

## 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), Gortázar *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)						
	P0LN	P1LN	P2LN	P3LN	P4LN	P5LN	P6LN
Standard Mediterranean mountain area vegetation	0,59	94,68	106,99	180,90	445,12	1161,40	146,33
Meadow/pasture including groves of holm-oaks	50,55	272,18	255,31	48,81	155,36	20,64	34,22
Reforested pinewood <i>P. pinea</i> , <i>P. pinaster</i>	394,97	728,66	726,43	1129,4	479,33	382,57	1158,7
Riparian vegetation	9,49	0,13	24,68	3,81	77,02	26,02	0,00
Thicket	0,00	183,36	0,78	11,51	11,51	0,00	0,00
Grassland	0,00	25,77	0,00	63,18	27,18	47,62	27,32
Mastic Tree forest and Olive woods	0,00	495,52	6,79	0,00	171,12	0,00	0,00
Other	0,00	0,00	17,11	7,61	6,09	16,65	0,03
Total	455,6	1800,3	1138,1	1445,2	1372,7	1654,9	1366,6

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.

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

Vegetation type	Area (ha)			
	P1SC	P2SC	P3SC	P4SC
Standard Mediterranean mountain area vegetation	736,27	916,79	310,09	324,49
Meadow/pasture including groves of holm-oaks	0,00	77,52	0,00	0,00
Reforested pinewood <i>P. pinea</i> , <i>P. pinaster</i>	927,15	1470,26	1427,81	1369,55
Cultivated area	0,00	0,00	3,91	0,00
Thicket	369,36	7,04	102,66	94,20
Grassland	54,44	12,44	170,21	29,38
Mastic Tree forest& Olive woods	748,29	40,85	808,69	21,10
Other	0,58	15,57	174,68	242,59
Total	2836,08	2540,47	2998,05	2081,31

### 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, corpse weight, 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 *Chlamydomphila psittaci*.

b. *Isolation and identification of causative agents*. Inoculation of liquid media (Peptone Broth, Brain Heart Infusion Broth) with specimens (lung, liver, 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 Strep and API CORYNE) were used. Preparation of Lowenstein-Jensen medium required supplementation with either tiophen-2-carbonic 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*

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

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

*haemolytica*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Chlamydomphila 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).

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

Tab. 5. Bacterial species detected in the studied areas

Bacterial species	Positive cases on its own	Positive cases associated with other species	Total
Lugar Nuevo			
<i>Mycobacterium avium</i> subsp.			
<i>Paratuberculosis</i> (MAP)	10	17	27
<i>Staphylococcus</i> spp.	1	0	1
<i>Mycobacterium bovis</i>	0	1	1
<i>Streptococcus pneumoniae</i>	2	6	8
<i>Mannheimia haemolytica</i>	2	13	15
<i>Pasteurella multocida</i>	0	5	5
<i>Pseudomonas aeruginosa</i>	1	3	4
<i>Brucella ovis</i>	0	1	1
<i>Corynebacterium</i> spp.	2	5	7
<i>Escherichia coli</i>	3	3	6
PLT	18	52	70
<i>Staphylococcus aureus</i>	0	0	0
<i>Clostridium</i> spp.	0	0	0
Selladores-Contadero			
<i>Mycobacterium avium</i> subsp.			
<i>Paratuberculosis</i> (MAP)	4	11	15
<i>Staphylococcus</i> spp.	0	1	1
<i>Mycobacterium bovis</i>	0	0	0
<i>Streptococcus pneumoniae</i>	0	5	5
<i>Mannheimia haemolytica</i>	2	5	7
<i>Pasteurella multocida</i>	0	1	1
<i>Pseudomonas aeruginosa</i>	0	2	2
<i>Brucella ovis</i>	0	0	0
<i>Corynebacterium</i> spp.	0	0	0
<i>Escherichia coli</i>	3	1	4
PLT	15	28	43
<i>Staphylococcus aureus</i>	0	1	1
<i>Clostridium</i> spp.	1	0	1

Tab. 6. Presence of different bacterial species along the year (both locations)

