENKEL (1970a): The *Toxoplasma gondii* oocyst from cat feces. J. Exp. Med. 132, 636–662.
DOI: 10.1084/jem.132.4.636.

DUBEY, J.P., F. H. A. MURATA, C. K. CERQUEIRA-CÉZAR, O. C. H. KWOK, C. SU (2020c): Economic and public health importance of *Toxoplasma gondii* infections in sheep: 2009–2020. Vet. Parasitol. 286, 109195. DOI: 10.1016/j.vetpar.2020.109195.

DUBEY, J. P., F. H. A. MURATA, C. K. CERQUEIRA-CÉZAR, O. C. H. KWOK (2020d): Public health and economic importance of *Toxoplasma gondii* infections in goats: The last decade. Res. Vet. Sci. 132, 292–307.
DOI: 10.1016/j.rvsc.2020.06.014.

DUBEY, J. P., F. H. A. MURATA, C. K. CERQUEIRA-CÉZAR, O. C. H. KWOK, M. E. GRIGG (2020b): Recent epidemiologic and clinical importance of *Toxoplasma gondii* infections in marine mammals: 2009–2020. Vet. Parasitol. 288, 109296.
DOI: 10.1016/j.vetpar.2020.109296.

DUBEY, J. P., F. H. A. MURATA, C. K. CERQUEIRA-CÉZAR, O. C. H. KWOK, I. VILLENA (2021a): Congenital toxoplasmosis in humans: An update of worldwide rate of congenital infections. Parasitology 148, 1406–1416.
DOI: 10.1017/S0031182021001013.

DUBEY, J. P., F. H. A. MURATA, C. K. CERQUEIRA-CÉZAR, O. C. H. KWOK (2021b): Recent epidemiologic and clinical *Toxoplasma gondii* infections in wild canids and other carnivores: 2009-2020. Vet. Parasitol. 290, 109337.
DOI: 10.1016/j.vetpar.2020.109337.

DUBEY, J. P., P. THULLIEZ, R. M. WEIGEL, C. D. ANDREWS, P. LIND, E. C. POWELL (1995): Sensitivity and specificity of various serologic tests for detection of *Toxoplasma gondii* infection in naturally infected sows. Am. J. Vet. Res. 56, 1030–1036. .

DUBEY, J. P., S. K. VERMA, L. R. FERREIRA, S. OLIVEIRA, A. B. CASSINELLI, Y. YING, O. C. H. KWOK, W. TUO, O. A. CHIESA, J. L. JONES (2014): Detection and survival of *Toxoplasma gondii* in milk and cheese from experimentally infected goats. J. Food Prot. 77, 1747–1753.
DOI: 10.4315/0362-028X.JFP-14-167.

DUBEY, J P, R. J. BRAKE, K. D. MURRELL, R. FAYER (1986): Effect of irradiation on the viability of *Toxoplasma gondii* cysts in tissues of mice and pigs. Am. J. Vet. Res. 47, 518–522. .

DUBEY, J P, J. K. FRENKEL (1973): Experimental toxoplasma infection in mice with strains producing oocysts. J. Parasitol. 59, 505–512.

DUBEY, J P, N. L. MILLER, J. K. FRENKEL (1970b): Characterization of the new fecal form of *Toxoplasma gondii*. J. Parasitol. 56, 447–56. .

DUBEY, J P, D. W. THAYER (1994): Killing of different strains of *Toxoplasma gondii* tissue cysts by irradiation under defined conditions. J. Parasitol. 80, 764–767. .

DUMÈTRE, A., C. LE BRAS, M. BAFFET, P. MENECEUR, J. P. DUBEY, F. DEROUIN, J. P. DUGUET, M. JOYEUX, L. MOULIN (2008): Effects of ozone and ultraviolet radiation treatments on the infectivity of *Toxoplasma gondii* oocysts. Vet. Parasitol. 153, 209–213.
DOI: 10.1016/j.vetpar.2008.02.004.

EFSA (2007): Scientific Opinion of the Panel on Biological Hazard on a request from EFSA on surveillance and monitoring of *Toxoplasma* in humans, food and animals. EFSA J. 5, 12. 583. pp 1–64.
DOI: 10.2903/j.efsa.2007.583.

EFSA (2011): Scientific Report on Technical specifications on harmonised epidemiological indicators for public health hazards to be covered by meat inspection of swine. EFSA J. 9, 10.
DOI: 10.2903/j.efsa.2011.2371.

EFSA BIOHAZ PANEL (EFSA PANEL ON BIOLOGICAL HAZARDS, K. KOUTSOUMANIS, A. ALLENDE, A. ALVAREZ-ORDÓÑEZ, D. BOLTON, S. BOVER-CID, M. CHEMALY, R. DAVIES, A. DE CESARE, L. HERMAN, F. HILBERT, R. LINDQVIST, M. NAUTA, L. PEIXE, G. RU, M. SIMMONS, P. SKANDAMIS, E. SUFFREDINI, S. CACCIÒ, R. CHALMERS, P. DEPLAZES, B.

DEVLEESSCHAUWER, E. INNES, T. ROMIG, J. VAN DER GIESSEN, M. HEMPEN, Y. VAN DER STEDE, L. ROBERTSON (2018): Scientific Opinion on the public health risks associated with food-borne parasites. EFSA J. 16, 113.
DOI: 10.2903/j.efsa.2018.5495.

EFSA and ECDC (2024): The European Union One Health 2023 Zoonoses report. EFSA J. 22, 12. DOI: 10.2903/j.efsa.2024.9106

EPPINK, D. M., M. BOUWKNEGT, J. W. B. VAN DER GIESSEN, M. SWANENBURG, D. OORBURG, B. A. P. URLINGS, C. P. A. VAN WAGENBERG, M. A. P. M. VAN ASSELDONK, H. J. WISSELINK (2022): Potential risk factors for the presence of anti-Toxoplasma gondii antibodies in finishing pigs on conventional farms in the Netherlands. Porc. Heal. Manag. 8, 27.
DOI: 10.1186/s40813-022-00272-z.

EUROPEAN COMMISSION (2023): Organic farming in the EU - A decade of organic growth. Available online: https://agriculture.ec.europa.eu/media/news/organic-farming-eu-decade-growth-2023-01-18_en

EUROPEAN COMMISSION (2025): Pigmeat Statistics. Available online: https://agriculture.ec.europa.eu/data-and-analysis/markets/overviews/market-observatories/meat/pigmeat-statistics_en

EUROPEAN COMMITTEE ON ORGAN TRANSPLANTATION (CD-P-TO) (2022): Guide to the quality and safety of organs for transplantation. Strasbourg; France: European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM). Available online: https://www.edqm.eu/en/guide-quality-and-safety-of-organs-for-transplantation

EUROSTAT (2024): Eurostat. Statistics Explained. Agricultural production - Livestock and Meat. Available online: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Agricultural_production_-_livestock_and_meat

FANIGLIULO, D., S. MARCHI, E. MONTOMOLI, C. M. TROMBETTA (2020): *Toxoplasma gondii* in women of childbearing age and during pregnancy: seroprevalence study in Central and Southern Italy from 2013 to 2017. Parasite 27, 2.
DOI: 10.1051/parasite/2019080.

FAO/WHO [FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED

NATIONS/WORLD HEALTH ORGANIZATION] (2014): Multicriteria-based ranking for risk management of food-borne parasites. Rome: FAO/WHO.

FELIN, E., O. HÄLLI, M. HEINONEN, E. JUKOLA, M. FREDRIKSSON-AHOMAA (2019): Assessment of the feasibility of serological monitoring and on-farm information about health status for the future meat inspection of fattening pigs. Prev. Vet. Med. 162, 76–82. DOI: 10.1016/j.prevetmed.2018.11.009.

FERGUSON, D. J. P. (2009): *Toxoplasma gondii*: 1908-2008, homage to Nicolle, Manceaux and Splendore. Mem. Inst. Oswaldo Cruz 104, 133–148.
DOI: 10.1590/S0074-02762009000200003.

FERNÁNDEZ-AGUILAR, X., V. ALZAGA, D. VILLANÚA, O. CABEZÓN, I. GARCÍA-BOCANEGRA, J. P. DUBEY, S. ALMERÍA (2013): Epidemiology and prevalence of *Toxoplasma gondii* infection in the Iberian hare (*Lepus granatensis*). Vet. Parasitol. 196, 194–8.
DOI: 10.1016/j.vetpar.2013.01.061.

FLORES, C. A., J. JIMENEZ, L. A. GOMEZ-PUERTA, C. PALACIOS, S. E. O'NEAL, C. MURO, A. E. GONZALEZ, R. H. GILMAN, M. CALDERÓN (2021): Seroprevalence of *Toxoplasma gondii* in free-range pigs in northern Peru. Vet. Parasitol. Reg. Stud. Reports 23, 100533.
DOI: 10.1016/j.vprsr.2021.100533.

FOROUTAN-RAD, M., H. MAJIDIANI, S. DALVAND, A. DARYANI, W. KOOTI, J. SAKI, F. HEDAYATI-RAD, E. AHMADPOUR (2016): Toxoplasmosis in Blood Donors: A Systematic Review and Meta-Analysis. Transfus. Med. Rev. 30, 116–122. DOI: 10.1016/j.tmrv.2016.03.002.

FOROUTAN, M., Y. FAKHRI, S. M. RIAHI, S. EBRAHIMPOUR, S. NAMROODI, A. TAGHIPOUR, A. SPOTIN, H. R. GAMBLE, A. ROSTAMI (2019): The global seroprevalence of *Toxoplasma gondii* in pigs: A systematic review and meta-analysis. Vet. Parasitol. 269, 42–52.
DOI: 10.1016/j.vetpar.2019.04.012.

FREDERICKS, J., D. S. HAWKINS-COOPER, D. E. HILL, J. LUCHANSKY, A. PORTO-FETT, H. R. GAMBLE, V. M. FOURNET, J. F. URBAN, R. HOLLEY, J. P. DUBEY (2019): Low salt exposure results in inactivation of *Toxoplasma gondii* bradyzoites during formulation of dry cured ready-to-eat pork sausage. Food Waterborne Parasitol. 15.

DOI: 10.1016/j.fawpar.2019.e00047.

FRENKEL, J. K., J. P. DUBEY (1973): Effects of freezing on the viability of *Toxoplasma* oocysts. J. Parasitol. 59, 587–588.
DOI: 10.2307/3278803.

FRENKEL, J. K., A. RUIZ, M. CHINCHILLA (1975): Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. Am. J. Trop. Med. Hyg. 24, 439–443.
DOI: 10.4269/ajtmh.1975.24.439.

GARCÍA-BOCANEGRA, I., M. SIMON-GRIFÉ, J. P. DUBEY, J. CASAL, G. E. MARTÍN, O. CABEZÓN, A. PEREA, S. ALMERÍA (2010): Seroprevalence and risk factors associated with *Toxoplasma gondii* in domestic pigs from Spain. Parasitol. Int. 59, 421–6.
DOI: 10.1016/j.parint.2010.06.001.

GARNAUD, C., H. FRICKER-HIDALGO, B. EVENGÅRD, M. J. ÁLVAREZ-MARTÍNEZ, E. PETERSEN, L. M. KORTBEEK, F. ROBERT-GANGNEUX, I. VILLENA, C. COSTACHE, M. PAUL, V. MERONI, E. GUY, P. L. CHIODINI, M. P. BRENIER-PINCHART, H. PELLOUX (2020): *Toxoplasma gondii*-specific IgG avidity testing in pregnant women. Clin. Microbiol. Infect. 26, 1155–1160.
DOI: 10.1016/j.cmi.2020.04.014.

