

Supplementary materials for Journal of Wildlife Diseases DOI: 10.7589/JWD-D-23-00027: Agustina Tammone Santos, Eliana Riva, Walter E. Condorí, Valentina Fernández, Marcelo G. Rodriguez, Mariana A. Rivero, Matias Faraco, Pablo Aguirre, Lorena Loyza, Andrea E. Caselli, Marcela M. Uhart, and Silvia M. Estein. *Trichinella* infection in culled wild boar (*Sus scrofa*) from El Palmar National Park, Argentina and exposure risk in humans and dogs consuming wild boar meat.

Online Resource 1 Informed consent form presented to dog owners.

INFORMED CONSENT FORM FOR INVESTIGATION ON ANIMALS

Title of studies:

- Contributions to the Invasive Exotic Mammal Control Plan in El Palmar National Park: zoonoses survey in wild boar (*Sus scrofa*) and axis deer (*Axis axis*) and impact on public health.
- Public health risks: lead from hunting ammunition in game meat.

Research team from Centro de Investigaciones Veterinarias de Tandil (CIVETAN) - School of Veterinary Sciences - Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA): Dr. Silvia M. Estein, Lic. Agustina Tammone and Med. Vet. student Valentina Fernández.

For the development of the aforementioned studies, owners are informed and invited to grant consent for the participation of their animals in this study, voluntarily and free of charge. Confidentiality of data provided is ensured.

Non-participation will not imply any loss of your rights and will not generate any type of sanction or penalty towards you.

You may contact the professionals named above for information about your rights as a research participant, or for details about the results of the tests.

The animal or animals authorized to participate in this study will undergo the following procedures, carried out by qualified personnel:

- Collection of 1 gram of hair, for lead level quantification.

- Extraction of 5 ml of blood by venipuncture, for lead level quantification, detection of hematological abnormalities (anemia) and detection of anti-*Brucella spp*, anti-*Leptospira spp* and anti-*Trichinella spp* antibodies (to determine if it has been exposed to these bacteria).

Risks

Hair removal is painless and does not cause damage or aesthetic alterations to your dog. Blood collection is generally painless, although it may cause slight discomfort when performed, but it does not cause harm to the animal either. If a small hematoma appears, it will be resolved by local compression and application of ice.

Benefits

Direct benefits are not contemplated for participation in this study. If through the performed tests we detect any abnormality in any of your animals, you will be notified and contacted with veterinary professional staff in your home area, who will indicate the corresponding treatment.

Results of tests will be available and communicated to you within approximately 45 days from extraction.

Consent / signature

This document is signed in 3 copies, 1 for the owner, 1 for the researcher in charge of the project and 1 for the Bioethics Committee of the UNCPBA – School of Veterinary Sciences for registration and filing.

1. Participant / owner

I have read this Informed Consent Form, I understand its content and freely and voluntarily give my consent to lend my animals to the research study, under conditions detailed in this document, knowing that if I wish I can withdraw them at any time and without consequences of any kind.

Owner's name and surname

ID number

Address

Phone number

Signature

Date

2. Researcher

I accept my responsibility for the care of the animals involved in this research, I promise to act according to the Animal Welfare Act of the Academic Council of the School of Veterinary Sciences – UNCPBA (res. n° 087/02), and to develop this study in compliance with the general guidelines and recommendations of the

Veterinary Medicine Best Practices Manual of the Argentina Veterinary Federation (Manual de Buenas Prácticas Médicas Veterinarias of the Federación Veterinaria Argentina).

I have carefully explained to the owner the characteristics of this research study. I hereby certify that, to the best of my knowledge, the owner understands the nature and scope of the study.

Researcher's name and surname

ID number

Signature

Date

Online Resource 2 Informed consent form presented to participants.

INFORMATION AND INFORMED CONSENT FORM FOR INVESTIGATION

Title of studies:

- Contributions to the *Plan de Control de Mamíferos Exóticos Invasores* from El Palmar National Park: zoonoses survey in wild boar (*Sus scrofa*) and axis deer (*Axis axis*) and their impact on public health.
- Public health risks: lead from hunting ammunition in game meat.

Research team from Centro de Investigaciones Veterinarias de Tandil (CIVETAN) - School of Veterinary Sciences - Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA): Dr. Silvia M. Estein and Lic. Agustina Tammone.

Brucellosis, leptospirosis, tuberculosis and trichinellosis are zoonotic diseases (diseases that are transmitted from domestic and wild animals to persons). In general, the infection is caused by improper handling and / or consumption of insufficiently cooked game meat. The sick person can present a flu-like state or chronic or serious manifestations that present with pain in different parts of the body and with affection of different organs.

