VETERINARIA ITALIANA
RIVISTA DI SANITÀ PUBBLICA VETERINARIA

Paper

Meat juice as a feasible alternative sample for tuberculosis surveillance in large game

Ana Carolina Abrantes[1*], David Risco[2], Miguel Carvalho[3], Rosario Cerrato[4], Pedro Fernández-Llario[4], Madalena Vieira-Pinto[1]

[1]*CECAV – Animal and Veterinary Research Centre, Trás-os-Montes e Alto Douro University, Vila Real, Portugal - PT*
[2]*Departamento de Medicina Animal, Universidad de Extremadura, Cáceres, Spain - ES*
[3]*Department of Veterinary Sciences, Trás-os-Montes e Alto Douro University, Vila Real, Portugal - PT*
[4]*INGULADOS – Innovación en Gestión y Conservación de Ungulados, Cáceres, Spain - ES*

[*]*Corresponding author at: CECAV – Animal and Veterinary Research Centre, Trás-os-Montes e Alto Douro University, Vila Real, Portugal - PT*
E-mail: carolina.psca@gmail.com

Veterinaria Italiana, Vol. 60 No. 1 (2024) DOI: 10.12834/VetIt.3132.22735.2

Abstract

In hunted animals, quality of blood samples may often be compromised. Alternative samples, such as meat juice, may offer an advantage to perform serological tests. This study evaluates if meat juice is a feasible alternative sample to perform the Tuberculosis ELISA test in hunted large game. Between 2017 and 2022, 175 samples were collected from 97 animals (14 red deer + 83 wild boar) in Portugal and Spain. Cohen's kappa coefficient was calculated at 0.71, pointing out a good agreement using 156 paired samples. The sensitivity of the ELISA test with serum was 37.6%, considering Tuberculosis-like lesions (TBL) detected during the initial examination (26 TBL+/ELISA+ in a total of 78 serum samples). Using meat juice as matrix, the sensitivity increased to 37.5% (33 TBL+/ELISA+ in 97 meat juice samples). According to the agreement score and sensitivity being so close between the two matrices tested, meat juice could be a feasible alternative matrix.

Keywords

Immunological diagnosis, Red deer, Wild boar, Serology

Introduction

Wild boar (*Sus scrofa*) and red deer (*Cervuselaphus*) are two of the most implicated wild species in the maintenance of Tuberculosis (TB) [infection with Mycobacterium tuberculosis complex agents (M. bovis, M. caprae, M. tuberculosis)] in Europe (Gortazar *et al.* 2012).

This multi-host zoonotic disease is listed in the New Animal Health Law (EU Regulation 2016/429) and falls into several categories according to eradication plan, control rules and target species.

Identifying TB infection is essential to limit the transmission and spread in the large game, sympatric species, and neighbouring livestock (Bernitz *et al.* 2021). Screening this disease in these wild populations can be challenging (Lyashchenko *et al.* 2008).

TB diagnosis in non-bovid species, as in some wild species like the wild boar and red deer, is based on conventional tests: bacteriological culture, histopathology, and post-mortem examination (Thomas & Chambers 2021). An adequate gold standard diagnostic test is necessary for the diagnosis since sensitivity (se) and specificity (sp) vary a lot (Alvarez et al. 2012). The most referenced and sensitive (approximately 80%) is the bacteriological culture, but their constraints condition the process. Time-consuming, cost and exposure risk can be avoided using other techniques such as PCR, histopathology, and ELISA, which are quicker, cheaper, and safer (Barandiaran et al. 2019; Richomme et al. 2019; Soares-Filho et al. 2019).