GAZZONIS, A. L., M. MARANGI, L. VILLA, M. E. RAGONA, E. OLIVIERI, S. A. ZANZANI, A. GIANGASPERO, M. T. MANFREDI (2018): *Toxoplasma gondii* infection and biosecurity levels in fattening pigs and sows: serological and molecular epidemiology in the intensive pig industry (Lombardy, Northern Italy). Parasitol. Res. 117, 539–546.
DOI: 10.1007/s00436-017-5736-z.

GAZZONIS, A. L., S. A. ZANZANI, L. VILLA, M. T. MANFREDI (2020): *Toxoplasma gondii* infection in meat-producing small ruminants: Meat juice serology and genotyping. Parasitol. Int. 76, 102060.
DOI: 10.1016/j.parint.2020.102060.

GEBREMEDHIN, E. Z., M. M. KEBETA, M. ASAYE, H. ASHENAFI, V. DI MARCO, M. VITALE (2015): First report on seroepidemiology of *Toxoplasma gondii* infection in pigs in Central Ethiopia. BMC Vet. Res. 11, 59.
DOI: 10.1186/s12917-015-0384-y.

GERHOLD, R. W., P. SARAF, A. CHAPMAN, X. ZOU, G. HICKLING, W. H. STIVER, A. HOUSTON, M. SOUZA, C. SU (2017): *Toxoplasma gondii* seroprevalence and genotype diversity in select wildlife species from the southeastern United States. Parasites and Vectors 10, 1–8.
DOI: 10.1186/s13071-017-2456-2.

GHONEIM, N. H., S. I. SHALABY, N. A. HASSANAIN, G. S. G. ZEEDAN, Y. A. SOLIMAN, A. M. ABDALHAMED (2010): Comparative study between serological and molecular methods for diagnosis of toxoplasmosis in women and small ruminants in Egypt. Foodborne Pathog. Dis. 7, 17–22.
DOI: 10.1089/fpd.2008.0223.

GLOR, S. B., R. EDELHOFER, F. GRIMM, P. DEPLAZES, W. BASSO (2013): Evaluation of a commercial ELISA kit for detection of antibodies against Toxoplasma gondii in serum, plasma and meat juice from experimentally and naturally infected sheep. Parasites and Vectors 6, 85.
DOI: 10.1186/1756-3305-6-85.

GONG, H., Q. WANG, Y. JIN, S. QIU, Z. CHEN, X. HAN, Z. CHEN, W. JIANG (2025): Fatal Toxoplasmosis in Red Kangaroos (*Macropus rufus*) in East China. Pathogens 14, 202.
DOI: 10.3390/pathogens14020202.

GRBAVAC, L., F. DÁMEK, S. THOUMIRE, A. MERCIER, K. PASSEBOSC-FAURE, N. KONSTANTINOVIĆ, M. KIŠ, Ž. MIHALJEVIĆ, T. ŽIVIČNJAK, R. BLAGA, D. LE ROUX (2025): Seroprevalence and genetic characterization of *Toxoplasma gondii* in hunted wild boars (*Sus scrofa*) from Croatia. Parasitol. Res. 124, 130.
DOI: 10.1007/s00436-025-08590-1.

HAMID, N. A., M. B. SADIQ, S. Z. RAMANOON, R. MANSOR, M. WATANABE, N. M. M. ISA, J. KAMALUDEEN, S. S. SYED-HUSSAIN (2020): Seroprevalence and risk factors of *Toxoplasma gondii* in ruminant meats from wet markets in klang valley and abattoirs in selangor, malaysia. Animals 10, 1–12.
DOI: 10.3390/ani10071139.

HASAN, T., Y. NISHIKAWA (2022): Advances in vaccine development and the immune response against toxoplasmosis in sheep and goats. Front. Vet. Sci. 9, 951584.
DOI: 10.3389/fvets.2022.951584.

HEBBAR, B. K., M. ROY, P. MITRA, K. CHAVHAN, S. CHAUDHARI, S. SHINDE, A. S.

DESHMUKH (2022): Seroprevalence, risk factors, and serological cross-reactivity for diagnosis of *Toxoplasma gondii* and *Neospora caninum* infections in goats in India. Microb. Pathog. 173, 105780.
DOI: 10.1016/j.micpath.2022.105780.

HERNÁNDEZ-CORTAZAR, I. B., K. Y. ACOSTA-VIANA, E. GUZMÁN-MARIN, A. ORTEGA-PACHECO, J. F. DE JESUS TORRES-ACOSTA, M. JIMENEZ-COELLO (2016): Presence of *Toxoplasma gondii* in Pork Intended for Human Consumption in Tropical Southern Mexico. Foodborne Pathog. Dis. 13, 695–699.
DOI: 10.1089/fpd.2016.2165.

HERRERO, L., M. J. GRACIA, C. PÉREZ-ARQUILLUÉ, R. LÁZARO, M. HERRERA, A. HERRERA, S. BAYARRI (2016): *Toxoplasma gondii*: Pig seroprevalence, associated risk factors and viability in fresh pork meat. Vet. Parasitol. 224, 52–59.
DOI: 10.1016/j.vetpar.2016.05.010.

HERRMANN, D. C., P. MAKSIMOV, A. MAKSIMOV, A. SUTOR, S. SCHWARZ, W. JASCHKE, A. SCHLIEPHAKE, N. DENZIN, F. J. CONRATHS, G. SCHARES (2012): *Toxoplasma gondii* in foxes and rodents from the German Federal States of Brandenburg and Saxony-Anhalt: Seroprevalence and genotypes. Vet. Parasitol. 185, 78–85.
DOI: 10.1016/j.vetpar.2011.10.030.

HILL, D. E., S. M. C. BENEDETTO, C. COSS, J. L. MCCRARY, V. M. FOURNET, J. P. DUBEY (2006): Effects of time and temperature on the viability of *Toxoplasma gondii* tissue cysts in enhanced pork loin. J. Food Prot. 69, 1961–1965.
DOI: 10.4315/0362-028X-69.8.1961.

HILL, D. E., J. LUCHANSKY, A. PORTO-FETT, H. R. GAMBLE, V. M. FOURNET, D. S. HAWKINS-COOPER, J. F. URBAN, A. A. GAJADHAR, R. HOLLEY, V. K. JUNEJA, J. P. DUBEY (2018): Rapid inactivation of *Toxoplasma gondii* bradyzoites during formulation of dry cured ready-to-eat pork sausage. Food Waterborne Parasitol. 12.
DOI: 10.1016/j.fawpar.2018.e00029.

HILL, D. E., C. SREEKUMAR, H. R. GAMBLE, J. P. DUBEY (2004): Effect of commonly used enhancement solutions on the viability of *Toxoplasma gondii* tissue cysts in pork loin. J. Food Prot. 67, 2230–2233.
DOI: 10.4315/0362-028X-67.10.2230.

HUERTAS-LÓPEZ, A., G. ÁLVAREZ-GARCÍA, R. SÁNCHEZ-SÁNCHEZ, A. CANTOS-

BARREDA, F. J. IBÁÑEZ-LÓPEZ, S. MARTÍNEZ-SUBIELA, J. J. CERÓN, C. MARTÍNEZ-CARRASCO (2023): A systematic review and meta-analysis of the serological diagnosis of *Toxoplasma gondii* infection highlight the lack of a One Health integrative research. Res. Vet. Sci. 155, 137–149.
DOI: 10.1016/j.rvsc.2023.01.005. Elsevier B.V.

IACOBUCCI, E., N. S. TAUS, M. W. UETI, L. SUKHBAATAR, Z. BASTSUKH, S. PAPAGEORGIOU, H. FRITZ (2019): Detection and genotypic characterization of *Toxoplasma gondii* DNA within the milk of Mongolian livestock. Parasitol. Res. 118, 2005–2008.
DOI: 10.1007/s00436-019-06306-w.

INNES, E. A. (2010): A brief history and overview of *Toxoplasma gondii*. Zoonoses Public Health 57, 1–7.
DOI: 10.1111/j.1863-2378.2009.01276.x.

INNES, E. A., C. HAMILTON, J. L. GARCIA, A. CHRYSSAFIDIS, D. SMITH (2019): A one health approach to vaccines against *Toxoplasma gondii*. Food Waterborne Parasitol. 15, e00053.
DOI: 10.1016/j.fawpar.2019.e00053.

JACOBS, L., R. J. S, M. L. MELTON (1960): The resistance of the encysted form of *Toxoplasma gondii*. J. Parasitol. 46, 11–21. .

JEREN, T., A. VINCE, I. ČREPINKO, B. KRŠNJAVI (1991): Toxoplasmosis - diferencijalno dijagnostički problem u nemalignim limfadenopatijama (Toxoplasmosis - a differential diagnostical problem in benign lymphadenopathy). Medica Jadertina 6, 1-4.

JOKELAINEN, P., M. ISOMURSU, A. NÄREAHO, A. OKSANEN (2011): Natural *Toxoplasma gondii* infections in european brown hares and mountain hares in finland: Proportional mortality rate, antibody prevalence, and genetic characterization. J. Wildl. Dis. 47, 154–163.
DOI: 10.7589/0090-3558-47.1.154.

KELBERT, L., R. STEPHAN, C. FURTWAENGLER, J. A. PINILLO, M. MORACH, M. NÜESCH-INDERBINEN (2021): Prevalence of *Toxoplasma gondii*, Hepatitis E virus, and *Salmonella* Antibodies in meat juice samples from pigs at slaughter in Switzerland. Acta Med. Port. 84, 1760–1764.
DOI: 10.4315/JFP-21-183.

KHURANA, S., N. BATRA (2016): Toxoplasmosis in organ transplant recipients: Evaluation, implication, and prevention. Trop. Parasitol. 6, pp. 123–128.
DOI: 10.4103/2229-5070.190814

KIM, M. J., S. J. PARK, H. PARK (2024): Trend in serological and molecular diagnostic methods for *Toxoplasma gondii* infection. Eur. J. Med. Res. 29, 520.
DOI: 10.1186/s40001-024-02055-4.

KIŠ, M., V. BADOVINAC, L. LOVRIĆ, N. ZDOLEC (2021): Meat juice serology as a potential tool in farm risk categorization – survey on *Toxoplasma gondii* antibodies in domestic pigs and wild boars in Karlovac county. p. p48 in: 9th Int. Congr. Vet. Sci. Prof. Zagreb: Faculty of Veterinary Medicine, University of Zagreb 10 000 Zagreb, Heinzelova 55.

KLUN, I., O. DJURKOVIĆ-DJAKOVIĆ, P. THULLIEZ (2007): Comparison of a commercial ELISA with the modified agglutination test for the detection of *Toxoplasma gondii* infection in naturally exposed sheep. Zoonoses Public Health 54, 165–168.
DOI: 10.1111/j.1863-2378.2007.01032.x.

KLUN, I., O. DJURKOVIĆ-DJAKOVIĆ, S. KATIĆ-RADIVOJEVIĆ, A. NIKOLIĆ (2006): Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: Seroprevalence and risk factors. Vet. Parasitol. 135, 121–131.
DOI: 10.1016/j.vetpar.2005.08.010.

