Comparative Bacteriological Study of Two Wild Boar Populations in Sierra Morena (Jaén, Spain)

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Abstract

The aim of this study was to investigate the presence of various bacterial species in the wild boar populations of Lugar Nuevo and Selladores-Contadero woodlands from Sierra Morena (Spain). Bacteriological analyses were carried out on a total of 229 wild boar individuals hunted in the period 2000-2003 in eleven experimental plots which are representative for the different biotopes of the area. The following species were detected: Brucella ovis, Clostridium sp., Corynebacterium sp., Streptococcus pneumoniae, Escherichia coli, Mycobacterium bovis, Mycobacterium avium subsp. paratuberculosis, Mannheimia haemolytica, Pasteurella multocida, Pseudomonas aeruginosa, Chlamydophila psittaci, Staphylococcus aureus and Staphylococcus sp. and the bacterial prevalence was estimated for each of them. The results provide useful indications of the health status of wild boar in both locations and highlight the potential of the wild boar populations to act as biological reservoirs of certain microorganisms that can be passed onto other vertebrate wild animals and humans.

Keywords: wild boar, post-mortem, bacteriology, infection prevalence

Introduction

Several studies on specific aspects of the bacteriology of wild boar have been recently published by the outstanding authors Acevedo-Whitehouse et al. (2005), Gortazar et al. (2003, 2005), Vicente et al. (2002, 2005, 2006), Segales et al. (2005).

According to Gortazar (2007), there are a number of diseases to be considered in the wild boar. It is the case for tuberculosis by strains of bovine and caprine origin included in the Mycobacterium tuberculosis Complex, widespread in game preserves and hunting-designated areas of Southern Spain and probably linked to a high density of ungulates in this territory. Brucellosis is also common as a result of the role of reservoirs of Brucella spp played by wild animals, which convey the potential infection to large domestic animals with significant implications in public health. Salmonellosis, a well known zoonosis has to be taken into account too, especially in those outbreaks with high mortality rates that can be wrongly diagnosed as either African swine fever or Hog cholera, both viral diseases. Analogous interest should be focused on other transmissible agents such as Escherichia coli and Campylobacter spp., present in the digestive tract of wild boars, Chlamydophila psittaci and Erysipelothrix rhusiopathiae, aetiological agent responsible of Swine erysipelas.

This study, subsidized by the Autonomous Body, Spanish National Parks, and carried out from 2000 to 2003, was designed to provide a thorough knowledge of the various bacterial species isolated from wild boar populations in Lugar Nuevo and Selladores-Contadero.

Materials and methods

The woodlands of Lugar Nuevo (9,233.55 hectares) and Selladores-Contadero (10,455.89 hectares), 60 km away from each other and managed by the autonomous body, Spanish National Parks, lie in the northwest of the province of Jaén, in a peripheral area of the southern side of Sierra Morena. The latter is a mountain range that forms the link between the Castilian Plateau and the River Guadalquivir Valley. Slopes are moderate to high and sunny. Most soils showed neutral or slightly acid pH values and medium texture. The climate can be classified as of Mediterranean type with mild winters, extremely dry and hot summers and a moderate rainfall regime (approx. 600 mm), characterized by an uneven distribution of rain throughout the year. The indigenous flora consists of the
standard vegetation of Mediterranean forests and reforested pinewoods.

**Land plot division**

Due to the difficulties associated with the size of the covered area under investigation, a subsequent division of land plots with similar or equivalent features in their habitats was performed, so that valid results could be obtained in different seasons.

**Tab. 1. Area and altitude of the studied plots (LN=Lugar Nuevo; SC=Selladores-Contadero)**

| Land plot identification          | Area (hectares) | Highest point above sea level (metres) | Lowest point above sea level (metres) |
|----------------------------------|-----------------|--------------------------------------|---------------------------------------|
| Coscojal (P0LN)                  | 455.6           | 197                                  | 706                                   |
| Fuencubierta y Solana (P1LN)     | 1800.31         | 235                                  | 648                                   |
| Estrecho (P2LN)                  | 1138.09         | 241                                  | 652                                   |
| Valdelipe (P3LN)                 | 1445.24         | 199                                  | 631                                   |
| Navalpacho (P4LN)                | 1372.73         | 214                                  | 559                                   |
| Centenera-Piedra bermeja (P5LN)  | 1654.95         | 199                                  | 674                                   |
| Cabrera (P6LN)                   | 1366.64         | 199                                  | 674                                   |
| Selladores (P1SC)                | 2700.39         | 372                                  | 990                                   |
| Navalcardo-Vallejones (P2SC)     | 2595.45         | 445                                  | 767                                   |
| Contadero (P3SC)                 | 3062.92         | 337                                  | 779                                   |
| Lentisquillo (P4SC)              | 2097.13         | 423                                  | 780                                   |