One way to monitor TB in wildlife is to perform the immunological diagnosis with the ELISA (Enzyme-Linked Immunosorbent Assay) test. These tests are a poor indicator of TB infection but can detect the asymptomatic animals and indicate the disease's progress and spread in specific populations (Richomme *et al*. 2019; Thomas & Chambers

How to cite: Abrantes et al. Meat juice as a feasible alternative sample for tuberculosis surveillance in large game. Veterinaria Italiana, Vol. 60 No. 1 (2024) DOI: 10.12834/VetIt.3132.22735.2

2021). The primary way to collect wild boar and red deer samples to perform a TB screening by ELISA is post-mortem collection after driven hunts. Nevertheless, the post-mortem collection of blood in the large game has many obstacles. Most of the blood collected is often haemolysed and/or degraded (Nielsen *et al.* 1998). Nonetheless, in the driven hunts, other samples may be collected systematically, such as muscle, mainly the diaphragm, to screen for other diseases, such as trichinellosis (Vieira-Pinto *et al.* 2021). With this muscle sample, it is possible to obtain meat juice that contains systemic antibodies and perform ELISA (Felin *et al.* 2015; Meemken *et al.* 2014; Lyashchenko *et al.* 2021).

In the Iberian Peninsula, Portugal and Spain, TB is an endemic disease in wild game species populations, notably wild boar and red deer (Gortazar *et al.* 2012; Gortazar *et al.* 2015; Santos *et al.* 2020). Although endemic, the distribution of TB in this wild population varies regionally, with areas of higher prevalence, such as the interior of Portugal (near the border with Spain) and the Extremadura and Andalucia regions of Spain, and others with almost zero prevalence, such as northern Portugal and Galicia in Spain (Aranha *et al.* 2021; Santos *et al.* 2022; Varela-Castro *et al.* 2020). High-risk areas are also conditioned by the risk factors that enhance the maintenance and spread of TB in these populations, such as the density of these hosts, climate and the presence of other domestic hosts (Aranha et al. 2021; Abrantes et al., 2021; Vicente et al. 2007). Considering that TB infection in these wild populations is difficult to detect, mostly based on post-mortem TBL detection (Aranha et al. 2021; Abrantes et al. 2021; Vicente et al. 2006). It is also one of the biggest constraints to eradicating the disease in domestic cattle (Naranjo et al. 2008). Therefore, it is necessary to optimise screening methods for target populations, such as the ELISA test.

This survey aims to evaluate if meat juice is a feasible alternative sample to perform the TB ELISA test by calculating the agreement score and the test's sensitivity in the two matrices, serum vs. meat juice, obtained from the large game from Iberian Peninsula.

Materials and methods

Ethics statement

All samples were collected from wild boars and red deer legally hunted. This study did not involve the deliberate killing of animals. No ethical approval was deemed necessary.

Sampling

A sampling design was implemented to collect samples from large game hunts between 2017 and 2022 in the Iberian Peninsula (Portugal and Spain).

After the initial examination *in loco*, 175 samples (97 muscle + 78 sera) were collected from 97 hunted animals (14 red deer + 83 wild boar). From those, in 78 hunted animals (11 red deer and 67 wild boars), both samples were collected, thus having a total of 156 paired samples (muscle and serum). In the remaining 19 animals (3 red deer and 16 wild boars), only meat sample was collected once the status of the carcass did not allow to obtain sera from these animals, neither the head sinus nor the inside of the carcass' thoracic cavity, due to head shooting, dog bites or very degraded and coagulated state of the blood.

Diagnostic tests

All muscle and blood collections from the hunted animals occurred after the in-charge veterinarian performed the initial examination on the spot. Hunted species were systematically examined according to the described procedure in Vieira-Pinto *et al.* (2014) to detect TBL (Tuberculosis-like lesions). Briefly, the systematic viscera's inspection includes incision and examination of heal, bronchial, mediastinal, mesenteric, precrural and pre-scapular lymph nodes, and visual examination and palpation of the lungs. It is considered TBL, all caseous or caseocalcareous tubercules with different sizes in any of these examined viscera (Zanella *et al.* 2008).

The initial examination data (TBL detection) was used as the gold standard TB diagnostic test in the field, as it is reported by some authors to be one of the most reliable methods of TB detection in the field for wild species, and TBL shows a significant correlation with bacteriology (Risco *et al.* 2013). In this work, molecular methods such as PCR or bacteriology were not used to confirm TBLs, and this topic is discussed at length in the discussion section.

All collected samples were correctly accommodated, labelled, and taken to the laboratory under appropriate refrigeration conditions. In the laboratory, meat juice to perform the ELISA test was obtained after the muscle pieces (such as diaphragm, heart or sternomastoideus muscles) were frozen and thawed, and the drained fluid was collected (Nielsen *et al.* 1998). Subsequently, with this drained meat juice fluid, ELISA test was performed.