Bacterial species	Month												Total
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
<i>Mycobacterium avium</i> subsp. <i>Paratuberculosis</i> (MAP)	3	2	4	4	1	2	8	5	4	5	4	0	42
<i>Staphylococcus</i> spp.	0	0	0	0	0	1	1	0	0	0	0	0	2
<i>Mycobacterium bovis</i>	0	0	0	0	0	0	0	1	0	0	0	0	1
<i>Streptococcus pneumoniae</i>	0	1	1	1	5	1	1	1	1	1	0	0	13
<i>Mannheimia haemolytica</i>	1	1	1	2	3	6	2	2	1	1	1	1	22
<i>Pasteurella multocida</i>	0	0	1	0	0	0	1	0	1	2	1	0	6
<i>Pseudomonas aeruginosa</i>	1	0	0	0	1	2	1	1	0	0	0	0	6
<i>Brucella ovis</i>	0	0	0	0	0	0	0	0	0	0	1	0	1
<i>Corynebacterium</i> spp.	0	1	0	0	1	1	1	1	0	1	0	1	7
<i>Escherichia coli</i>	2	0	1	0	1	2	1	1	0	1	1	0	10
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	1	0	0	0	0	0	1
<i>Clostridium</i> spp	0	0	0	0	0	0	0	0	0	0	1	0	1
PLT	8	7	11	13	11	13	9	6	11	8	10	6	113
Total number of isolations													
	15	12	19	20	23	28	26	18	18	19	19	8	225
N° of negative samples													
	10	5	6	7	6	3	7	6	6	7	9	3	75
Total number of analyzed samples													
	24	14	17	20	20	22	25	18	18	19	21	11	229
Monthly infection prevalence													
*	58	64	65	65	70	86	72	67	67	63	57	73	67

\*Monthly infection prevalence rate, calculated by dividing the number of positive cases by the total number of investigated animals. It is not a biohazard indicator but a monthly prevalence rate

Tab. 7. Plot distribution and level of presence (%) of bacterial species in different locations of Lugar Nuevo

Bacterial species	Number of positive cases								Level of presence(%)
	P0 LN	P1 LN	P2 LN	P3 LN	P4 LN	P5 LN	P6 LN	Total	
<i>Mycobacterium avium</i> subsp. <i>Paratuberculosis</i> (MAP)	0	7	7	3	4	5	1	27	18,62
<i>Staphylococcus</i> spp.	0	0	0	1	0	0	0	1	0,69
<i>Mycobacterium bovis</i>	0	1	0	0	0	0	0	1	0,69
<i>Streptococcus pneumoniae</i>	1	0	2	1	2	2	0	8	5,52
<i>Mannheimia haemolytica</i>	1	4	3	2	3	1	1	15	10,34
<i>Pasteurella multocida</i>	0	1	0	3	0	1	0	5	3,54
<i>Pseudomonas aeruginosa</i>	0	2	0	1	0	1	0	4	2,76
<i>Brucella ovis</i>	0	0	1	0	0	0	0	1	0,69
<i>Corynebacterium</i> spp.	0	1	1	2	1	0	2	7	4,83
<i>Escherichia coli</i>	0	3	0	0	2	1	0	6	4,14
PLT	3	15	7	15	9	13	8	70	48,27
Total number of isolations	5	34	21	28	21	24	12	145	100

