



A holistic approach model to food safety surveillance: detecting *Toxoplasma gondii* in meat

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ABSTRACT

Prevalences of infections caused by food- and waterborne parasites, some of them causing a significant disease burden, are on the increase. In the absence of systematic surveillance programmes for many of these parasites, particularly in extensive or free-range husbandry settings, research efforts are under way to design monitoring procedures and improve food safety, beyond the current standard of practice or of food safety legislation. An example is given of the commencement of these efforts, by presenting the experimental work package in a research project which entails testing of meat (heart tissue) of backyard poultry and game birds for the presence of *Toxoplasma gondii* gDNA. The high molecular prevalence of infection detected in some bird species, particularly in backyard chickens raised in rural areas, points to the need for comprehensive and timely surveillance programmes to be applied to all extensively farmed, or game animals and birds used for human consumption. Surveillance should include testing of animal feed as well as the environment (water and soil). However, until risk-based surveillance programmes are operational, meat safety will principally depend on adherence to prevention practices, such as pre-market product processing, as well as on educating consumers in hygienic handling and proper meat preparation practices.

1. Introduction

The prevalence of infections caused by food- and waterborne parasites (FWP) in humans and animals in Europe is increasing (Bouwknegt *et al.*, 2018), prompting needs for a holistic approach to their surveillance. There is a need to quickly identify reservoirs of infection, recognise emerging threats, and warn public health authorities in good time. Currently, there are no active surveillance programs

for most of the FWP, and the parasitoses they cause are not notifiable for the most part.

In controlled husbandry conditions of intensive farming, parasitic diseases are easily surveyed, prevented, or treated. For instance, surveillance programmes for *Toxoplasma gondii* infection for pig farms that supply around 50% of all slaughter pigs in the Netherlands have been in place since 2012 (Swanenburg *et al.*, 2019). In addition to on-farm risk factor management (such as neutering all

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cats and covering feed storage), control of *T. gondii* infection includes a large-scale serological monitoring programme endorsed by a private food producing company. This risk-based active surveillance combines farm audits and serological testing to identify high, medium and low risk farms. A random sample of slaughter pigs is serologically monitored for *T. gondii*-specific antibodies at each delivery to abattoirs, depending on the farm status (fewer animals are sampled from low-risk farms, as opposed to those from high risk, or from a new supplier's farm; also, in the case of continuously negative serological results on repeated testing, the number sampled is decreased). This method has proven effective for *T. gondii* surveillance, as well as being affordable both in cost and time. However, this is still an unregulated programme, as opposed to that for *Trichinella* surveillance, Europe-wide. Provisions of a 2022 Serbian bylaw, harmonized with the European Union regulation from 2015, allow for the implementation of a risk-based approach in the testing for *Trichinella* infection in Serbia. Namely, pig farms could be designated 'Trichinella-free', whereupon only 10% of animals from those farms would be subject to mandatory post-mortem *Trichinella* inspection; the enactment of this bylaw is nonetheless slow to begin (Betić *et al.*, 2025 – *this Special Issue*).

In Serbia, *T. gondii* infection in food animals has been monitored to the greatest extent in pigs through individual research efforts since 2002. Seroprevalence in intensively farmed market-weight pigs over the years has been in the range of 8.3–17% (Klun *et al.*, 2006; Kuruca *et al.*, 2017; Betić *et al.*, 2022), while a much higher seroprevalence of 30–41% has been determined for intensively and semi-extensively farmed sows (Klun *et al.*, 2006, 2011), and particularly for extensively farmed or free-range pigs, such as in Mangalitza breed droves – 67% (Kuruca *et al.*, 2016). Understandably, controlling parasitic infections in an extensive husbandry setting is much more challenging; it would perhaps be practically impossible to create a uniform one-fits-all control programme that could enable circumvention of post-harvest mitigation measures. When using meat from free-range animals, vigilance in adhering to these measures is still crucial, be it freezing of meat for 48–72 hours, proper salting or curing, vacuum packing for a minimum of 21 days, cooking to an internal temperature of 66 °C (Dubey *et al.*, 1990), or any other means confirmed to kill

all viable parasites (tissue cysts) within the meat or meat product.