How to cite: Abrantes et al. Meat juice as a feasible alternative sample for tuberculosis surveillance in large game. Veterinaria Italiana, Vol. 60 No. 1 (2024) DOI: 10.12834/VetIt.3132.22735.2

The serological diagnosis with the detection of specific IgG antibodies against TB was performed using a commercial indirect ELISA test (INgezim multispecies Tuberculosis DR) according to the manufacturer's instructions.

According to the instructions of the ELISA kit, the dilution for serum from wild boar is 1/250, and for other species, such as red deer, it is 1/25. To carry out the assay with meat juice, a dilution of 1/25 was used for wild boar samples and 1/2.5 for red deer samples, according to the testing carried out by Fabisiak *et al.* (2013) with an ELISA kit of the same manufacturer , which shows that the meat juice dilution should be ten times lower.

Statistical analysis

To measure the agreement between the two ELISA parallel assays (in sera vs. meat juice), Cohen's kappa coefficient *(k)* was used. The proportion of positives observed in each group (e.g. several tested samples positive to ELISA test in the TBL positive group) was compared by Fisher's exact test to calculate the sensitivity of the carried tests, with a 95% confidence interval (p-value < 0,05). With the data obtained, the sensitivity of the test with the two matrices compared to the TBL occurrence data in the carcasses was calculated, and no other values, such as specificity, were calculated. The statistical analysis was performed using the EpiTools Epidemiological Calculators (Ausvet2022©).

Results

Agreement score

In the two parallel ELISA assays in 156 paired samples (both samples from 78 animals), seropositivity was obtained from 21 hunted animals. Forty-seven animals were seronegative for both samples, and ten animals were positive only for one of the samples (5 for serum and 5 for meat juice). Cohen's kappa coefficient *(k)* value was 0.71, considered a good agreement score for the parallel assays (McHugh 2012).

ELISA's sensitivity with two matrices

To calculate the sensitivity of ELISA test, TBL detected during initial examination *in loco* was considered the gold standard diagnostic test for TB in these hunted species, as previously referred by Risco *et al.* (2013). This assumption occur due to TBL occurrence was considered an indicator of TB status in target populations. After all, TBL shows a great correlation with the bacteriological culture (the proper gold standard). Of the 97 animals sampled, 88 showed TBL on initial examination on-spot, and nine did not.

Sensitivity of the ELISA test with serum as the matrix was 37.6% (26 TBL+/ELISA+ in 78 serum samples). With meat juice as a matrix, the sensitivity was 37.5% (33 TBL+/ELISA+ in 97 meat juice samples). Considering only wild boar samples, the sensitivity of the ELISA test with serum was 34.3% (23 TBL+/ELISA+ in a total of 73 samples), and with meat, juice increased to 38.9% (30 TBL+/ELISA+ in a total of 83 samples). The sensitivity of the few red deer sampled with serum was 37.5%, and meat juice decreased to 18.8%.

Meat juice as an alternative matrix

Sensitivity of the ELISA test with meat juice is similar to that of the ELISA with the standard serum sample. One of this study's main results is highlighted by the highest sensitivity of the TB ELISA with wild boar meat juice as an alternative matrix (38.9%).

When separating samples by wild boar's sex (male vs. female), the sensitivity is 28.5% for males and 47.6% for females. In the case of the age analysis (adult vs. subadult), the sensitivity is 39.6% for adults and 37.9% for subadults.

Discussion

Limitative diagnostic tests in wild hosts of TB are considered a confounding factor in the problematic control of this disease (Barandiaran *et al.* 2019).