*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. *Pseudomonas aeruginosa* shows equivalent prevalence rates of 2.67% 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	
<i>Mycobacterium avium</i> subsp. <i>Paratuberculosis</i> (MAP)	4	0	5	6	15	18,75
<i>Staphylococcus</i> spp.	0	0	1	0	1	1,25
<i>Mycobacterium bovis</i>	0	0	0	0	0	0
<i>Streptococcus pneumoniae</i>	2	1	1	1	5	6,25
<i>Mannheimia haemolytica</i>	5	0	1	1	7	8,75
<i>Pasteurella multocida</i>	0	1	0	0	1	1,25
<i>Pseudomonas aeruginosa</i>	1	0	1	0	2	2,50
<i>Brucella ovis</i>	0	0	0	0	0	0
<i>Corynebacterium</i> spp.	0	0	0	0	0	0
<i>Escherichia coli</i>	0	2	1	1	4	5,00
<i>Staphylococcus aureus</i>	0	1	0	0	1	1,25
<i>Clostridium</i> spp	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
<i>Mycobacterium avium</i> subsp. <i>Paratuberculosis</i> (MAP)	14,29	18,03	12,50	27,27	11,54	33,33	33,33
<i>Staphylococcus</i> spp.	1,79	0,00	0,00	3,03	0,00	0,00	0,00
<i>Mycobacterium bovis</i>	0,00	0,00	3,13	0,00	0,00	0,00	0,00
<i>Streptococcus pneumoniae</i>	3,57	8,20	9,38	3,03	3,85	11,11	0,00
<i>Mannheimia haemolytica</i>	12,50	13,11	12,50	0,00	3,85	0,00	16,67
<i>Pasteurella multocida</i>	5,36	0,00	0,00	6,06	0,00	0,00	8,33
<i>Pseudomonas aeruginosa</i>	3,57	3,28	3,13	3,03	0,00	0,00	0,00
<i>Brucella ovis</i>	0,00	0,00	0,00	0,00	3,85	0,00	0,00
<i>Corynebacterium</i> spp.	3,57	3,28	3,13	0,00	3,85	0,00	8,33
<i>Escherichia coli</i>	5,36	3,28	12,50	3,03	0,00	0,00	0,00
<i>Staphylococcus aureus</i>	0,00	0,00	0,00	0,00	0,00	0,00	8,33
<i>Clostridium</i> spp	0,00	0,00	0,00	3,03	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
									(%)
<i>Mycobacterium avium</i> subsp. <i>Paratuberculosis</i> (MAP)	0	7	7	3	4	5	1	27	19,01
<i>Staphylococcus</i> spp.	0	0	0	1	0	0	0	1	0,70
<i>Mycobacterium bovis</i>	0	1	0	0	0	0	0	1	0,70
<i>Streptococcus pneumoniae</i>	1	0	2	1	2	2	0	8	5,63
<i>Mannheimia haemolytica</i>	1	4	3	2	3	1	1	15	10,56
<i>Pasteurella multocida</i>	0	1	0	3	0	1	0	5	3,52
<i>Pseudomonas aeruginosa</i>	0	2	0	1	0	1	0	4	2,82
<i>Brucella ovis</i>	0	0	1	0	0	0	0	1	0,70
<i>Corynebacterium</i> spp.	0	1	1	2	1	0	2	7	4,93
<i>Escherichia coli</i>	0	3	0	0	2	1	0	6	4,23
PLT	3	15	7	15	9	13	8	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	

Tab. 11. Bacterial prevalence rate (percentage relative to total) in the wild boar population of Selladores-Contadero

Bacterial species	P1 SC	P2 SC	P3 SC	P4 SC	Total	Prevalence(%)
<i>Mycobacterium avium</i> subsp. <i>Paratuberculosis</i> (MAP)	4	0	5	6	15	17,24
<i>Staphylococcus</i> spp.	0	0	1	0	1	1,15
<i>Mycobacterium bovis</i>	0	0	0	0	0	0
<i>Streptococcus pneumoniae</i>	2	1	1	1	5	5,75
<i>Mannheimia haemolytica</i>	5	0	0	1	7	8,05
<i>Pasteurella multocida</i>	0	1	0	0	1	1,15
<i>Pseudomonas aeruginosa</i>	1	0	1	0	2	2,30
<i>Brucella ovis</i>	0	0	0	0	0	0
<i>Corynebacterium</i> spp.	0	0	0	0	0	0
<i>Escherichia coli</i>	0	2	1	1	4	4,60
<i>Staphylococcus aureus</i>	0	1	0	0	1	1,15
<i>Clostridium</i> spp	0	1	0	0	1	1,15
PLT	15	9	10	9	43	49,43
Total number of isolations	27	15	20	18	80	
Negative samples	6	6	9	9	30	

*Escherichia coli* shows an analogous prevalence rate in both woodlands. *Mannheimia haemolytica* and *Escherichia coli* prevalence dramatically increases in young animals (up to 3 years). *Chlamydomphila psittaci* is widely distributed and has a higher prevalence in Selladores-Contadero.

### 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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