2. The chicken connection

Similarly, consuming undercooked meat from chickens raised in extensive husbandry conditions, such as free-range or backyard, or from game birds, poses a public health risk. Although it is commonly accepted that chicken meat in general does not pose a risk since it is usually consumed properly cooked, or at least the risk is lower than from other types of meat, specifically from pigs, sheep or goats (Tenter *et al.*, 2000), in certain situations the risk may be much higher. Only in one type of dish is chicken meat purposefully used uncooked, and that is in the southern Kyushu region of Japan, where traditional 'torisashi' (e.g. sashimi, or 'carpaccio') is prepared from thinly sliced meat, seared on the surface while remaining raw within, and served with various seasonings. However, in Europe and other parts of the world, chicken meat may still accidentally remain undercooked (with viable *T. gondii* tissue cysts reaching the consumer), due to undue haste in preparing food, for instance when catering for large numbers of people, or in some cases of street served food. Eating undercooked poultry is a known risk factor for developing campylobacteriosis (rev. in Varrone *et al.*, 2020). Alternatively, poor standards of hygiene may lead to the use of meat cutting boards for salad (raw vegetables or fruit) preparation immediately afterwards, allowing for cross-contamination, i.e., transfer of particles of infected meat (or tissue cysts) to other food that is naturally eaten raw. Also, chicken liver is sometimes prepared and eaten quickly seared, without thorough cooking to reach an internal temperature of 66 °C; consumption of chicken liver pâté has been implicated in numerous cases of campylobacteriosis and/or salmonellosis outbreaks (Wensley and Coole, 2013; Lahti *et al.*, 2017). Therefore, it can be inferred that this mode of transmission is also highly probable for *T. gondii*.

Due to their feeding habits, with scratching the ground and pecking, backyard and free-range chickens are frequently exposed to the parasite. They have been used as sources of *T. gondii* strains around the world, and they make for good indicators as well as sentinels for detecting environmental contamination with *T. gondii* oocysts (Dubey, 2010; Dubey *et al.*, 2020). However, no surveillance for *T. gondii* infection is performed (nor is it mandatory) on chicken

farms, let alone in free-range poultry or wild-caught game birds.

3. Hunting for parasites

The possibilities of setting the stage for a novel and comprehensive surveillance programme for FWP are being explored in a 3-year project funded by the Science Fund of the Republic of Serbia – Environmental Monitoring of Food and Waterborne Parasites – PARASITE_HUNTER (DIPIS, 2025). It aims to establish a data-driven surveillance framework for FWP to improve food safety beyond the current standard, by including monitoring of vulnerable groups (humans and animals) not covered by the current food safety legislation. The holistic approach applied is expected to produce information on the presence of FWP of public health significance; therefore, the species targeted by the project are: *Ascaris* spp., *Toxocara* spp., *Trichuris* spp., *Taenia* spp., *Diphyllobothrium* spp., *Echinococcus* spp., *Spirometra* spp., *Opisthorchis felinus*, *Fasciola hepatica*, *Cyclospora cayetanensis*, *Cryptosporidium* spp., *T. gondii*, *Entamoeba* spp., and *Giardia* spp.

The PARASITE_HUNTER project comprises six experimental work packages, and one each of analytical and coordination packages. Experimental packages entail analysis of food (domestic animal tissues, leafy vegetables and herbs), feed (hay, silage), environmental matrices (water and soil), freshwater crustaceans, and wild animals inhabiting urban areas, for the presence of zoonotic para-

sites using standardised parasitological and molecular techniques. The work also includes exploring the likelihood of interaction of golden jackals as parasite reservoirs with food and feed. In parallel, data is collected on information and knowledge of food producers and suppliers (farmers, fishermen, hunters) about FWP, as well as parasitic diseases and prevention measures. The impact of ecosystem health and wildlife on food safety will be evaluated. All data thus collected will be analysed in the context of assessing food safety and public health risks, while considering potential means of preventing parasite transmission either at the production or distribution level.