Game meat can also be contaminated with lead (Pb) from hunting ammunition, which represents one of the main routes of exposure to this metal. Careless removal of tissue surrounding the bullet path and wounds results in the ingestion of high amounts of Pb. This metal causes acute poisoning after brief exposures at high concentrations, but it is often responsible for chronic toxicity after long-term exposure to small doses which is also concerning. This metal is primarily neurotoxic, but it can also damage kidneys, bones, cardiovascular and immune systems, and can cause infertility.

For the development of the aforementioned studies, people are informed and invited to participate voluntarily, free of charge, and the confidentiality of data provided is ensured. In the first place, the

participant is asked to answer a structured questionnaire (Online Resource 2), in which the possible factors associated with the problems detected (zoonosis and lead from hunting ammunition in game meat) are asked. On one hand, a blood sample will be obtained for the detection of anti-*Brucella* spp, anti-*Leptospira* spp and anti-*Trichinella* spp antibodies, which is the way to determine if the person was ever in contact with these bacterias or parasite. The serological results of the study take approximately 2 weeks. On the other hand, the tuberculin skin test (PPD) indicated for the diagnosis of tuberculosis and a chest x-ray will be performed. The PPD reading will be done between 48 and 72 hours, and the results of an X-ray can be available almost immediately for review by the physician. Likewise, a blood sample will be obtained for the determination of lead and these results will be available approximately 2 weeks after the extraction.

The aim of the study

The aim is to evaluate the risks on Public Health and their links with environmental and animal health, focusing on zoonoses and lead from hunting ammunition in game meat. For that, the purpose is to evaluate the presence of zoonotic diseases such as brucellosis, leptospirosis, tuberculosis and trichinellosis, through the determination of specific antibodies against each pathogen, added to the analysis of the tuberculin test and a chest X-ray. Likewise, it is intended to evaluate and quantify lead levels, through the determination of the concentration of this metal in blood samples. In this way, it is intended to contribute to the knowledge of these problems and of the risk factors associated with the activity.

Expected period for your participation

It will take approximately fifteen minutes of your time to respond to the mentioned structured questionnaire. This research work does not interfere with the actions of the attending physician.

1. Costs / refunds

This analysis is free of charge. You will not receive compensation or payment for your participation.

2. Risks and annoyances

The blood extraction will be carried out by qualified personnel, the procedure is generally painless and does not cause damage. Sometimes it can cause a slight discomfort when performed. If a small hematoma appears, it will be resolved by local compression and application of ice.

PPD consists of an injection performed in the front of the forearm, where the area of the papule is marked with a circle. It is not contraindicated in pregnant women or immunosuppressed people. Only the induration will be measured and not the erythema produced, considering the diameter of the induration transverse to the long axis of the forearm.

X-ray is a non-invasive medical test that helps diagnose and treat medical conditions. X-ray imaging involves exposing a part of the body to a small dose of ionizing radiation to produce images of the inside of the body. This procedure has no side effects but is contraindicated for pregnant women.

3. Possible benefits

If you give your Consent to participate in this study, direct medical benefits will not be contemplated but you will collaborate with the health authorities to develop activities for the surveillance and prevention of zoonoses as well as lead from hunting ammunition in game meat. Likewise, if you have any pathology, you will be notified in a personal way and you will be given the appropriate support to achieve your correct medical care. For this, you will be contacted with qualified health personnel who will indicate and ensure the corresponding treatment.

4. Patient involvement

Your participation is voluntary and you must sign this Consent Form. This will not affect the medical care you receive. Non-participation will not imply any loss of your rights and will not generate any type of sanction or penalty towards you.

5. Confidentiality of records

The confidentiality and privacy of your personal record will be respected in compliance with the provisions of National Law 25,326 on the Protection of Personal Data. A numerical code will be assigned to each of the participants.

The following groups may access or obtain a copy of the research records for the purpose of quality control or study of the data collected

- *Ministerio de Salud de la Provincia de Buenos Aires*
- *Administración Nacional de Alimentos, Medicamentos y Tecnología Médica*
- *Corresponding Ethics Committee(s)*

By signing this document, you give your Consent so that the personnel of the institutions mentioned above can carry out a review and authorize access to your registry.

6. Contacts

At any time during the course of the study, you may contact the professionals named above whose phone number is on the first page to get answers to any questions or concerns you may have about your rights as a research participant or about the analysis results.

This research work has been evaluated by the *Comité Central de Bioética en la Práctica y en la Investigación Biomédica* on August 01 2019. If you have any questions related to your rights as a participant in this study, you can also contact this committee through email (ccbioetica.entrerios@gmail.com) or telephone (+54 3434209607).