KLUN, I., M. VUJANIĆ, H. YERA, A. NIKOLIĆ, V. IVOVIĆ, B. BOBIĆ, S. BRADONJI, J. DUPOUY-CAMET, O. DJURKOVIĆ-DJAKOVIĆ (2011): *Toxoplasma gondii* infection in slaughter pigs in Serbia: Seroprevalence and demonstration of parasites in blood. Vet. Res. 42.
DOI: 10.1186/1297-9716-42-17.

KNIEL, K. E., D. S. LINDSAY, S. S. SUMNER, C. R. HACKNEY, M. D. PIERSON, J. P. DUBEY (2002): Examination of attachment and survival of *Toxoplasma gondii* oocysts on raspberries and blueberries. J. Parasitol. 88, 790–793.
DOI: 10.1645/0022-3395(2002)088[0790:EOAASO]2.0.CO;2.

KOFOED, K. G., M. VORSLUND-KIÆR, H. V. NIELSEN, L. ALBAN, M. V. JOHANSEN (2017): Sero-prevalence of *Toxoplasma gondii* in Danish pigs. Vet. Parasitol. Reg. Stud. reports 10, 136–138.
DOI: 10.1016/j.vprsr.2017.10.004.

KORNACKA, A., B. MOSKWA, A. WERNER, P. NOWOSAD, W. JANKOWSKA, A. CYBULSKA, A. C. MAJEWSKA (2020): The Seroprevalence of *Toxoplasma gondii* in Wild Boars from Three Voivodeships in Poland, MAT Analyses. Acta Parasitol. 65, 490–495.
DOI: 10.2478/s11686-020-00185-3.

KOUAM, M. K., A. DIAKOU, V. KANTZOURA, E. PAPADOPOULOS, A. A. GAJADHAR, G. THEODOROPOULOS (2010): A seroepidemiological study of exposure to *Toxoplasma*, *Leishmania*, *Echinococcus* and *Trichinella* in equids in Greece and analysis of risk factors. Vet. Parasitol. 170, 170–175.
DOI: 10.1016/j.vetpar.2010.02.004.

KUNIC, J. M., M. BERNSTEIN, M. C. VENTURINI, L. PARDINI, I. E. SOMMERFELT (2022): Risk factors associated with *Toxoplasma gondii* seroprevalence in domestic pig farms in Argentina. Vet. Parasitol. Reg. Stud. Reports 30, 100710.
DOI: 10.1016/j.vprsr.2022.100710.

KURUCA, L., I. KLUN, A. UZELAC, A. NIKOLIĆ, B. BOBIĆ, S. SIMIN, V. LALOŠEVIĆ, D. LALOŠEVIĆ, O. DJURKOVIĆ-DJAKOVIĆ (2017): Detection of *Toxoplasma gondii* in naturally infected domestic pigs in Northern Serbia. Parasitol. Res. 116, 3117–3123.
DOI: 10.1007/s00436-017-5623-7.

KUTIČIĆ, V. (1992): Resistance of *Toxoplasma gondii* tissue cysts to freezing. Vet. Arh. 62, 217–222.

KUTICIC, V., T. WIKERHAUSER (1996): Studies of the effect of various treatments on the viability of Toxoplasma gondii tissue cysts and oocysts. Curr. Top. Microbiol. Immunol. 219, 261–265.
DOI: 10.1007/978-3-642-51014-4_23.

KUTIČIĆ, V., T. WIKERHAUSER (1994): Effects of some chemical and physical factors on the viability of *Toxoplasma gondii*. Vet. Arh. 64, 89–93.

KUTIČIĆ, V., T. WIKERHAUSER, L. ORŠANIĆ (1989): Effects of X-irradiation on the infectivity of *Toxoplasma gondii* cysts in the flesh of pigs. Vet. Arh. 59, 113–116. .

LACOMBE, A., A. BREARD, C. A. HWANG, D. HILL, X. FAN, L. HUANG, B. K. YOO, B. A. NIEMIRA, J. B. GURTLER, V. C. H. WU (2017): Inactivation of *Toxoplasma gondii* on blueberries using low dose irradiation without affecting quality. Food Control

73, 981–985.
DOI: 10.1016/j.foodcont.2016.10.011.

LAYTON, J., D. C. THEIOPOULOU, D. RUTENBERG, A. ELSHEREYE, Y. ZHANG, J. SINNOTT, K. KIM, J. G. MONTOYA, D. G. CONTOPOULOS-IOANNIDIS (2023): Clinical Spectrum, Radiological Findings, and Outcomes of Severe Toxoplasmosis in Immunocompetent Hosts: A Systematic Review. Pathogens 12, 543.
DOI: 10.3390/pathogens12040543.

LIMON, G., W. BEAUVAIS, N. DADIOS, I. VILLENA, C. COCKLE, R. BLAGA, J. GUITIAN (2017): Cross-Sectional Study of *Toxoplasma gondii* Infection in Pig Farms in England. Foodborne Pathog. Dis. 14, 269–281.
DOI: 10.1089/fpd.2016.2197.

LIND, P., J. HAUGEGAARD, A. WINGSTRAND, S. A. HENRIKSEN (1997): The time course of the specific antibody response by various elisas in pigs experimentally infected with *Toxoplasma gondii*. Vet. Parasitol. 71, 1–15.
DOI: 10.1016/S0304-4017(97)00010-1.

LINDSAY, D. S., B. L. BLAGBURN, J. P. DUBEY (2002): Survival of nonsporulated *Toxoplasma gondii* oocysts under refrigerator conditions. Vet. Parasitol. 103, 309–313.
DOI: 10.1016/S0304-4017(01)00554-4.

LINDSAY, D. S., M. V. COLLINS, S. M. MITCHELL, R. A. COLE, G. J. FLICK, C. N. WETCH, A. LINDQUIST, J. P. DUBEY (2003): Sporulation and Survival of *Toxoplasma gondii* Oocysts in Seawater. J. Eukaryot. Microbiol. 50, pp. 687–688.
DOI: 10.1111/j.1550-7408.2003.tb00688.x

LIU, P., H. TANG, Q. XU, Y. DONG, F. CHEN, D. ZHAO, B. TANG, X. SUN, X. LIU, M. LIU, Y. WANG (2025 (1. December)): The seroprevalence and distribution of *Toxoplasma gondii* in pigs in China from 2000 to 2023: a systematic review and meta-analysis. Anim. Dis. 5, 1–16.
DOI: 10.1186/s44149-025-00173-y.

LIU, Q., Z. D. WANG, S. Y. HUANG, X. Q. ZHU (2015): Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. Parasites and Vectors 8, 292.
DOI: 10.1186/s13071-015-0902-6.

LIYANAGE, K. L. D. T. D., A. WIETHOELTER, J. HUFSCHMID, A. JABBAR (2021):

Descriptive comparison of elisas for the detection of *Toxoplasma gondii* antibodies in animals: A systematic review. Pathogens 10, 605.
DOI: 10.3390/pathogens10050605.

LOPES, A. P., J. P. DUBEY, F. NETO, A. RODRIGUES, T. MARTINS, M. RODRIGUES, L. CARDOSO (2013): Seroprevalence of *Toxoplasma gondii* infection in cattle, sheep, goats and pigs from the North of Portugal for human consumption. Vet. Parasitol. 193, 266–269.
DOI: 10.1016/j.vetpar.2012.12.001.

LÓPEZ-UREÑA, N. M., R. CALERO-BERNAL, N. GONZÁLEZ-FERNÁNDEZ, R. BLAGA, B. KOUDELA, L. M. ORTEGA-MORA, G. ÁLVAREZ-GARCÍA (2023): Optimization of the most widely used serological tests for a harmonized diagnosis of *Toxoplasma gondii* infection in domestic pigs. Vet. Parasitol. 322, 110024.
DOI: 10.1016/j.vetpar.2023.110024.

LUPU, M. A., R. LIGHEZAN, A. A. PADURARU, A. DRAGOMIR, R. PAVEL, S. GRADA, A. G. MIHU, S. URSONIU, T. R. OLARIU (2022): Seroepidemiology of *Toxoplasma gondii* Infection in Blood Donors from Western Romania. Microorganisms 10, 973. DOI: 10.3390/microorganisms10050973.

MAMIZADEH, M., F. MALEKI, M. R. MOHAMMADI, L. SHAMSI, A. ASGHARI, A. POURYOUSEF (2025): Seroprevalence and risk factors for *Toxoplasma gondii* infection in solid organ transplant patients: A global systematic review and meta-analysis. Parasite Epidemiol. Control 29, e00421.
DOI: 10.1016/j.parepi.2025.e00421.

MANCIANTI, F., S. NARDONI, R. PAPINI, L. MUGNAINI, M. MARTINI, I. ALTOMONTE, F. SALARI, C. D'ASCENZI, J. P. DUBEY (2014): Detection and genotyping of *Toxoplasma gondii* DNA in the blood and milk of naturally infected donkeys (Equus asinus). Parasites and Vectors 7, 165.
DOI: 10.1186/1756-3305-7-165.

MARINCULIĆ, A., S. BOSNIĆ, R. RAJKOVIĆ-JANJE (1997): [Seroepizootiological studies of toxoplasmosis among sheep from central Croatia]. pp. 311–314 in: Proceedings. Veterinarski dani '97, Cavtat, Croatia, October 15-18.

MARKOVIĆ-DENIĆ, L., M. STOPIĆ, B. BOBIĆ, V. NIKOLIĆ, I. DJILAS, S. J. SRZENTIĆ, T. ŠTAJNER (2023): Factors Associated with *Toxoplasma gondii* Seroprevalence in

Pregnant Women: A Cross-Sectional Study in Belgrade, Serbia. Pathogens 12, 1240. DOI: 10.3390/pathogens12101240.

MATEUS-PINILLA, N. E., J. P. DUBEY, L. CHOROMANSKI, R. M. WEIGEL (1999): A Field Trial of the Effectiveness of a Feline *Toxoplasma gondii* Vaccine in Reducing T. gondii Exposure for Swine. J. Parasitol. 85, 855.
DOI: 10.2307/3285821.

MCCALL, J., L. ROTHFELDT, K. GIESBRECHT, A. HUNT, L. BAUCK, J. SCHEFTEL, R. BIRN, B. BUSS, B. SCHROEDER, T. E. HAUPT, R. KLOS, A. STRAILY (2022): Public Health Surveillance and Reporting for Human Toxoplasmosis — Six States, 2021. MMWR. Morb. Mortal. Wkly. Rep. 71, 889–893.
DOI: 10.15585/mmwr.mm7128a1.

MÉVÉLEC, M. N., Z. LAKHRIF, I. DIMIER-POISSON (2020): Key Limitations and New Insights Into the *Toxoplasma gondii* Parasite Stage Switching for Future Vaccine Development in Human, Livestock, and Cats. Front. Cell. Infect. Microbiol. 10, 1.
DOI: 10.3389/fcimb.2020.607198.

MIHALJEVIĆ, I., M. MILETIĆ LOVRIĆ, M. BALIJA, I. JUKIĆ, M. BUŠIĆ (2013): Analysis and the results of serological testing of Croatian organ donors from 2006 to 2012. Croat. J. Infect. 33, 117–125.

MILLÁN, J., O. CABEZÓN, M. PABÓN, J. P. DUBEY, S. ALMERÍA (2009): Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in feral cats (Felis silvestris catus) in Majorca, Balearic Islands, Spain. Vet. Parasitol. 165, 323–326.
DOI: 10.1016/j.vetpar.2009.07.014.