In this perspective, the serological diagnosis, as the ELISA test performed in the presented assay, is a complementary, simple, rapid, and cheap valuable technique for screening TB in certain wildlife species (Infantes-Lorenzo *et al.* 2019; Lyashchenko *et al*. 2008) mainly in the wild boar, which is considered a biological vector of TB and considered a sentinel host (Ferreras-Colino *et al.* 2022; Richomme *et al.* 2019). TB as a chronic disease produces a delayed response. However, suid species, such as wild boar, show a consistent immunological response, which

How to cite: Abrantes et al. Meat juice as a feasible alternative sample for tuberculosis surveillance in large game. Veterinaria Italiana, Vol. 60 No. 1 (2024) DOI: 10.12834/VetIt.3132.22735.2

helps in this case to detect the circulating antibodies in the analysed animals (Thomas & Chambers 2021). The results of this presented assay performed in hunted animals suggest that the sensitivity of the ELISA test was higher in this species than in cervids in all analyses performed.

Validation of the diagnostic test in these wild animals is limited by access to many high-quality samples from confirmed infected and uninfected animals (Bernitz *et al.* 2021). Presumably, the difficulty with samples provided of hunted animals is having good conditions in which these samples arrive at the laboratory (Richomme *et al.* 2019). Most blood samples come degraded and haemolysed without quality, and the test cannot be carried out. With that assumption, as Felin *et al.* (2015) have shown, using meat juice as a sample is a valuable tool, as proven in this assay.

As reported in the present assay, the major constraint is using the post-mortem examination as the standard diagnostic test to calculate the sensitivity of the ELISA test, which obtained a sensitivity of around 35% and not up to 80% as indicated in the kit. TBL does not have laboratory confirmation and can result from other infectious agents, such as Actinomyces, *Rodoccocusequi* or other non-tuberculous mycobacteria (Vicente *et al*. 2007). This proves that some of the carcasses that were considered positive for TB because they have TBL may indeed be infected with TB. On the other hand, some publications/research proved that TBL was regarded as an indicator of TB status (Risco*et al.* 2013; Vicente *et al.* 2007) due to the high correlation between TBL detection in loco and TB confirmed with bacteriology. The ideal procedure involves running the TBL by PCR or culture them (Barandiaran *et al*. 2019; Ferreras-Colino *et al.* 2022). However, the consistent sensitivity and agreement score of the presented results of tests with standard and alternative matrices can also be compared and considered a valid assay.

Conclusions

With these results, agreement score and sensitivity being so close between these two matrices tested to perform the ELISA test, meat juice could be considered the alternative matrix in this serological diagnosis. More studies are needed to assess the effectiveness. The authors conclude that more studies should be carried out to better assess the sensitivity and specificity of this ELISA test in hunted animals, taking into account the constraints that the samples of these animals present.

In cases where lesions compatible with tuberculosis are not observed or expensive tests such as bacteriological culture and PCR are used, the authors believe that the use of serological tests is useful in order to screen for an infectious pathology in a target population. It is also expected that in future, meat juice can be routinely used as a feasible sample for TB infection screening in wildlife. This type of sample (meat juice from diafragm and heart muscles) is widely collected routinely to scan other game zoonotic diseases. With this, it could provide in future information more practically and efficiently on long-time and spatial trends of TB infection in target large game, such as the wild boar, a sentinel host. And thus, provide that hunters help in the surveillance of infectious diseases in circulation in large game populations. Hunters may be responsible for routinely collect a sample that is easy to collect, such as a piece of muscle, contributing to the study and TB screening.

References

Abrantes A.C., Serejo J., Vieira-Pinto M. 2021. The Association between Palmer Drought Severity Index Data and Tuberculosis-like Lesions Occurrence in Mediterranean Hunted Wild Boars. Animals. 11(7), 2060.

Álvarez J., Perez A., Bezos J., Marqués S., Grau A., Saez J.L., Mínguez O., Juan L., Domínguez L. 2012. Evaluation of the sensitivity and specificity of bovine tuberculosis diagnostic tests in naturally infected cattle herds using a Bayesian approach. Veterinary Microbiology. 155(1), 38-43.

Aranha J., Abrantes A.C., Gonçalves R., Miranda R., Serejo J., Vieira-Pinto M. 2021. GIS as an epidemiological tool to monitor the spatial–temporal distribution of tuberculosis in large game in a high-risk area in Portugal. Animals, 11(8), 2374.