For instance, for *T. gondii* surveillance, hearts of backyard or free-range chickens, waterfowl, game birds, as well as lambs have been collected and tested for the presence of *T. gondii* gDNA. The results showed a high molecular prevalence of *T. gondii* in hunted pheasants and backyard chickens, particularly rural (80%), while those raised in peri-urban areas had a considerably lower prevalence of infection (21%), similar to mallards (Table 1) (Penezić et al., 2025, *in press*). *T. gondii* has previously been detected and isolated from chickens in Serbia, with two strains successfully propagated in mice (Uzelac et al., 2021), so the current results were hardly surprising. However, they show the need for comprehensive and timely surveillance programmes to be applied to extensively farmed, or wild (hunted) animals and birds used for human consumption, as well as to include testing of animal feed, and water and soil from the animals' habitats and environments.

Table 1. *Toxoplasma gondii* infection in edible birds (poultry and game) in Serbia, based on detection of parasite gDNA in heart tissue.

Bird species (type)	<i>T. gondii</i> infection (%)	Reference
Domestic chicken (backyard poultry)	41	Penezić et al., 2025 (<i>in press</i>)
Ring-necked pheasant (game bird)	29	
Mallard (duck game)	23	

PARASITE_HUNTER is in its second implementation year, and by its completion, it is envisaged that it will give a new viewpoint as well as recommendations that could enhance food safety standards. The resulting proposals are aimed for inclusion in a white paper which will be put forward to the stakeholders – policy makers in Serbia, in the

hope of instigating a country-wide, systematic surveillance programme for FWP. Ideally, risk-based surveillance programmes would pinpoint the necessary intervention strategies and keep the costs to a minimum, while not compromising the outcome of effective food safety control measures to reduce the disease burden.

4. Conclusion

Until researched, evidence- and risk-based surveillance programmes for most of FWP are set up, food as well as public health safety will essentially depend on diligent adherence to post-harvest pre-

vention practices to control FWP. These practices involve pre-market processing of meat and meat products by using procedures that inactivate the parasites, as well as educating consumers in safe and hygienic ways of handling and preparing the meat at home.