MIRZA ALIZADEH, A., S. JAZAERI, B. SHEMSHADI, F. HASHEMPOUR-BALTORK, Z. SARLAK, Z. PILEVAR, H. HOSSEINI (2018): A review on inactivation methods of *Toxoplasma gondii* in foods. Pathog. Glob. Health 112, 306–319.
DOI: 10.1080/20477724.2018.1514137.

MONTOYA, J. G., O. LIESENFELD (2004): Toxoplasmosis. Lancet. 363, pp. 1965–1976. DOI: 10.1016/S0140-6736(04)16412-X.

MOUDGIL, P., S. PANDITA, R. KUMAR, V. KHASA, S. S. DASH, Y. C. BANGAR, N. JINDAL (2024): Seroprevalence and risk factors associated with *Toxoplasma gondii* in pigs in Haryana, India. Zoonoses Public Health 71, 136–143.

DOI: 10.1111/zph.13091.

OLARIU, T. R., S. URSONIU, I. HOTEA, V. DUMITRASCU, D. ANASTASIU, M. A. LUPU (2020): Seroprevalence and Risk Factors of *Toxoplasma gondii* Infection in Pregnant Women from Western Romania. Vector-Borne Zoonotic Dis. 20, 763–767.
DOI: 10.1089/vbz.2019.2599.

OLSEN, A., L. ALBAN, M. DENWOOD, H. HOUE, T. BIRK JENSEN, H. VEDEL NIELSEN (2021): A longitudinal study of *Toxoplasma gondii* seroconversion on four large Danish sow farms. Vet. Parasitol. 295, 109460.
DOI: 10.1016/j.vetpar.2021.109460.

OLSEN, A., R. BERG, M. TAGEL, K. MUST, G. DEKSNE, H. L. ENEMARK, L. ALBAN, M. V. JOHANSEN, H. V. NIELSEN, M. SANDBERG, A. LUNDÉN, C. R. STENSVOLD, S. M. PIRES, P. JOKELAINEN (2019): Seroprevalence of *Toxoplasma gondii* in domestic pigs, sheep, cattle, wild boars, and moose in the Nordic-Baltic region: A systematic review and meta-analysis. Parasite Epidemiol. Control 5.
DOI: 10.1016/j.parepi.2019.e00100.

OLSEN, A., M. SANDBERG, H. HOUE, H. V. NIELSEN, M. DENWOOD, T. B. JENSEN, L. ALBAN (2020): Seroprevalence of *Toxoplasma gondii* infection in sows and finishers from conventional and organic herds in Denmark: Implications for potential future serological surveillance. Prev. Vet. Med. 185, 105149.
DOI: 10.1016/j.prevetmed.2020.105149.

OPSTEEGH, M., M. MAAS, G. SCHARES, J. VAN DER GIESSEN (2016): Relationship between seroprevalence in the main livestock species and presence of *Toxoplasma gondii* in meat (GP/EFSA/BIOHAZ/2013/01) An extensive literature review. Final report. EFSA Support. Publ. 13, 996E.
DOI: 10.2903/sp.efsa.2016.EN-996.

PAPINI, R., P. DI CICCIO, M. MARANGI, S. GHIDINI, E. ZANARDI, A. VERGARA, A. GIANGASPERO, S. NARDONI, G. ROCCHIGIANI, F. MANCIANTI, A. IANIERI (2017): Occurrence of *Toxoplasma gondii* in carcasses of pigs reared in intensive systems in Northern Italy. J. Food Prot. 80, 515–522.
DOI: 10.4315/0362-028X.JFP-16-314.

PAŞTIU, A. I., A. GYÖRKE, R. BLAGA, V. MIRCEAN, B. M. ROSENTHAL, V. COZMA (2013): In Romania, exposure to *Toxoplasma gondii* occurs twice as often in swine raised

for familial consumption as in hunted wild boar, but occurs rarely, if ever, among fattening pigs raised in confinement. Parasitol. Res. 112, 2403–2407.
DOI: 10.1007/s00436-013-3353-z.

PENA, H. F. J., T. M. PINHEIRO, H. S. SOARES, S. OLIVEIRA, B. F. ALVES, M. N. FERREIRA, S. M. GENNARI (2018): Typical Brazilian genotype of *Toxoplasma gondii* isolated from a horse destined for human consumption in Europe from a slaughterhouse. Parasitol. Res. 117, 3305–3308.
DOI: 10.1007/s00436-018-5999-z.

PERICA, B., A. RALJEVIĆ BRADARIĆ, B. TOMČIĆ (2016): Prevalencija protutijela na *Toxoplasma gondii* u Zadarskoj županiji. Hrvat. časopis za javno Zdr. 12, 91–95. .

PICONE, O., F. FUCHS, G. BENOIST, C. BINQUET, F. KIEFFER, M. WALLON, K. WEHBE, L. MANDELBROT, I. VILLENA (2020): Toxoplasmosis screening during pregnancy in France: Opinion of an expert panel for the CNGOF. J. Gynecol. Obstet. Hum. Reprod. 49, 101814.
DOI: 10.1016/j.jogoh.2020.101814.

PIPIA, A. P., A. VARCASIA, G. DESSÌ, R. PANZALIS, C. GAI, F. NONNIS, F. VERONESI, C. TAMPONI, A. SCALA (2018): Seroepidemiological and biomolecular survey on *Toxoplasma gondii* infection on organic pig farms. Parasitol. Res. 117, 1637–1641.
DOI: 10.1007/s00436-018-5823-9.

PLAZA, J., F. DÁMEK, I. VILLENA, E. A. INNES, F. KATZER, C. M. HAMILTON (2020): Detection of *Toxoplasma gondii* in retail meat samples in Scotland. Food Waterborne Parasitol. 20, e00086.
DOI: 10.1016/j.fawpar.2020.e00086.

PUCHALSKA, M., J. WIŚNIEWSKI, D. KLICH, E. GOŁĄB, D. JAŃCZAK, J. SOKOŁOWSKA, K. URBAŃSKA, K. ANUSZ (2022): A serological survey of *Toxoplasma gondii* in polish pigs from organic farms, other housing systems and in pigs of different age groups. Acta Vet. Scand. 64, 3.
DOI: 10.1186/s13028-022-00623-4.

PUNDA-POLIĆ, V., M. TONKIĆ, V. ČAPKUN (2000): Prevalence of antibodies to *Toxoplasma gondii* in the female population of the County of Split Dalmatia, Croatia. Eur. J. Epidemiol. 16, 875–877.
DOI: 10.1023/A:1007606501923.

RAJKOVIĆ-JANJE, R., A. MARINCULIĆ, V. JOVANOVIĆ-BUNTA, T. ŽIVIČNJAK (1993): Seroepidemiological survey of toxoplasmosis in goats in the Republic of Croatia. Vet. Arh. 63, 125–129.

RAJKOVIĆ-JANJE, R., A. MARINCULIĆ, Č. PAUKOVIĆ, Z. KOVAČ, J. HORVAT (1994): Nalaz protutijela za protozoon *Toxoplasma gondii* u krvi ovaca u Republici Hrvatskoj [Antibody findings for the *Toxoplasma gondii* protozoon in the sheep blood in the Republic of Croatia]. Vet. Stanica 25, 145–148. .

REITER-OWONA, I., E. PETERSEN, D. JOYNSON, H. ASPÖCK, M. L. DARDÉ, R. DISKO, O. DREAZEN, H. DUMON, R. GRILLO, U. GROSS, M. HAYDE, R. HOLLIMAN, D. O. HO-YEN, K. JANITSCHKE, P. A. JENUM, K. NASER, M. OLSZEWSKI, P. THULLIEZ, H. M. SEITZ (1999): The past and present role of the Sabin-Feldman dye test in the serodiagnosis of toxoplasmosis. Bull. World Health Organ. 77, 929–935. .

RIBAS, M. P., S. ALMERÍA, X. FERNÁNDEZ-AGUILAR, G. DE PEDRO, P. LIZARRAGA, O. ALARCIA-ALEJOS, R. MOLINA-LÓPEZ, E. OBÓN, H. GHOLIPOUR, C. TEMIÑO, J. P. DUBEY, O. CABEZÓN (2018): Tracking *Toxoplasma gondii* in freshwater ecosystems: interaction with the invasive American mink (*Neovison vison*) in Spain. Parasitol. Res. 117, 2275–2281.
DOI: 10.1007/s00436-018-5916-5.

RICHOMME, C., E. AFONSO, V. TOLON, C. DUCROT, L. HALOS, A. ALLIOT, C. PERRET, M. THOMAS, P. BOIREAU, E. GILOT-FROMONT (2010): Seroprevalence and factors associated with *Toxoplasma gondii* infection in wild boar (*Sus scrofa*) in a Mediterranean island. Epidemiol. Infect. 138, 1257–1266.
DOI: 10.1017/S0950268810000117.

ROBERT-GANGNEUX, F., V. MERONI, D. DUPONT, F. BOTTEREL, J. M. AGUADO GARCIA, M. P. BRENIER-PINCHART, I. ACCOCEBERRY, H. AKAN, I. ABBATE, K. BOGGIAN, F. BRUSCHI, J. CARRATALÀ, M. DAVID, L. DRGONA, O. DJURKOVIĆ-DJAKOVIĆ, M. C. FARINAS, F. GENCO, E. GKRANIA-KLOTSAS, A. H. GROLL, E. GUY, C. HIRZEL, N. KHANNA, Ö. KURT, L. M. JUNIE, T. LAZZAROTTO, O. LEN, N. J. MUELLER, P. MUNOZ, Z. D. PANA, E. ROILIDES, T. STAJNER, C. VAN DELDEN, I. VILLENA, H. PELLOUX, O. MANUEL (2018): Toxoplasmosis in transplant recipients, Europe, 2010–2014. Emerg. Infect. Dis. 24,

1497-1504.
DOI: 10.3201/eid2408.180045.

ROCHA, D. de S., R. L. d. S. MOURA, B. M. MACIEL, L. A. GUIMARÃES, H. N. S. O'DWYER, A. D. MUNHOZ, G. R. ALBUQUERQUE (2015): Detection of *Toxoplasma gondii* DNA in naturally infected sheep's milk. Genet. Mol. Res. 14, 8658–8662.
DOI: 10.4238/2015.July.31.14.

ROQUEPLO, C., R. BLAGA, J. Lou MARIÉ, I. VALLÉE, B. DAVOUST (2017): Seroprevalence of *Toxoplasma gondii* in hunted wild boars (S*us scrofa*) from southeastern France. Folia Parasitol. 64.
DOI: 10.14411/fp.2017.003.

ROSTAMI, A., S. M. RIAHI, H. R. GAMBLE, Y. FAKHRI, M. NOUROLLAHPOUR SHIADEH, M. DANESH, H. BEHNIAFAR, S. PAKTINAT, M. FOROUTAN, A. H. MOKDAD, P. J. HOTEZ, R. B. GASSER (2020): Global prevalence of latent toxoplasmosis in pregnant women: a systematic review and meta-analysis. Clin. Microbiol. Infect. 26, 673–683.
DOI: 10.1016/j.cmi.2020.01.008.