Barandiaran S., Pérez-Aguirreburualde M.S., Marfil M.J., Martínez-Vivot M., Aznar N., Zumárraga M., Perez A.M. 2019. Bayesian Assessment of the Accuracy of a PCR-Based Rapid Diagnostic Test for Bovine Tuberculosis in Swine. Frontiers in Veterinary Sciences. 6, 204.

How to cite: Abrantes et al. Meat juice as a feasible alternative sample for tuberculosis surveillance in large game. Veterinaria Italiana, Vol. 60 No. 1 (2024) DOI: 10.12834/VetIt.3132.22735.2

Bernitz N., Kerr T.J., Goosen W.J., Chileshe J., Higgitt R.L., Roos E.O., Meiring C., Gumbo R., de Waal C., Clarke C., Smith K., Goldswain S., Sylvester T.T., Kleynhans L., Dippenaar A., Buss P.E., Cooper D.V., Lyashchenko K.P., Warren R.M., van Helden P.D., Parsons S.D.C., Miller M.A. 2021. Review of Diagnostic Tests for Detection of Mycobacterium bovis Infection in South African Wildlife. Frontiers in Veterinary Sciences, 8, 588697.

Fabisiak M., Podgórska K., Skrzypiec E., Szczotka A., Stadejek T. 2013. Detection of porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) antibodies in meat juice samples from Polish wild boar (Sus scrofa L.). Acta Veterinaria Hungarica. 61(4), 529-536.

Felin E., Jukola E., Raulo S., Fredriksson-Ahomaa M. 2015. Meat juice serology and improved food chain information as control tools for pork-related public health hazards. Zoonoses and Public Health. 62(6), 456-464.

Ferreras-Colino E., Moreno I., Cruz-Arnal M., Balseiro A., Acevedo P., Domínguez M., Fernández de Luco D., Gortázar C., Risalde M.A. 2022. Is serology a realistic approach for monitoring red deer tuberculosis in the field? Preventive Veterinary Medicine. 202, 105612.

Gortázar C., Che Amat A., O'Brien D.J. 2015. Open questions and recent advances in the control of a multi-host infectious disease: Animal tuberculosis. Mammal review. 45(3), 160-175.

Gortázar C., Delahay R.J., Mcdonald R.A., Boadella M., Wilson G.J., Gavier-Widen D., Acevedo P. 2012. The status of tuberculosis in European wild mammals. Mammal Review. 42(3), 193-206.

Infantes-Lorenzo J.A., Moreno I., Roy A., Risalde M.A., Balseiro A., de Juan L., Romero B., Bezos J., Puentes E., Åkerstedt J., Tessema G.T., Gortázar C., Domínguez L., Domínguez M. 2019. Specificity of serological test for detection of tuberculosis in cattle, goats, sheep and pigs under different epidemiological situations. BMC Veterinary Reseach. 15, 70.

Lyashchenko K., Sikar-Gang A., Sridhara A.A., Johnathan-Lee A., Elahi E., Greenwald S., Lambotte P., Esfandiari J., Roos E.O., Kerr T.J., Miller M.A., Thacker T.C., Palmer M.V., Waters W.R. 2021. Use of blood matrices and alternative biological fluids for antibody detection in animal tuberculosis. Veterinary Immunology and Immunopathology. 239, 110303.

Lyashchenko K.P., Greenwald R., Esfandiari J., Chambers M.A., Vicente J., Gortazar C., Santos N., Correia-Neves M., Buddle B.M., Jackson R., O'Brien D.J., Schmitt S., Palmer M.V., Delahay R.J., Waters W.R. 2008. Animal-side serologic assay for rapid detection of Mycobacterium bovis infection in multiple species of free-ranging wildlife. Veterinary microbiology, 132(3-4), 283-292.

McHugh M.L. 2012. Interrater reliability: the kappa statistic. Biochemia medica. 22(3), 276-282.

Meemken D., Tangemann A.H., Meermeier D., Gundlach S., Mischok D., Greiner M., Klein G., Blaha T. 2014. Establishment of serological herd profiles for zoonoses and production diseases in pigs by "meat juice multi-serology". Preventive Veterinary Medicine. 113(4), 589-598.

Naranjo V., Gortazar C., Vicente J., de La Fuente, J. 2008. Evidence of the role of European wild boar as a reservoir of Mycobacterium tuberculosis complex. Veterinary microbiology, 127(1-2), 1-9.