**Tab. 2. Vegetation characteristics of the Lugar Nuevo area**

| Vegetation type                                      | Area (ha) |
|------------------------------------------------------|-----------|
| Standard Mediterranean mountain area vegetation      | 0.59      |
| Meadow/pasture including groves of holm-oaks         | 50.55     |
| Reforested pinewood *P. pinea, P. pinaster*          | 394.97    |
| Riparian vegetation                                  | 9.49      |
| Thicket                                              | 0.00      |
| Grassland                                            | 0.00      |
| Mastic Tree forest and Olive woods                    | 0.00      |
| Other                                                | 0.00      |
| Total                                                | 455.6     |

**Tab. 3. Vegetation characteristics of the Selladores-Contadero area**

| Vegetation type                                      | Area (ha) |
|------------------------------------------------------|-----------|
| Standard Mediterranean mountain area vegetation      | 736.27    |
| Meadow/pasture including groves of holm-oaks         | 0.00      |
| Reforested pinewood *P. pinea, P. pinaster*          | 927.15    |
| Cultivated area                                      | 0.00      |
| Thicket                                              | 369.36    |
| Grassland                                            | 54.44     |
| Mastic Tree forest and Olive woods                    | 748.29    |
| Other                                                | 0.58      |
| Total                                                | 2836.08   |

A division of woodlands in eleven plots (seven in the woodlands of Lugar Nuevo and four in those of Selladores-Contadero) following patterns of homogeneity of habitat and behavioural basis was made so as to make proper investigation feasible and statistically valid. Tab. 1, 2 and 3 depict some of the studied parameters (i.e. total surface, perimeter, lowest and highest points above the sea level, vegetation type).

**Duration of sanitary on-spot checks and sampling**

Collection of 229 samples (142 from Lugar Nuevo and 87 from Selladores-Contadero) took place from 2000 to 2003. Animals were randomly selected and immediate post-mortem examinations were done at local sanitary units/facilities equipped for that specific purpose. Specimens were aseptically submitted for analysis within 2-5 hours after death.
Sample collection and processing

A system of protocols containing relevant information was used. Details reflected a vast range of indicators, such as:

1. Identification chart-Sample number, plot identification, sex, estimated age, carcass weight, height to the withers, head-rump length, thoracic circumference.
2. Macroscopic study-External abnormalities and anatomopathological findings in offals and tissues of carcasses (lymph nodes, abscesses, presence of endoparasites).
3. Sampling for bacteriological tests - Representative portions of lung, spleen, liver, intestines, lymph nodes as well as abscesses.
4. Laboratory diagnosis
   a. Direct microscopy. Diverse techniques were used alone or in conjunction with cultural methods to stain smears from lesions and specimens. This is the case for the Gram stain, acid-fast stains such as Thiam-Hok and Kinyoun besides those like either the Giemsa stain or the Stamp stain capable of revealing Brucella spp. and Chlamyphila.psittaci.
   b. Isolation and identification of causative agents. Inoculation of liquid media (Peptone Broth, Brain Heart Infusion Broth) with specimens (lung, spleen, intestinal mucosa and/or lymph nodes) was carried out in those samples positive to reactions specified in section 4a. The isolation of strains was performed through plate streaking of selective agars (Columbia Agar enriched with 5% of sheep blood, Endo-Columbia Agar to which 5% sheep blood was incorporated, Triple Sugar Iron Agar, and MacConkey Agar) from those cultures in liquid media showing turbidity as a way of demonstrating growth. For identification of species CTA medium with cystine and trypticase as well as commercial strips (API 20 E, API Staph, API ID 32 Staph, API 20 A, API 20 Strept and API CORYNE) were used. Preparation of Lowenstein-Jensen medium required supplementation with either thiophen-2-carbolic acid hydrazide (TCH) or mycobactin, as specified by Fuentes Pérez (1988). The choice of ready-to-use media, was based on the results obtained in the Gram reaction. All products, including the miniaturized identification systems were supplied by Bio-Mérieux.