7. Consent / signature

a) Participant

Statement that you understand that you can withdraw without prejudice of any kind (at FCI)

I confirm that I have had sufficient time to read and consider the information presented above and to seek advice if I so desire. In addition, I state that I was sufficiently informed by the researcher whose name appears, the technical language used was clarified to my satisfaction and I received the appropriate answers to the questions I asked. I have read this Informed Consent Form, I understand its content and I freely and voluntarily give my consent to participate in the research study under the conditions detailed in this document, knowing that if I wish, I can withdraw without prejudice of any kind.

Name and surname

ID number

Signature

Date

b) Witness

The undersigned below states that he/she has been present during the act of explanation, presentation and taking of Consent and that, in my opinion, the participant in this study has received a complete, detailed

explanation and clearly understands the nature and potential benefit for the community linked to the *Plan de Control de Mamíferos Exóticos Invasores* from El Palmar National Park to participate in this study.

Name and surname

ID number

Signature

Date

c) Researcher

I accept my responsibility for the care and well-being of the aforementioned patient, I promise to respect their rights and wishes, and to develop this study in compliance with the general guidelines and regulations of Good Clinical Practice.

I have carefully explained to the participants the characteristics of this research study. I hereby certify that, to the best of my knowledge, the participant understands the nature and scope of the study and does not present additional risk.

Name and surname

ID number

Signature

Date

Online Resource 3 Questionnaire for dog owners.

DATA REGISTRATION QUESTIONNAIRE

Identification number: 1 Corresponds to: EDTA tube n°: 1

Heparin tube n°: 1

Cryovial n°: Pb 1 and ALAD 1

Slides n°: 1.1, 1.2 and 1.3

Ziploc bag n°: 1

Owner: _____

Name: _____ Breed: _____ Age: _____ Sex: _____

Weight: _____ kg Body condition: emaciated – thin – good – exceeded – obese

Vaccines: _____

Usual diet: _____

Do you feed it with game products? - Meat YES / NO

- Offal YES / NO

- Do you cook them? YES / NO

How often do you feed it with game products?

- High frequency (10 to 14 meals per week)

- Medium frequency (5 to 9 meals per week)

- Low frequency (1 to 4 meals per week)

- Other (specify): _____

When was the last time you fed it with game products? _____

Has this dog participated in hunting activities? YES / NO

If yes, when was the last time it participated? _____

If yes, do you remember if it has been hurt? YES / NO

Other relevant data: _____

Online Resource 4. Structured questionnaire for participants

This interview contributes to the following projects: **“Contributions to the Plan de Control de Mamíferos Exóticos Invasores (PCMEI) from El Palmar National Park: zoonoses survey in wild boar (*Sus scrofa*) and axis deer (*Axis axis*) and impact on public health” and “Public health risks: lead from hunting ammunition in game meat”.**

Your answers, used in complete privacy, will help us to complement the studies related to these topics that have been carried out in El Palmar National Park since 2017.

Thank you very much for your collaboration, which is essential for this work carried out by park staff, Universidad Nacional del Centro de la Provincia de Buenos Aires and CONICET.

Date: / /

PERSONAL INFORMATION

Name: _____ **Date of birth:** _____

Address: _____ **Town:** _____

Telephone: _____ **Email:** _____

GENERAL INFORMATION

1. **Do you have contact with the following animals? Circle the type of contact.**

	YES	NO	Type of contact	Comments
Wild boar			Hunt - Slaughter - Cook - Consumption	
Axis deer			Hunt - Slaughter - Cook - Consumption	
Cow			Slaughter - Cook - Consumption – Parturition assistance	
Domestic pig			Slaughter - Cook - Consumption - Parturition assistance	
Dog			Coexistence	
Cat			Coexistence	
Other				

2. *Indicate with a cross the task that you perform and for which you are related to the PCMEI:*

A. Park ranger or park staff

B. Hunter

C. Hunter companion

D. Others (specify): _____

If the answer was Hunter, indicate:

Watchtower number: _____

HANDLING

1. *During slaughter, you wear:*

<i>Gloves</i>	<i>Yes</i>	<i>No</i>	<i>Boots</i>	<i>Yes</i>	<i>No</i>	<i>Overalls</i>	<i>Yes</i>	<i>No</i>
<i>Face mask</i>	<i>Yes</i>	<i>No</i>	<i>Glasses</i>	<i>Yes</i>	<i>No</i>	<i>Others (specify):</i> _____		

2. *During slaughter, do you remember cutting yourself? Yes No*

If your answer is Yes, how long ago? _____

3. After each hunting activity, how do you clean ...

knives and other elements? _____

your truck? _____

your clothes? _____

4. Do you cut game meat on the same table that you use for other foods? Yes No

5. Name the 3 most frequent meals where game meat is used.

6. ¿ How often do you eat game meat?