ROY, M., V. MISHRA, P. MITRA, Y. UMBARDAND, V. SAPATE, W. KHAN, A. S. DESHMUKH (2024): Serological and Molecular Detection of *Toxoplasma gondii* in Domestic Pigs Intended for Human Consumption and Potential Occupational Hazard to Pig Farmers in India. Foodborne Pathog. Dis. 21, 99–108.
DOI: 10.1089/fpd.2023.0073.

SABIN, A. B., H. A. FELDMAN (1948): Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (*Toxoplasma*). Science 80. 108, 660–663.
DOI: 10.1126/science.108.2815.660.

SANTORO, A., M. TAGEL, K. MUST, M. LAINE, B. LASSEN, P. JOKELAINEN (2017): *Toxoplasma gondii* seroprevalence in breeding pigs in Estonia. Acta Vet. Scand. 59, 82.
DOI: 10.1186/s13028-017-0349-1.

SAWERS, L., M. WALLON, L. MANDELBROT, I. VILLENA, E. STILLWAGGON, F. KIEFFER (2022): Prevention of congenital toxoplasmosis in France using prenatal screening: A decisionanalytic economic model. PLoS One 17, e0273781.
DOI: 10.1371/journal.pone.0273781.

SHAMS, F., M. JOKAR, A. ABDOUS, P. MOHAMMADI, A. ABBASSIOUN, T. SEUBERLICH, V. RAHMANIAN (2024): Seroprevalence of *Toxoplasma gondii* and *Neospora* spp. in horse population of Tehran, Iran. Sci. Rep. 14.
DOI: 10.1038/s41598-024-61999-z.

SHAPIRO, K., L. BAHIA-OLIVEIRA, B. DIXON, A. DUMÈTRE, L. A. DE WIT, E. VANWORMER, I. VILLENA (2019): Environmental transmission of *Toxoplasma gondii*: Oocysts in water, soil and food. Food Waterborne Parasitol. 15.
DOI: 10.1016/j.fawpar.2019.e00049.

SINI, M. F., M. MANCONI, A. VARCASIA, G. MASSEI, R. SANDU, N. MEHMOOD, F. AHMED, C. CARTA, C. CANTACESSI, C. SCARANO, A. SCALA, C. TAMPONI (2024): Seroepidemiological and biomolecular survey on *Toxoplasma gondii* in Sardinian wild boar (*Sus scrofa*). Food waterborne Parasitol. 34, e00222.
DOI: 10.1016/j.fawpar.2024.e00222.

SLANY, M., R. DZIEDZINSKA, V. BABAK, P. KRALIK, M. MORAVKOVA, I. SLANA (2019): *Toxoplasma gondii* in vegetables from fields and farm storage facilities in the Czech Republic. FEMS Microbiol. Lett. 366, 170.
DOI: 10.1093/femsle/fnz170.

SLANY, M., N. RESLOVA, V. BABAK, A. LORENCOVA (2016): Molecular characterization of *Toxoplasma gondii* in pork meat from different production systems in the Czech Republic. Int. J. Food Microbiol. 238, 252–255.
DOI: 10.1016/j.ijfoodmicro.2016.09.020.

SOBANSKI, V., D. AJZENBERG, L. DELHAES, N. BAUTIN, N. JUST (2013): Severe toxoplasmosis in immunocompetent hosts: Be aware of atypical strains. Am. J. Respir. Crit. Care Med. 187, 1143–1145.
DOI: 10.1164/rccm.201209-1635LE.

SOUSA, R. Á. de, J. da F. LEMOS, L. A. FARIAS, C. D. LOPES, K. R. dos SANTOS (2014): Seroprevalence and risk factors for *Toxoplasma gondii* infection in pigs in southern Piauí. Rev. Bras. Parasitol. Veterinária 23, 98–100.
DOI: 10.1590/s1984-29612014015.

SPEER, C. A., S. CLARK, J. P. DUBEY (1998): Ultrastructure of the oocysts, sporocysts, and sporozoites of *Toxoplasma gondii*. J. Parasitol. 84, 505–12.

SROKA, J., J. KARAMON, J. DUTKIEWICZ, A. W. FATLA, V. ZAJĄC, T. CENCEK (2018): Prevalence of *Toxoplasma gondii* infection in cats in southwesternpoland. Ann. Agric. Environ. Med. 25, 576–580.
DOI: 10.26444/aaem/94675.

SROKA, J., J. KARAMON, A. WÓJCIK-FATLA, W. PIOTROWSKA, J. DUTKIEWICZ, E. BILSKA-ZAJA&CEDIL;C, V. ZAJA&CEDIL;C, M. KOCHANOWSKI, J. DA&CEDIL;BROWSKA, T. CENCEK (2020): *Toxoplasma gondii* infection in slaughtered pigs and cattle in Poland: Seroprevalence, molecular detection and characterization of parasites in meat. Parasites and Vectors 13.
DOI: 10.1186/s13071-020-04106-1.

SROKA, J., P. KUSYK, E. BILSKA-ZAJÁC, J. KARAMON, J. DUTKIEWICZ, A. WÓJCIK-FATLA, V. ZAJÁC, K. STOJECKI, M. RÓZYCKI, T. CENCEK (2017): Seroprevalence of *Toxoplasma gondii* infection in goats from the south-west region of Poland and the detection of T. gondii DNA in goat milk. Folia Parasitol. 64.
DOI: 10.14411/fp.2017.023.

STELZER, S., W. BASSO, J. BENAVIDES SILVÁN, L. M. ORTEGA-MORA, P. MAKSIMOV, J. GETHMANN, F. J. CONRATHS, G. SCHARES (2019): *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. Food Waterborne Parasitol. 15, e00037.
DOI: 10.1016/j.fawpar.2019.e00037.

STOPIĆ, M., T. ŠTAJNER, L. MARKOVIĆ-DENIĆ, V. NIKOLIĆ, I. DJILAS, S. JOVANOVIĆ SRZENTIĆ, O. DJURKOVIĆ-DJAKOVIĆ, B. BOBIĆ (2022): Epidemiology of Toxoplasmosis in SERBIA: A Cross-Sectional Study on Blood Donors. Microorganisms 10, 492.
DOI: 10.3390/microorganisms10030492.

SUARÉZ-ARANDA, F., A. J. GALISTEO, R. M. HIRAMOTO, R. P. A. CARDOSO, L. R. MEIRELES, O. MIGUEL, H. F. ANDRADE (2000): The prevalence and avidity of *Toxoplasma gon*dii IgG antibodies in pigs from Brazil and Peru. Vet. Parasitol. 91, 23–32.
DOI: 10.1016/S0304-4017(00)00249-1.

SVIBEN, M., K. BARBIĆ, M. BOGDANIĆ, E. REICHER, S. GLAVAŠ, D. NAVOLAN, A. SANKOVIĆ, T. MEŠTROVIĆ, I. MLINARIĆ, S. VLĂDĂREANU, R. VLĂDĂREANU, T. VILIBIĆ-ČAVLEK (2025): Emerging Trends in Toxoplasmosis Seroepidemiology in

Childbearing-Aged Women in Croatia, 2015–2024. Pathogens 14.
DOI: 10.3390/pathogens14080796.

SWANENBURG, M., J. L. GONZALES, M. BOUWKNEGT, G. J. BOENDER, D. OORBURG, L. HERES, H. J. WISSELINK (2019): Large-scale serological screening of slaughter pigs for *Toxoplasma gondii* infections in The Netherlands during five years (2012–2016): Trends in seroprevalence over years, seasons, regions and farming systems. Vet. Parasitol. 2, 100017.
DOI: 10.1016/j.vpoa.2019.100017.

TEIMOURI, A., S. MOHTASEBI, E. KAZEMIRAD, H. KESHAVARZ (2020): Role of *Toxoplasma gondi*i IgG avidity testing in discriminating between acute and chronic toxoplasmosis in pregnancy. J. Clin. Microbiol. 58.
DOI: 10.1128/JCM.00505-20.

TENTER, A. M., A. R. HECKEROTH, L. M. WEISS (2000): *Toxoplasma gondii*: From animals to humans. Int. J. Parasitol. 30, 1217–1258.
DOI: 10.1016/S0020-7519(00)00124-7.

THEBAULT, A., P. KOOH, V. CADAVEZ, U. GONZALES-BARRON, I. VILLENA (2021): Risk factors for sporadic toxoplasmosis: A systematic review and meta-analysis. Microb. Risk Anal. 17, 100133.
DOI: 10.1016/j.mran.2020.100133.

TIDY, A., S. FANGUEIRO, J. P. DUBEY, L. CARDOSO, A. P. LOPES (2017): Seroepidemiology and risk assessment of *Toxoplasma gondii* infection in captive wild birds and mammals in two zoos in the North of Portugal. Vet. Parasitol. 235, 47–52. DOI: 10.1016/j.vetpar.2017.01.004.

TONKIĆ, M., V. PUNDA-POLIĆ, S. SARDELIĆ, V. CAPKUN (2002): Učestalost protutijela za *Toxoplasmu gondii* u populaciji splitsko-dalmatinske županije [Occurrence of *Toxoplasma gondii* antibodies in the population of Split-Dalmatia County]. Lijec. Vjesn. 124, 19–22. .

TSOKANA, C. N., C. SOKOS, A. GIANNAKOPOULOS, P. BIRTSAS, G. VALIAKOS, V. SPYROU, L. V. ATHANASIOU, A. RODI BURRIEL, C. BILLINIS (2020): European Brown hare (Lepus europaeus) as a source of emerging and re-emerging pathogens of Public Health importance: A review. Vet. Med. Sci. 6, 550–564.
DOI: 10.1002/vms3.248. Wiley-Blackwell.

UREÑA, N. M. L., U. CHAUDHRY, R. C. BERNAL, S. C. ALSUA, D. MESSINA, F. EVANGELISTA, M. BETSON, M. LALLE, P. JOKELAINEN, L. M. O. MORA, G. Á. GARCÍA (2022): Contamination of Soil, Water, Fresh Produce, and Bivalve Mollusks with *Toxoplasma gondii* Oocysts: A Systematic Review. Microorganisms 10, 517.
DOI: 10.3390/microorganisms10030517.

VERONESI, F., D. RANUCCI, R. BRANCIARI, D. MIRAGLIA, R. MAMMOLI, D. P. FIORETTI (2011): Seroprevalence and Risk Factors for *Toxoplasma gondii* Infection on Finishing Swine Reared in the Umbria Region, Central Italy. Zoonoses Public Health 58, 178–184.
DOI: 10.1111/j.1863-2378.2010.01336.x.

VILIBIĆ-ČAVLEK, T., B. KOLARIĆ, M. SVIBEN, T. VILIBIC-CAVLEK, S. LJUBIN-STERNAK, M. BAN, B. KOLARIC, G. MLINARIC-GALINOVIC, M. SVIBEN, G. MLINARIC-GALINOVIC (2011): Seroprevalence of TORCH infections in women of childbearing age in Croatia. 24, 280–283.
DOI: 10.3109/14767058.2010.485233.

VILLARD, O., B. CIMON, C. L'OLLIVIER, H. FRICKER-HIDALGO, N. GODINEAU, S. HOUZE, L. PARIS, H. PELLOUX, I. VILLENA, E. CANDOLFI (2016): Serological diagnosis of *Toxoplasma gondii* infection. Recommendations from the French National Reference Center for Toxoplasmosis. Diagn. Microbiol. Infect. Dis. 84, 22–33.
DOI: 10.1016/j.diagmicrobio.2015.09.009.