Results and discussion

The results of the 229 analyzed samples are summarized in Tab. 4 to 11.

While in 67% of the sampled population at least one microorganism was isolated, in 33% of the population a lack of presence of viable strains was evident (Tab. 4).

The following species were detected: Brucella ovis, Clostridium spp, Corynebacterium spp., Streptococcus pneumoniae, Escherichia coli, Mycobacterium bovis, Mycobacterium avium subsp. paratuberculosis, Mannheimia haemolytica, Pasteurella multocida, Pseudomonas aeruginosa, Chlamyphila.psittaci (PLT), Staphylococcus aureus and Staphylococcus spp.

A wider variety of bacterial pathogens is clearly observed during May, June and July (Tab. 6).

"Fuencubierta-Solana" and "Valdelipe" in Lugar Nuevo and "Selladores" in Selladores-Contadero were the land plots data depicting populations with a higher infection rate (Tab. 7 and 8).

Chlamyphila.psittaci and Mycobacterium avium subsp. paratuberculosis (MAP) affect wild boars of all ages and conditions.

Tab. 4. Sample distribution, reflecting positive and negative cases, for each location

| Location          | Number of samples | Negative samples | Positive samples |
|-------------------|-------------------|------------------|------------------|
| Monte             |                   |                  |                  |
| Lugar Nuevo       | 142               | 45               | 97               |
| Selladores-Contadero | 87            | 30               | 57               |
| Total             | 229               | 75               | 154              |

Tab. 5. Bacterial species detected in the studied areas

| Bacterial species                          | Positive cases on its own | Positive cases associated with other species | Total |
|--------------------------------------------|---------------------------|---------------------------------------------|-------|
| Mycobacterium avium subsp. Paratuberculosis (MAP) | 4                         | 11                                          | 15    |
| Staphylococcus spp.                        | 0                         | 1                                           | 1     |
| Mycobacterium bovis                        | 0                         | 0                                           | 0     |
| Streptococcus pneumoniae                   | 0                         | 5                                           | 5     |
| Mannheimia haemolytica                     | 2                         | 5                                           | 7     |
| Pasteurella multocida                      | 0                         | 1                                           | 1     |
| Pseudomonas aeruginosa                     | 0                         | 2                                           | 2     |
| Brucella ovis                              | 0                         | 0                                           | 0     |
| Corynebacterium spp.                       | 0                         | 0                                           | 0     |
| Escherichia coli                           | 3                         | 3                                           | 6     |
| PLT                                         | 18                        | 52                                          | 70    |
| Staphylococcus aureus                      | 0                         | 0                                           | 0     |
| Clostridium spp.                           | 0                         | 0                                           | 0     |

| Lugar Nuevo       |                   |                  |                  |
| Selladores-Contadero |                 |                  |                  |
| Mycobacterium avium subsp. Paratuberculosis (MAP) | 4                         | 11                                          | 15    |
| Staphylococcus spp.                        | 0                         | 1                                           | 1     |
| Mycobacterium bovis                        | 0                         | 0                                           | 0     |
| Streptococcus pneumoniae                   | 0                         | 5                                           | 5     |
| Mannheimia haemolytica                     | 2                         | 5                                           | 7     |
| Pasteurella multocida                      | 0                         | 1                                           | 1     |
| Pseudomonas aeruginosa                     | 0                         | 2                                           | 2     |
| Brucella ovis                              | 0                         | 0                                           | 0     |
| Corynebacterium spp.                       | 0                         | 0                                           | 0     |
| Escherichia coli                           | 3                         | 1                                           | 4     |
| PLT                                         | 15                        | 28                                          | 43    |
| Staphylococcus aureus                      | 0                         | 1                                           | 1     |
| Clostridium spp.                           | 1                         | 0                                           | 1     |
Mycobacterium avium subsp. Paratuberculosis (MAP) is present in both woodlands with an equivalent prevalence of 19%. Mycobacterium bovis is not relevant as solely detected in one sample. Staphylococcus sp. and S. aureus show a low prevalence with a slightly higher value in Selladores-Contadero. Brucella ovis and Mycobacterium bovis are not significant having only been isolated in one case in Lugar Nuevo.

Streptococcus pneumoniae is present in both woodlands with a similar prevalence rate of 6%, whilst Mannheimia haemolytica shows a slightly higher prevalence in Lugar Nuevo.