High frequency (10 to 14 meals per week)

Medium (5 - 9 meals per week)

Low (1 - 4 meals per week)

Other (specify): _____

7. When was the last time you ate game meat?

8. Do you make sausages with game meat? Yes No

If the answer is Yes, of what animal species? _____

Do you consume them? Yes No (specify): _____

9. During slaughter, do you discard the meat of the bullet-struck portions? Yes No

If the answer is Yes, what do you do with it? _____

If the answer is No, what do you do with that meat?

WASTE

1. How do you discard the leftover game pieces?

2. Do you feed your dogs with game meat?

Viscera Yes No

Meat Yes No

Do you cook game meat before feeding dogs? Yes No

3. If your answer is Yes, how often do you feed your dogs with game meat?

High frequency (10 to 14 meals per week)

Medium (5 - 9 meals per week)

Low (1 - 4 meals per week)

Other (specify): _____

4. **Have you participated in dog-hunting? Yes No**

If Yes, have they been injured by the activity? _____

5. **Do you feed other animals with game meat? Yes No**

Which? _____

Viscera Yes No

Meat Yes No

Do you cook them? Yes No

CLINICAL DATA

1. **During slaughter, have you observed suspicious lesions of any disease in the last 10 years? Yes
No**

If the answer is Yes, in which species? _____

When (year)? _____

What did you do with those pieces of game meat? _____

Did you report it? _____

2. Did you get tested for any of these zoonoses in the last 10 years?

Brucellosis Yes No

Leptospirosis Yes No

Tuberculosis Yes No

Trichinellosis Yes No

3. During your lifetime, have you been ill with any of the aforementioned zoonoses? Yes No

If your answer is Yes, which one? _____

Do you remember when? _____

Do you know the cause? _____

4. Do you know someone who became ill with any of the aforementioned zoonoses? Yes No

Which one? _____

5. Have you ever used non-lead bullets? Yes No

If your answer is Yes, which ones? _____

To hunt which species? _____

Table S1. Summary of epidemiological data and artificial digestion results for the detection of *Trichinella* spp. larvae in wild boar (*Sus scrofa*) hunted in El Palmar National Park, Entre Ríos, Argentina, 2018-2019.

	Positive		Total	
	<i>n</i> (%)		<i>N</i>	
Sample size	5 (10%)		49	
Sex				
Female	3 (17%)		18	
Male	2 (6%)		31	
Age category				
Juvenile	1 (8%)		12	
Young adult	3 (20%)		15	
Mature adult	0		7	
Older adult	1 (25%)		4	
Sampling Area				
North	2 (9%)		22	
South	3 (11%)		27	
	Negative		Positive	
	mean ± S.E.	<i>N</i>	mean ± S.E.	<i>N</i>
Weight (kg)	39.48 ± 2.804	31	44.80 ± 9.609	5
Body length (cm)	110 ± 2.833	31	111.6 ± 9.928	5

Table S2. Summary of epidemiological data and seropositivity to *Trichinella* spp. in the dogs (*Canis familiaris*) fed game meat and offal from culled wild boar (*Sus scrofa*) from El Palmar National Park, Entre Ríos, Argentina, or from private lands neighboring the park (2018-2019).

	Positive	Total
	n (%)	N
Sample size	12 (35%)	34
Sex		
Female	7 (47%)	15
Male	5 (26%)	19
Age category		
Pup (≤ 6 mo)	0	3
Young (6–18 mo)	1 (33%)	3
Adult (> 18 mos)	11 (39%)	28
Dog owner		
A	1 (20%)	5
B	4 (27%)	15
C	3 (50%)	6
D	3 (75%)	4
E	1 (25%)	4
Frequency of feeding with game meat		
High (10–14 times a wk)	4 (27%)	15
Medium (5–9 times a wk)	2 (22%)	9
Low (1–4 times a wk)	3 (50%)	6
Very low (once every 15 d)	3 (75%)	4
Time since last feeding with game prior to sampling:		
Very recent (1 d prior)	3 (25%)	12
Recent (approximately 1 wk prior)	1 (20%)	5
Not recent (approximately 2 wk prior)	6 (60%)	10
Feeding with cooked meat		
Sometimes	7 (33%)	21
Never	5 (38%)	13
	Negative	Positive
	mean \pm S.E.	n mean \pm S.E. n
Weight (kg)	29.09 \pm 225	2 24.17 \pm 104 12