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References

- Betić, N., Karabasil, N., Djurković-Djaković, O., Ćirković, V., Bobić, B., Branković Lazić, I., Djordjević, V. & Klun, I. (2022). Seroprevalence, direct detection and risk factors for *Toxoplasma gondii* infection in pigs in Serbia, and influence of biosecurity measures. *Microorganisms*, 10, 1069. <https://doi.org/10.3390/microorganisms10051069>
- Betić, N., Klun, I., Djordjević, V., Velebit, B., Uzelac, A. & Karabasil, N. (2025). Transitioning to a risk-based approach in pork inspection in Serbia: Are we ready for *Trichinella* control reform? *Meat Technology – this Special Issue*.
- Bouwknegt, M., Devleeschauwer, B., Graham, H., Robertson, L. J., van der Giessen, J. W. & Euro-FBP workshop participants. (2018). Prioritisation of food-borne parasites in Europe, 2016. *Eurosurveillance*, 23, 17-00161. <https://doi.org/10.2807/1560-7917.ES.2018.23.9.17-00161>
- DIPIS – Digital Platform for Intelligent Search of Information on beneficiaries of the Science Fund of the Republic of Serbia. Retrieved from <https://dipis.fondznanauku.gov.rs/dipis/#/projekti/266>. Accessed July 29, 2025.
- Dubey, J. P., Kotula, A. W., Sharar, A., Andrews, C. D. & Lindsay, D. S., (1990). Effect of high temperature on infectivity of *Toxoplasma gondii* tissue cysts in pork. *Journal of Parasitology*, 76(2), 201-204.
- Dubey, J. P. (2010). *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): prevalence, clinical disease, diagnosis and public health significance. *Zoonoses and Public Health*, 57(1), 60-73. <https://doi.org/10.1111/j.1863-2378.2009.01274.x>
- Dubey, J. P., Pena, H. F. J., Cerqueira-Cézar, C. K., Murata, F. H. A., Kwok, O. C. H., Yang, Y. R., Gennari, S. M. & Su, C. (2020). Epidemiologic significance of *Toxoplasma gondii* infections in chickens (*Gallus domesticus*): the past decade. *Parasitology*, 147(12), 1263-1289. <https://doi.org/10.1017/S0031182020001134>
- Klun, I., Djurković-Djaković, O., Katić-Radivojević, S. & Nikolić, A. (2006). Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: Seroprevalence and risk factors. *Veterinary Parasitology*, 135, 121–131. <https://doi.org/10.1016/j.vetpar.2005.08.010>
- Klun, I., Vujančić, M., Yera, H., Nikolić, A., Ivović, V., Bobić, B., Bradonjić, S., Dupouy-Camet, J. & Djurković-Djaković, O. (2011). *Toxoplasma gondii* infection in slaughter pigs in Serbia: Seroprevalence and demonstration of parasites in blood. *Veterinary Research*, 42, 1, 17. <https://doi.org/10.1186/1297-9716-42-17>
- Kuruca, Lj., Klun, I., Uzelac, A., Nikolić, A., Bobić, B., Simin, S., Djurković-Djaković, O. & Lalošević, V. (2016). Detection of viable *Toxoplasma gondii* in free-range pigs from the special nature reserve of Zasavica. *Contemporary Agriculture*, 65, 1–6. <https://doi.org/10.1515/contagri-2016-0010>
- Kuruca, Lj., Klun, I., Uzelac, A., Nikolić, A., Bobić, B., Simin, S., Lalošević, V., Lalošević, D. & Djurković-Djaković O. (2017). Detection of *Toxoplasma gondii* in naturally infected domestic pigs in Northern Serbia. *Parasitology Research*, 116, 3117–3123. <https://doi.org/10.1007/s00436-017-5623-7>
- Lahti, E., Löfdahl, M., Ågren, J., Hansson, I. & Olsson Engvall, E. (2017). Confirmation of a campylobacteriosis outbreak associated with chicken liver pâté using PFGE and WGS. *Zoonoses and Public Health*, 64(1), 14-20. <https://doi.org/10.1111/zph.12272>
- Penezić, A., Uzelac, A., Breka, K., Simin, S., Ovari, K., Pantelić, I., Ćirković, V., Ćirović, D. & Klun, I. (2025). Understanding *Toxoplasma gondii* transmission in an ecological context – the contribution of wild avian species from urban environments. *Frontiers in Veterinary Science*, (in press).
- Swanenburg, M., Gonzales, J. L., Bouwknegt, M., Boender, G. J., Oorburg, D., Heres, L. & Wisselink, H. J. (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. *Veterinary Parasitology*, 276S, Article 100017. <https://doi.org/10.1016/j.vpoa.2019.100017>
- Tenter, A. M., Heckeroth, A. R., & Weiss, L. M. (2001). *Toxoplasma gondii*: From animals to humans. *International Journal of Parasitology*, 30, 1217–1258. [https://doi.org/10.1016/s0020-7519\(00\)00124-7](https://doi.org/10.1016/s0020-7519(00)00124-7). Erratum in: *International Journal of Parasitology*, 31, 217–220.
- Uzelac, A., Klun, I., Ćirković, V., Bauman, N., Bobić, B., Štajner, T., Srbljanović J., Lijeskić, O. & Djurković-Djaković, O. (2021). *Toxoplasma gondii* genotypes circulating in Serbia – insight into the population structure

and diversity of the species in Southeastern Europe, a region of intercontinental strain exchange. *Microorganisms*, 9(12), 2526. <https://doi.org/10.3390/microorganisms9122526>

Varrone, L., Glass K., Stafford, R. J., Kirk, M. D., Selvey, L. & CampySource Project Team. (2020). A meta-analysis of case-control studies examining sporadic campylobacteriosis in Australia and New Zealand from 1990 to 2016.

Australian and New Zealand Journal of Public Health, 44(4), 313-319. <https://doi.org/10.1111/1753-6405.12998>

Wensley, A. & Coole, L. (2013). Cohort study of a dual-pathogen point source outbreak associated with the consumption of chicken liver pâté, UK, October 2009. *Journal of Public Health (Oxford)*, 35(4), 585-589. <https://doi.org/10.1093/pubmed/ftd020>

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