Pasteurella multocida has a significantly lower prevalence rate with higher values in Lugar Nuevo (2.24%) and absence in Selladores-Contadero (0%). Pseudomonas aeruginosa shows equivalent prevalence rates of 0.87% in both locations.

In the case of Corynebacterium spp. remarkable differences can be seen between prevalence rates in Lugar Nuevo (4.83%) and absence in Selladores-Contadero (0%). Clostridium spp. is only present in Selladores-Contadero.
Tab. 8. Plot distribution and level of presence (%) of bacterial species in different locations of Selladores-Contadero

| Bacterial species                                      | Number of positive cases | Level of presence (%) |
|--------------------------------------------------------|--------------------------|-----------------------|
|                                                        | P1 SC | P2 SC | P3 SC | P4 SC | Total |                         |
| *Mycobacterium avium* subsp. *Paratuberculosis* (MAP)  | 4     | 0     | 5     | 6     | 15    | 18.75                  |
| *Staphylococcus* spp.                                  | 0     | 0     | 1     | 0     | 1     | 1.25                   |
| *Mycobacterium bovis*                                  | 0     | 0     | 0     | 0     | 0     | 0                      |
| *Streptococcus pneumoniae*                             | 2     | 1     | 1     | 1     | 5     | 6.25                   |
| *Mannheimia haemolytica*                               | 5     | 0     | 1     | 1     | 7     | 8.75                   |
| *Pasteurella multocida*                                | 0     | 1     | 0     | 0     | 1     | 1.25                   |
| *Pseudomonas aeruginosa*                               | 1     | 0     | 1     | 0     | 2     | 2.50                   |
| *Brucella ovis*                                        | 0     | 0     | 0     | 0     | 0     | 0                      |
| *Corynebacterium* spp.                                 | 0     | 2     | 1     | 1     | 4     | 5.00                   |
| *Escherichia coli*                                     | 0     | 1     | 0     | 0     | 1     | 1.25                   |
| *Paeturella multocida*                                 | 0     | 1     | 0     | 0     | 1     | 1.25                   |
| PLT                                                    | 15    | 9     | 10    | 9     | 43    | 53.75                  |
| Total number of isolations                             | 27    | 15    | 20    | 18    | 80    | 100                    |

Tab. 9. Summary of bacterial prevalence rates (%) in the different age cohorts (both locations)

| Bacterial species                                      | Months       | 0-12 | 13-24 | 25-36 | 37-48 | 49-60 | 61-72 | 73  |
|--------------------------------------------------------|--------------|------|-------|-------|-------|-------|-------|-----|
| *Mycobacterium avium* subsp. *Paratuberculosis* (MAP)  | 14.29        | 18.03| 12.50 | 27.27 | 11.54 | 33.33 | 33.33 |
| *Staphylococcus* spp.                                  | 1.79         | 0.00 | 0.00  | 3.03  | 0.00  | 0.00  | 0.00  |
| *Mycobacterium bovis*                                  | 0.00         | 0.00 | 3.13  | 0.00  | 0.00  | 0.00  | 0.00  |
| *Streptococcus pneumoniae*                             | 3.57         | 8.20 | 9.38  | 3.03  | 3.85  | 11.11 | 0.00  |
| *Mannheimia haemolytica*                               | 12.50        | 13.11| 12.50 | 0.00  | 3.85  | 0.00  | 16.67 |
| *Pasteurella multocida*                                | 5.36         | 0.00 | 6.06  | 0.00  | 0.00  | 8.33  |       |
| *Pseudomonas aeruginosa*                               | 3.57         | 3.28 | 3.13  | 3.03  | 0.00  | 0.00  | 0.00  |
| *Brucella ovis*                                        | 0.00         | 0.00 | 0.00  | 3.85  | 0.00  | 0.00  | 0.00  |
| *Corynebacterium* spp.                                 | 3.57         | 3.28 | 3.13  | 3.03  | 0.00  | 0.00  | 8.33  |
| *Escherichia coli*                                     | 5.36         | 3.28 | 12.50 | 3.03  | 0.00  | 0.00  | 0.00  |
| *Staphylococcus aureus*                                | 0.00         | 0.00 | 0.00  | 3.03  | 0.00  | 0.00  | 8.33  |
| *Clostridium* spp.                                     | 0.00         | 0.00 | 3.03  | 0.00  | 0.00  | 0.00  | 0.00  |
| PLT                                                    | 42.86        | 59.02| 46.88 | 42.42 | 53.85 | 44.44 | 50.00 |
| Negative samples                                       | 32.12        | 27.87| 34.38 | 30.30 | 42.31 | 55.56 | 25.00 |