Nielsen B., Ekeroth L., Bager F., Lind P. 1998. Use of muscle fluid as a source of antibodies for serologic detection of Salmonella infection in slaughter pig herds. Journal of Veterinary Diagnostic Investigation. 10(2), 158-163.

Richomme C., Courcoul A., Moyen J.L., Reveillaud E., Maestrini O., de Cruz K., Drapeau A., Boschiroli M.L. 2019. Tuberculosis in the wild boar: Frequentist and Bayesian estimations of diagnostic test parameters when Mycobacterium bovis is present in wild boars but at low prevalence. PLoS ONE, 14(9): e0222661.

Risco D., Fernández-Llario P., García-Jiménez W.L., Gonçalves P., Cuesta J.M., Martínez R., Sanz C., Sequeda M., Gómez L., Carranza J., de Mendoza J.H. 2013. Influence of Porcine Circovirus Type 2 Infections on Bovine Tuberculosis in Wild Boar Populations. Transboundary and Emerging Diseases. 60, 121-127.

Santos N., Colino E.F., Arnal M.C., de Luco D.F., Sevilla I., Garrido J.M., Fonseca E., Valente A., Balseiro A., Queiros

J., Almeida V., Vicente J., Gortazar C., Alves, P.C. 2022. Complementary roles of wild boar and red deer to animal tuberculosis maintenance in multi-host communities. Epidemics, 41, 100633.

Santos N., Richomme C., Nunes T., Vicente J., Alves P.C., de la Fuente J., Correia-Neves M., Boschiroli M.L., Delahay R., Gortázar C. 2020. Quantification of the Animal Tuberculosis Multi-Host Community Offers Insights for Control. Pathogens. 9(6), 421.

Soares-Filho P., Ramalho A.K., de Moura Silva A., Hodon M.A., de Azevedo Issa M., Júnior A.A., Mota P., Silva C.H., dos Reis J.K., Leite R. 2019. Evaluation of post-mortem diagnostic tests' sensitivity and specificity for bovine tuberculosis using Bayesian latent class analysis. Research in Veterinary Science. 125, 14-23.

Thomas R. & Chambers M. 2021. Review of Methods Used for Diagnosing Tuberculosis in Captive and Free-Ranging Non-Bovid Species (2012–2020). Pathogens, 10, 584.

Varela-Castro L., Alvarez V., Sevilla I.A., Barral, M. 2020. Risk factors associated to a high Mycobacterium tuberculosis complex seroprevalence in wild boar (Sus scrofa) from a low bovine tuberculosis prevalence area. PLoS One, 15(4), e0231559.

Vicente J., Höfle U., Garrido J., Fernández-De-Mera I.G., Juste R., Barral M., Gortazar, C. 2006. Wild boar and red deer display high prevalences of tuberculosis-like lesions in Spain. Veterinary research, 37(1), 107-119.

Vicente J., Höfle U., Garrido J.M., Fernández-de-Mera I.G., Acevedo P., Juste R., Barral M., Gortazar C. 2007. Risk factors associated with the prevalence of tuberculosis-like lesions in fenced wild boar and red deer in south central Spain. Veterinary Research. 38(3), 451-64.

Vieira-Pinto M., Fernandes A.R.G., Santos M.H., Marucci G. 2021. Trichinella britovi infection in wild boar in Portugal. Zoonoses and Public Health. 68, 103– 109.

Vieira-Pinto M., Vinhas B., Coelho C. 2014. Initial Examination of Wild Large Game on the Spot—Importance and Rules. Journal of Nutritional Ecology and Food Research. 1, 1–3.

Zanella G., Duvauchelle A., Hars J., Moutou F., Boschiroli M.L., Durand B. 2008. Patterns of lesions of bovine tuberculosis in wild red deer and wild boar. Veterinary Record. 163, 43-47.

How to cite: Abrantes et al. Meat juice as a feasible alternative sample for tuberculosis surveillance in large game. Veterinaria Italiana, Vol. 60 No. 1 (2024) DOI: 10.12834/VetIt.3132.22735.2