VILLENA, I., B. DURAND, D. AUBERT, R. BLAGA, R. GEERS, M. THOMAS, C. PERRET, A. ALLIOT, S. ESCOTTE-BINET, A. THÉBAULT, P. BOIREAU, L. HALOS (2012): New strategy for the survey of *Toxoplasma gondii* in meat for human consumption. Vet. Parasitol. 183, 203–208.
DOI: 10.1016/j.vetpar.2011.08.001.

VLATKOVIC, S., M. SAGUD, D. SVOB STRAC, M. SVIBEN, M. ZIVKOVIC, M. VILIBIC, B. VUKSAN-CUSA, A. MIHALJEVIC-PELES, N. PIVAC (2018): Increased prevalence of *Toxoplasma gondii* seropositivity in patients with treatment-resistant schizophrenia. Schizophr. Res. 193, 480–481.
DOI: 10.1016/j.schres.2017.08.006.

VOYIATZAKI, C., A. D. ZARE CHORMIZI, M. E. TSOUMANI, A. EFSTATHIOU, K. KONSTANTINIDIS, D. CHANIOTIS, G. CHRYSOS, A. ARGYRAKI, V.

PAPASTAMOPOULOS, M. KOTSIANOPOULOU (2024): Seroprevalence of *Toxoplasma gondii* among HIV Positive Patients under Surveillance in Greek Infectious Disease Units: A Screening Study with Comparative Evaluation of Serological Methods. Pathogens 13, 375.
DOI: 10.3390/pathogens13050375.

WAAP, H., T. NUNES, Y. VAZ, A. LEITÃO (2016): Serological survey of *Toxoplasma gondii* and *Besnoitia besnoiti* in a wildlife conservation area in southern Portugal. Vet. Parasitol. Reg. Stud. Reports 3–4, 7–12.
DOI: 10.1016/j.vprsr.2016.05.003.

WAINWRIGHT, K. E., M. LAGUNAS-SOLAR, M. A. MILLER, B. C. BARR, I. A. GARDNER, C. PINA, A. C. MELLI, A. E. PACKHAM, N. ZENG, T. TRUONG, P. A. CONRAD (2007): Physical inactivation of *Toxoplasma gondii* oocysts in water. Appl. Environ. Microbiol. 73, 5663–5666.
DOI: 10.1128/AEM.00504-07.

WAINWRIGHT, K. E., M. A. MILLER, B. C. BARR, I. A. GARDNER, A. C. MELLI, T. ESSERT, A. E. PACKHAM, T. TRUONG, M. LAGUNAS-SOLAR, P. A. CONRAD (2007): Chemical inactivation of *Toxoplasma gondii* oocysts in water. J. Parasitol. 93, 925–931.
DOI: 10.1645/GE-1063R.1.

WALLANDER, C., J. FRÖSSLING, I. VÅGSHOLM, A. BURRELLS, A. LUNDÉN (2015): “Meat juice” is not a homogeneous serological matrix. Foodborne Pathog. Dis. 12, 280–288.
DOI: 10.1089/fpd.2014.1863.

WANG, Z. D., H. H. LIU, Z. X. MA, H. Y. MA, Z. Y. LI, Z. Bin YANG, X. Q. ZHU, B. XU, F. WEI, Q. LIU (2017): *Toxoplasma gondii* infection in immunocompromised patients: A systematic review and meta-analysis. Front. Microbiol. 8.
DOI: 10.3389/fmicb.2017.00389.

WEISS, L. M., J. P. DUBEY (2009): Toxoplasmosis: A history of clinical observations. Int. J. Parasitol. 39, 895–901.
DOI: 10.1016/j.ijpara.2009.02.004.

WIKERHAUSER, T., V. KUTIČIĆ, A. MARINCULIĆ, L. ORŠANIĆ (1988): Effect of γ-irradiation on the infectivity of *Toxoplasma gondii* cysts in mouse brains. Vet. Arh. 58, 257–260.

YBAÑEZ, R. H. D., A. P. YBAÑEZ, Y. NISHIKAWA (2020): Review on the Current Trends of Toxoplasmosis Serodiagnosis in Humans. Front. Cell. Infect. Microbiol. 10, 204. DOI: 10.3389/fcimb.2020.00204.

YILMAZ, S. M., S. H. HOPKINS (1972): Effects of different conditions on duration of infectivity of *Toxoplasma gondii* oocysts. J. Parasitol. 58, 938–939.
DOI: 10.2307/3286589.

ZULPO, D. L., A. S. SAMMI, J. R. DOS SANTOS, J. P. SASSE, T. A. MARTINS, A. F. MINUTTI, S. T. CARDIM, L. D. DE BARROS, I. T. NAVARRO, J. L. GARCIA (2018): *Toxoplasma gondii*: A study of oocyst re-shedding in domestic cats. Vet. Parasitol. 249, 17–20.
DOI: 10.1016/j.vetpar.2017.10.021.

9. APPENDICES

Appendix 1. Questionnare for risk factor assessment – 8 extracted questions from original biosecurity questionnare

TITLE		QUESTION	ANSWER
C. Number of animals on the farm	C. 1.	Total number of pigs on the farm at the time of categorization	_____
		Other animals on the farm	
		Are there pets?	Yes/No
6. Biosecurity	C6.1.	Is protection against the entry of rodents, birds, and other animals into pig facilities being implemented?	Yes/No
	6.15	Are the pigs permanently kept in enclosed facilities without outdoor access?	Yes/No
	6.17	Are the pigs kept outdoors?	Yes/No
	F6.29	Is footwear disinfected when entering and exiting pig facilities?	Yes/No
	F6.33	Is there a foot dip at the entrance to the farm for personnel?	Yes/No
F. Other conditions	7.5	Does the owner produce their own feed for pig feeding?	Yes/No
	G 7.8	Is the feed stored properly and protected from pests?	Yes/No

Appendix 2. Simulation table for the required number of samples per group based on the difference in proportions

Alfa	Power	N[1]	n[2]	Difference in porportions	P1[3]	P2[4]
.05	.8	855.9	427.9	.05	.05	.1
.05	.8	271.2	135.6	.1	.05	.15
.05	.8	92.81	46.41	.2	.05	.25
.05	.8	50.69	25.34	.3	.05	.35
.05	.8	32.98	16.49	.4	.05	.45
.05	.8	23.42	11.71	.5	.05	.55
.05	.8	17.48	8.742	.6	.05	.65
.05	.8	13.41	6.706	.7	.05	.75
.05	.8	10.4	5.198	.8	.05	.85
.05	.8	.	.	.9	.05	.95
.05	.8	1365	682.7	.05	.1	.15
.05	.8	393.8	196.9	.1	.1	.2
.05	.8	121	60.51	.2	.1	.3
.05	.8	62.01	31	.3	.1	.4
.05	.8	38.57	19.28	.4	.1	.5
.05	.8	26.45	13.22	.5	.1	.6
.05	.8	19.14	9.57	.6	.1	.7
.05	.8	14.25	7.125	.7	.1	.8
.05	.8	10.66	5.331	.8	.1	.9
.05	.8	393.8	196.9	-.1	.2	.1
.05	.8	1808	904	-.05	.2	.15
.05	.8	585.6	292.8	.1	.2	.3
.05	.8	162.5	81.23	.2	.2	.4
.05	.8	77.45	38.72	.3	.2	.5
.05	.8	45.47	22.74	.4	.2	.6
.05	.8	29.62	14.81	.5	.2	.7
.05	.8	20.36	10.18	.6	.2	.8
.05	.8	14.25	7.125	.7	.2	.9
.05	.8	121	60.51	-.2	.3	.1
.05	.8	585.6	292.8	-.1	.3	.2
.05	.8	2753	1376	.1	.3	.35
.05	.8	712.4	356.2	.1	.3	.4
.05	.8	186.8	93.41	.2	.3	.5
.05	.8	84.94	42.47	.3	.3	.6
.05	.8	47.69	23.85	.4	.3	.7
.05	.8	29.62	14.81	.5	.3	.8
.05	.8	19.14	9.57	.6	.3	.9
.05	.8	62.01	31	-.3	.4	.1
.05	.8	162.5	81.23	-.2	.4	.2
.05	.8	712.4	356.2	-.1	.4	.3
.05	.8	3067	1534	.05	.4	.45
.05	.8	775.7	387.8	.1	.4	.5
.05	.8	194.9	97.45	.2	.4	.6
.05	.8	84.94	42.47	.3	.4	.7

.05	.8	45.47	22.74	.4	.4	.8
.05	.8	26.45	13.22	.5	.4	.9
.05	.8	38.57	19.28	-.4	.5	.1
.05	.8	77.45	38.72	-.3	.5	.2
.05	.8	186.8	93.41	-.2	.5	.3
.05	.8	775.7	387.8	-.1	.5	.4
.05	.8	3130	1565	.05	.5	.55
.05	.8	775.7	387.8	.1	.5	.6
.05	.8	186.8	93.41	.2	.5	.7
.05	.8	77.45	38.72	.3	.5	.8
.05	.8	38.57	19.28	.4	.5	.9
.05	.8	26.45	13.22	-.5	.6	.1
.05	.8	34.51	17.26	-.45	.6	.15
.05	.8	45.47	22.74	-.4	.6	.2
.05	.8	84.94	42.47	-.3	.6	.3
.05	.8	194.9	97.45	-.2	.6	.4
.05	.8	775.7	387.8	-.1	.6	.5
.05	.8	2942	1471	.05	.6	.65
.05	.8	712.4	356.2	.1	.6	.7
.05	.8	162.5	81.23	.2	.6	.8
.05	.8	62.01	31	.3	.6	.9
.05	.8	19.14	9.57	-.6	.7	.1
.05	.8	23.77	11.89	-.55	.7	.15
.05	.8	29.62	14.81	-.5	.7	.2
.05	.8	47.69	23.85	-.4	.7	.3
.05	.8	84.94	42.47	-.3	.7	.4
.05	.8	186.8	93.41	-.2	.7	.5
.05	.8	712.4	356.2	-.1	.7	.6
.05	.8	2502	1251	.05	.7	.75
.05	.8	585.6	292.8	.1	.7	.8
.05	.8	121	60.51	.2	.7	.9
.05	.8	14.25	7.125	-.7	.8	.1
.05	.8	17.04	8.519	-.65	.8	.15
.05	.8	20.36	10.18	-.6	.8	.2
.05	.8	29.62	14.81	-.5	.8	.3
.05	.8	45.47	22.74	-.4	.8	.4
.05	.8	77.45	38.72	-.3	.8	.5
.05	.8	162.5	81.23	-.2	.8	.6
.05	.8	585.6	292.8	-.1	.8	.7
.05	.8	1808	904	.05	.8	.85
.05	.8	393.8	196.9	.1	.8	.9
.05	.8	10.66	5.331	-.8	.9	.1
.05	.8	12.34	6.17	-.75	.9	.15
.05	.8	14.25	7.125	-.7	.9	.2
.05	.8	19.14	9.57	-.6	.9	.3
.05	.8	26.45	13.22	-.5	.9	.4
.05	.8	38.57	19.28	-.4	.9	.5
.05	.8	62.01	31	-.3	.9	.6

.05	.8	121	60.51	-.2	.9	.7
.05	.8	393.8	196.9	-.1	.9	.8
.05	.8	855.9	427.9	.05	.9	.95