Tab. 10. Bacterial prevalence rate (percentage related to total) in the wild boar population of Lugar Nuevo

| Bacterial species                                      | P0 LN | P1 LN | P2 LN | P3 LN | P4 LN | P5 LN | P6 LN | Total | Prevalence (%) |
|--------------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|----------------|
| *Mycobacterium avium* subsp. *Paratuberculosis* (MAP)  | 0     | 7     | 7     | 3     | 4     | 5     | 1     | 27    | 19.01         |
| *Staphylococcus* spp.                                  | 0     | 0     | 0     | 1     | 0     | 0     | 0     | 1     | 0.70          |
| *Mycobacterium bovis*                                  | 0     | 1     | 0     | 0     | 0     | 0     | 0     | 1     | 0.70          |
| *Streptococcus pneumoniae*                             | 1     | 0     | 2     | 1     | 2     | 0     | 8     | 5     | 5.63          |
| *Mannheimia haemolytica*                               | 1     | 4     | 3     | 2     | 3     | 1     | 1     | 15    | 10.56         |
| *Pasteurella multocida*                                | 0     | 1     | 0     | 3     | 0     | 1     | 0     | 5     | 3.52          |
| *Pseudomonas aeruginosa*                               | 0     | 2     | 0     | 1     | 0     | 1     | 0     | 4     | 2.82          |
| *Brucella ovis*                                        | 0     | 0     | 1     | 0     | 0     | 0     | 0     | 1     | 0.70          |
| *Corynebacterium* spp.                                 | 0     | 1     | 1     | 2     | 1     | 0     | 2     | 7     | 4.93          |
| *Escherichia coli*                                     | 0     | 3     | 0     | 0     | 2     | 1     | 0     | 6     | 4.23          |
| PLT                                                    | 3     | 15    | 7     | 15    | 9     | 13    | 9     | 70    | 49.30         |
| Total number of isolations                             | 5     | 34    | 21    | 28    | 21    | 24    | 12    | 145   |               |
| Negative samples                                       | 2     | 7     | 8     | 7     | 8     | 7     | 6     | 45    |               |
Conclusions

From this study it can be stated that bacterial species present in domestic swine are similarly distributed in boar populations from Lugar Nuevo and Selladores-Contadero, acting as biological reservoirs of certain microorganisms that can be passed onto other vertebrate wild animals and humans. So, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) which is not relevant in adult boars, may well be pointed out as major pathogen in certain ungulates such as red deer (*Cervus elaphus*), causing clinical conditions closely resembling that of tuberculosis by *Mycobacterium bovis*.

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Tabla 11. Prevalencia de bacterias en el colectivo de cerdos salvajes de Selladores-Contadero

| Bacterial species                        | P1 SC | P2 SC | P3 SC | P4 SC | Total | Prevalence(%) |
|-----------------------------------------|-------|-------|-------|-------|-------|---------------|
| *Mycobacterium avium* subsp. *paratuberculosis* (MAP) | 4     | 0     | 5     | 6     | 15    | 17,24         |
| *Staphylococcus* spp.                   | 0     | 0     | 1     | 0     | 1     | 1.15          |
| *Mycobacterium bovis*                   | 0     | 0     | 0     | 0     | 0     | 0             |
| *Streptococcus pneumoniae*              | 2     | 1     | 1     | 1     | 5     | 5.75          |
| *Pasteurella multocida*                 | 5     | 0     | 0     | 1     | 7     | 8.05          |
| *Pseudomonas aeruginosa*                | 0     | 0     | 0     | 0     | 0     | 0             |
| *Brucella ovis*                         | 0     | 0     | 0     | 0     | 0     | 0             |
| *Corynebacterium* spp.                  | 0     | 0     | 0     | 0     | 0     | 0             |
| *Escherichia coli*                      | 0     | 2     | 1     | 1     | 4     | 4.60          |
| *Staphylococcus aureus*                 | 0     | 1     | 0     | 0     | 1     | 1.15          |
| *Clostridium* spp.                      | 0     | 1     | 0     | 0     | 1     | 1.15          |
| **PLT**                                 |       |       |       |       |       | **49.43**     |
| Total number of isolations              | 27    | 15    | 20    | 18    | 80    | 49.43         |
| Negative samples                        | 6     | 6     | 9     | 9     | 30    | 30            |