Alfa	Power	N	n	Difference in porportions	P1	P2
.05	.8	855.9	427.9	.05	.05	.1
.05	.8	143.7	71.85	.15	.05	.2
.05	.8	66.47	33.24	.25	.05	.3
.05	.8	40.29	20.15	.35	.05	.4
.05	.8	27.57	13.79	.45	.05	.5
.05	.8	20.14	10.07	.55	.05	.6
.05	.8	15.28	7.639	.65	.05	.7
.05	.8	11.81	5.903	.75	.05	.8
.05	.8	855.9	427.9	.05	.05	.1
.05	.8	143.7	71.85	.15	.05	.2

[1]N-total number, [2]n-number per group if the groups are equal, [3]P1 – no risk group, [4]P2 – risk group

Appendix 3. Raw data on 79 seropositive pigs from biosecurity category 3 with all corresponding information on risk factors evaluated in this study

PART I

No.	Sample ID	Pig category (0=fattening, 1=sows)	Age category (0=<12 m, 1=≥12 m)	Sex (0=male, 1=female)	Total number of pigs (1=up to 20, 2=21-100, 3=>100)	Pets on the farm (0=No, 1=Yes)	Protection against entry of other animals (0=No, 1=Yes)	Indoor housing without outdoor access (0=No, 1=Yes)	Outdoor housing (0=No, 1=Yes)	Footwear disinfection upon entry into the facility (0=No, 1=Yes)	A foot dip for staff at the farm entrance (0=No, 1=Yes)	Feed production on farm (0=No, 1=Yes)	Proper feed storage (0=No, 1=Yes)	Titre	Farm ID	County
1	108	1	1	1	2	1	1	1	0	1	1	1	1	1/6	1	Vukovarsko-srijemska
2	126	1	1	1	2	0	1	1	0	1	1	1	1	1/96	2	Osječko-baranjska
3	129	1	1	1	1	1	1	1	0	1	1	1	1	1/768	3	Vukovarsko-srijemska
4	130	1	1	1	1	1	1	1	0	1	1	1	1	1/192	3	Vukovarsko-srijemska
5	131	1	1	1	1	1	1	1	0	1	1	1	1	1/24	3	Vukovarsko-srijemska
6	212	1	1	1	1	1	1	1	0	1	0	1	1	1/768	4	Krapinsko-zagorska
7	213	1	1	1	1	1	1	1	0	1	0	1	1	1/24	4	Krapinsko-zagorska
8	240	1	1	1	1	1	1	0	0	1	0	1	1	1/384	5	Sisačko-moslavačka
9	253	1	1	1	2	0	1	1	0	1	1	1	1	1/96	6	Sisačko-moslavačka
10	281	1	1	1	2	1	1	1	0	1	1	1	1	1/192	7	Krapinsko-zagorska
11	282	1	1	1	2	1	1	1	0	1	1	1	1	1/192	7	Krapinsko-zagorska
12	283	1	1	1	1	0	1	1	0	0	0	1	1	1/24	8	Krapinsko-zagorska
13	284	1	1	1	1	0	1	1	0	0	0	1	1	1/24	8	Krapinsko-zagorska
14	326	1	1	1	1	1	1	1	0	1	1	1	1	1/12	9	Sisačko-moslavačka
15	334	1	1	1	1	0	1	1	0	1	0	1	1	1/192	10	Varaždinska
16	335	1	1	1	1	0	1	1	0	1	0	1	1	1/24	10	Varaždinska
17	403	1	1	1	1	0	1	0	0	1	0	1	1	1/96	11	Sisačko-moslavačka
18	455	1	1	1	3	0	1	1	0	1	1	0	1	1/6	12	Vukovarsko-srijemska
19	554	0	0	1	1	1	1	1	0	1	0	1	1	1/12	13	Osječko-baranjska
20	555	0	0	1	1	1	1	1	0	1	0	1	1	1/96	13	Osječko-baranjska
21	556	0	0	1	1	1	1	1	0	1	0	1	1	1/24	13	Osječko-baranjska
22	557	0	0	1	1	1	1	1	0	1	0	1	1	1/192	13	Osječko-baranjska
23	558	0	0	1	1	1	1	1	0	1	0	1	1	1/24	13	Osječko-baranjska
24	559	0	0	1	1	1	1	1	0	1	0	1	1	1/48	13	Osječko-baranjska
25	562	0	0	1	1	1	1	1	0	1	0	1	1	1/12	13	Osječko-baranjska
26	613	1	1	1	1	0	1	1	0	1	1	1	1	1/48	14	Krapinsko-zagorska
27	614	1	1	1	1	0	1	1	0	1	1	1	1	1/384	14	Krapinsko-zagorska
28	615	1	1	1	1	0	1	1	0	1	1	1	1	1/48	14	Krapinsko-zagorska
29	650	1	1	1	1	1	1	1	0	1	0	1	1	1/96	15	Sisačko-moslavačka
30	651	1	1	1	1	1	1	1	0	1	0	1	1	1/96	15	Sisačko-moslavačka
31	654	1	1	1	1	1	1	1	0	1	0	1	1	1/96	15	Sisačko-moslavačka
32	738	1	1	1	2	0	1	0	0	1	1	1	1	1/48	16	Osječko-baranjska
33	741	1	1	1	2	0	1	0	0	1	1	1	1	1/192	16	Osječko-baranjska
34	806	1	1	1	1	0	1	1	0	1	0	0	1	1/384	17	Brodsko-posavska
35	808	1	1	1	1	1	1	1	0	0	0	1	1	1/192	18	Osječko-baranjska
36	936	0	0	0	3	0	1	1	0	1	1	0	1	1/12	19	Osječko-baranjska
37	938	0	0	0	3	0	1	1	0	1	1	0	1	1/12	19	Osječko-baranjska
38	939	0	0	0	3	0	1	1	0	1	1	0	1	1/12	19	Osječko-baranjska
39	988	0	0	0	3	0	1	1	0	1	1	0	1	1/6	20	Međimurska
40	1007	0	0	1	3	0	1	1	0	1	1	1	1	1/12	21	Osječko-baranjska
41	1017	0	0	0	3	0	1	1	0	1	1	1	1	1/12	21	Osječko-baranjska

PART II

No.	Sample ID	Pig category (0=fattening, 1=sows)	Age category (0=<12 m, 1=≥12 m)	Sex (0=male, 1=female)	Total number of pigs (1=up to 20, 2=21-100, 3=>100)	Pets on the farm (0=No, 1=Yes)	Protection against entry of other animals (0=No, 1=Yes)	Indoor housing without outdoor access (0=No, 1=Yes)	Outdoor housing (0=No, 1=Yes)	Footwear disinfection upon entry into the facility (0=No, 1=Yes)	A foot dip for staff at the farm entrance (0=No, 1=Yes)	Feed production on farm (0=No, 1=Yes)	Proper feed storage (0=No, 1=Yes)	Titre	Farm ID	County
42	1032	0	0	1	3	0	1	1	0	1	1	1	1	1/6	21	Osječko-baranjska
43	1113	0	0	0	3	0	1	1	0	1	1	0	1	1/12	22	Vukovarsko-srijemska
44	104	1	1	1	3	0	1	1	0	1	1	1	1	1/6	23	Vukovarsko-srijemska
45	1120	0	0	1	3	0	1	1	0	1	1	1	1	1/12	23	Vukovarsko-srijemska
46	1128	0	0	1	3	0	1	1	0	1	1	1	1	1/12	23	Vukovarsko-srijemska
47	1151	0	0	1	3	0	1	1	0	1	1	1	1	1/12	24	Zagrebačka
48	1153	0	0	0	3	0	1	1	0	1	1	1	1	1/384	24	Zagrebačka
49	1171	0	0	1	3	0	1	1	0	1	1	1	1	1/12	24	Zagrebačka
50	1197	0	0	1	3	0	1	1	0	1	1	1	1	1/24	25	Osječko-baranjska
51	1207	0	0	0	3	0	1	1	0	1	1	1	1	1/24	25	Osječko-baranjska
52	1219	0	0	0	3	0	1	1	0	1	1	1	1	1/12	25	Osječko-baranjska
53	1223	0	0	0	3	0	1	1	0	1	1	1	1	1/24	25	Osječko-baranjska
54	1337	1	1	1	1	0	1	0	0	1	1	1	1	1/24	26	Sisačko-moslavačka
55	1339	1	1	1	1	1	1	0	0	1	0	1	1	1/6	27	Sisačko-moslavačka
56	1341	1	1	1	1	1	1	0	0	1	0	1	1	1/48	27	Sisačko-moslavačka
57	1342	1	1	1	1	1	1	0	0	1	0	1	1	1/6	27	Sisačko-moslavačka
58	1343	1	1	1	1	1	1	0	0	1	0	1	1	1/24	27	Sisačko-moslavačka
59	1366	1	1	1	1	1	1	1	0	1	0	1	1	1/12	28	Grad Zagreb
60	1368	1	1	1	1	1	1	1	0	1	0	1	1	1/12	28	Grad Zagreb
61	1369	1	1	1	1	1	1	1	0	1	0	1	1	1/48	28	Grad Zagreb
62	1370	1	1	1	1	1	1	1	0	1	0	1	1	1/12	28	Grad Zagreb
63	1371	1	1	1	1	1	1	1	0	1	0	1	1	1/6	28	Grad Zagreb
64	1373	1	1	1	1	1	1	1	0	1	0	1	1	1/12	29	Sisačko-moslavačka
65	1408	1	1	1	3	0	1	0	0	1	1	0	1	1/24	30	Zagrebačka
66	1409	1	1	1	3	0	1	0	0	1	1	0	1	1/24	30	Zagrebačka
67	1412	1	1	1	3	0	1	0	0	1	1	0	1	1/12	30	Zagrebačka
68	1413	1	1	1	3	0	1	0	0	1	1	0	1	1/12	30	Zagrebačka
69	1415	1	1	1	3	0	1	0	0	1	1	0	1	1/24	30	Zagrebačka
70	1416	1	1	1	3	0	1	0	0	1	1	0	1	1/48	30	Zagrebačka
71	1417	1	1	1	3	0	1	0	0	1	1	0	1	1/48	30	Zagrebačka
72	1419	1	1	1	3	0	1	0	0	1	1	0	1	1/48	30	Zagrebačka
73	53	0	0	0	3	0	1	0	0	1	1	1	1	1/6	30	Zagrebačka
74	64	0	0	1	3	0	1	0	0	1	1	1	1	1/6	30	Zagrebačka
75	599	1	1	1	3	0	1	0	0	1	1	0	1	1/6	30	Zagrebačka
76	603	1	1	1	3	0	1	0	0	1	1	0	1	1/24	30	Zagrebačka
77	1422	0	1	1	2	1	1	1	0	1	1	1	1	1/96	31	Zagrebačka
78	1423	0	1	0	2	1	1	1	0	1	1	1	1	1/24	31	Zagrebačka
79	1424	0	1	0	2	1	1	1	0	1	1	1	1	1/24	31	Zagrebačka

10. BIOGRAPHY OF THE AUTHOR WITH BIBLIOGRAPHY OF PUBLISHED WORK

Lea Grbavac (née Lovrić) was born on 20 September 1991 in Zagreb, Croatia. She is married and has two children. She completed her primary and secondary education in Velika Gorica. In 2017, she graduated from the Faculty of Veterinary Medicine at the University of Zagreb. As a student, she received the Rector's Award for scientific work in parasitology. Immediately after graduation, she began working as a veterinarian at the Veterinarska stanica Velika Gorica, primarily focusing on companion animals. In 2018, she was employed as a substitute assistant at the Microbiology and Infectious Diseases Unit at the Faculty of Veterinary Medicine. Since 2019, she has been working at the Parasitology and Invasive Diseases Unit. In 2020, she enrolled in the doctoral programme in Veterinary Sciences. She participates in teaching one compulsory and one elective course in the integrated undergraduate and graduate study of veterinary medicine, delivered in both Croatian and English. As an author or co-author, she has published six scientific papers (five cited in Web of Science) and one case report (also cited in Web of Science),and has actively participated in ten national and international conferences through poster or oral presentations. She completed a two-month Erasmus+ traineeship at the École Nationale Vétérinaire d'Alfort (EnvA) and Agence Nationale Sécurité Sanitaire Alimentaire Nationale (ANSES) - Laboratoire de Santé Animale in Maisons-Alfort, France, focused on toxoplasmosis in wild and domestic pigs. She is a member of the Croatian Microbiological Society and is fluent in English and German.

Scientific articles in WoS journals

GRBAVAC, L., F. DÁMEK, S. THOUMIRE, A. MERCIER, K. PASSEBOSC-FAURE, KARINE, N. KONSTANTINOVIĆ, M. KIŠ, Ž. MIHALJEVIĆ, T. ŽIVIČNJAK, R. BLAGA, D. LE ROUX (2025): Seroprevalence and genetic characterization of *Toxoplasma gondii* in hunted wild boars (*Sus scrofa*) from Croatia. Parasitol. Res. 124 (11), 130. doi: 10.1007/s00436-025-08590-1

ŠIMONJI, K., D. KONJEVIĆ, M. BUJANIĆ, I. RUBIĆ, V. FARKAŠ, A. BELETIĆ, ANĐELO, **L. GRBAVAC**, J. KULEŠ (2022): Liver Proteome Alterations in Red Deer (*Cervus elaphus*) Infected by the Giant Liver Fluke *Fascioloides magna*. Pathogens, 11 (12): 1503, 16. doi: 10.3390/pathogens11121503

LOVRIĆ, L., V. VAVŽIK, T. ŽIVIČNJAK (2022): Subclinical dirofilariosis in dogs in Croatia – results of retrospective research based on archived blood samples. Vet. Arhiv., 92 (3), pp. 323-330. doi: 10.24099/vet.arhiv.1453

KULEŠ, J., **L. LOVRIĆ**, A. GELEMANOVIĆ, B. BEER LJUBIĆ, I. RUBIĆ, M. BUJANIĆ, D. KONJEVIĆ (2021): Complementary liver and serum protein profile in wild boars infected by the giant liver fluke *Fascioloides magna* using tandem mass tags quantitative approach. J. Proteomics. 247, 104332, 10. doi: 10.1016/j.jprot.2021.104332

LOVRIĆ, L., M. KRESZINGER, M. PEĆIN (2020): Surgical Treatment of Canine Femoral Fractures - a Review. World. Vet. J., 10 (2), pp. 137-145. doi: 10.36380/scil.2020.wvj18

Scientific article in Scopus journals

LOVRIĆ, L., M. KIŠ, S. LUČINGER, T. ŽIVIČNJAK (2015): Parasitological Examination of Dog Faeces Samples Collected from Grassy Areas in the Cities of Zagreb and Velika Gorica – Contamination Intensity and its Significance. Vet. Stn. 46 (6), pp. 447-457.

Case report in WoS

GRBAVAC, L., A. ŠIKIĆ, P. KOSTEŠIĆ, I.-C. ŠOŠTARIĆ-ZUCKERMANN, V. MOJČEC PERKO, J. BORAS, I. BATA, A. MUSULIN, T. KOSTANJŠAK, T. ŽIVIČNJAK (2024): Comprehensive Diagnosis, Treatment, and Outcome of *Taenia crassice*ps Cysticercosis in a Ring-Tailed Lemur (*Lemur catta*) from a Croatian Zoo: No Longer Unusual? Pathogens. 13 (4), 283. doi: 10.3390/pathogens13040283

Other articles

KONSTANTINOVIĆ, N., **GRBAVAC, L.** (2022): Parazitologija konja u novom ruhu. Hrvatski veterinarski vjesnik. 30, 1. pp. 28-37.

Conference papers (in Proceedings)

KIŠ, M., V. BADOVINAC, **L. LOVRIĆ**, N. ZDOLEC (2021): The use of meat juice serological surveillance for the control of important zoonotic agents in pigs at slaughter. Proceedings of lectures and posters HYGIENA ALIMENTORUM XLI. 23rd November 2021, Vysoké Tatry, Slovakia, pp. 244-251.

LOVRIĆ, L., A. MARINCULIĆ, M. KIŠ, S. LEGEN, SAŠA (2021): Trichinellosis in Croatia - past and present. Proceedings of lectures and posters HYGIENA ALIMENTORUM XLI. 23rd November 2021, Vysoké Tatry, Slovakia, 2021. pp. 288-295.

LOVRIĆ, L., T. ŽIVIČNJAK, S. LUČINGER (2021): Tko ima podstanare – važnost parazitološke koprološke pretrage. Zbornik radova znanstveno-stručnog skupa s međunarodnim sudjelovanjem Veterinarski dani 2021, 26-29 September 2021, Vodice, Croatia, pp. 107-110.

LOVRIĆ, L. (2021): FLOTAC – novi dijagnostički alat u kliničkoj parazitologiji. Zbornik radova znanstveno-stručnog skupa s međunarodnim sudjelovanjem Veterinarski dani 2021, 26-29 September 2021, Vodice, Croatia, pp. 285-288.

LOVRIĆ, L., T. ŽIVIČNJAK (2021): *Toxoplasma gondii* – gdje ćemo ju pronaći? Zbornik radova znanstveno-stručnog skupa s međunarodnim sudjelovanjem Veterinarski dani 2021, 26-29 September 2021, Vodice, Croatia, pp. 359-361.

MARINCULIĆ, A., **L. LOVRIĆ**, N. KONSTANTINOVIĆ (2021): Što to gmiže u plućima pasa i mačaka? Zbornik radova znanstveno-stručnog skupa s međunarodnim sudjelovanjem Veterinarski dani 2021, 26-29 September 2021, Vodice, Croatia, pp. 99-106.

MARINCULIĆ, A., **L. LOVRIĆ**, N. KONSTANTINOVIĆ (2021): Očni nematodi pasa kao potencijalne zoonoze. Zbornik radova znanstveno-stručnog skupa s međunarodnim sudjelovanjem Veterinarski dani 2021, 26-29 September 2021, Vodice, Croatia, pp. 91-98.

MARINCULIĆ, A., D. ŽUBČIĆ, **L. LOVRIĆ**, N. KONSTANTINOVIĆ (2021): Zbornik radova znanstveno-stručnog skupa s međunarodnim sudjelovanjem Veterinarski dani 2021, 26-29 September 2021, Vodice, Croatia, pp. 253-258.

Conference abstracts (Book of abstracts)

GRBAVAC, L., F. DÁMEK, S. THOUMIRE, I. VILLENA, R. BLAGA, T. ŽIVIČNJAK, D. LE ROUX (2023): *Toxoplasma gondii* seroprevalence in pigs in Croatia. European Veterinary Parasitology College (EVPC) Abstract book. 29-30 June 2023, Paris, France. p.102.

GRBAVAC, L., F. DÁMEK, S. THOUMIRE, I. VILLENA, R. BLAGA, T. TATJANA, D. LE ROUX DELPHINE Seroprevalence de *Toxoplasma gondii* chez les porcs en Croatie. Symposium du Club d'Immunologie et de Vaccinologie Veterinaires. 23-24 May 2023, Toulouse, France.

GRBAVAC, L., T. ŽIVIČNJAK (2022): *Toxoplasma gondii* – gdje je pronaći. Aktualne parazitske zoonoze. 17th November 2022, Zagreb, Croatia, pp. 19-20.

MENČIK, S., I.-C. ŠOŠTARIĆ-ZUCKERMANN, B. ARTUKOVIĆ, I. MIHOKOVIĆ BUHIN, **L. GRBAVAC**, M. OSTOVIĆ, A. EKERT KABALIN (2022): Leg weakness, osteomalacia and hypocalcemia in weaned Black Slavonian piglet: A case report. Book of Abstract XI. International Symposium on the Mediterranean Pig. 11-14 October 2022, Vodice, Croatia, pp. 67-68.

ŠIMONJI, K., D. KONJEVIĆ, M. BUJANIĆ, I. RUBIĆ, V. FARKAŠ, A. BELETIĆ, **L. GRBAVAC**, J. KULEŠ. Liver proteome profile in red deer (*Cervus elaphus*) infected by the liver fluke *Fascioloides magna* (2022): 10th International Deer Biology Congress (IDBC) Abstract Book. 4-9 September 2022, Osijek, Croatia. pp. 142-143.

KONSTANTINOVIĆ, N., **L. LOVRIĆ** (2021): *Paraspidodera uncinata* in guinea pig. 9th International Congress Veterinary Science and Profession Abstract Book. 9th October 2021, Zagreb, Croatia, p. 78.

LOVRIĆ, L., I. MIHOKOVIĆ BUHIN, V. MOJČEC PERKO, T. ŽIVIČNJAK, Z. ŠTRITOF, R. BECK (2021): Co-infection with *Dirofilaria repens* and *Dirofilaria immitis* in a dog - a case report 9th International Congress Veterinary Science and Profession Abstract Book. 9th October 2021, Zagreb, Croatia, p. 84.

MIHOKOVIĆ BUHIN, I., **L. LOVRIĆ**, I.-C. ŠOŠTARIĆ- ZUCKERMANN, B. ARTUKOVIĆ, T. ŽIVIČNJAK, D. VLAHOVIĆ, A. GUDAN KURILJ, L. MEDVEN ZAGRADIŠNIK, M. KIŠ, M. HOHŠTETER (2021): Differential diagnosis of intestinal emphysema and cysticercosis (Cysticercus tenuicollis) in pigs. 9th International Congress Veterinary Science and Profession Abstract Book. 9th October 2021, Zagreb, Croatia, p. 51.

KIŠ, M., V. BADOVINAC, **L. LOVRIĆ**, N. ZDOLEC (2021): Meat juice serology as a potential tool in farm risk categorization - survey on *Toxoplasma gondii* antibodies in domestic pigs and wild boars in Karlovac county. 9th International Congress Veterinary Science and Profession Abstract Book. 9th October 2021, Zagreb, Croatia, p. 48.

BROZIĆ, D., T. ŽIVIČNJAK, **L. LOVRIĆ**, J. HABUŠ, S. HAĐINA, S. LUČINGER, H. VALPOTIĆ, M. PERHARIĆ, Z. ŠTRITOF (2019): The use of raw meat based dietary regimes (BARF) in dogs in Croatia. 8th International Congress Veterinary Science and Profession Abstract Book. 10-12 October 2019, Zagreb, Croatia, p. 89.

LOVRIĆ, L., S. LUČINGER, V. MATIJATKO, I. KIŠ, T. ŽIVIČNJAK (2019): Prevalence of *Dirofilaria* spp. in randomly chosen dogs. 8th International Congress Veterinary Science and Profession Abstract Book. 10-12 October 2019, Zagreb, Croatia, p. 86.