Faecal *Escherichia coli* as biological indicator of spatial interaction between domestic pigs and wild boar (*Sus scrofa*) in Corsica.

Running title: *E. coli* as field indicator for wild boar-domestic pig interaction

Stefanie A. Barth[1], Sandra Blome[2], Daniel Cornelis[3], Jana Pietschmann[1,2], Morgane Laval[4], Oscar Maestrini[4], Lutz Geue[1], François Charrier[4], Eric Etter[3,5], Christian Menge[1], Martin Beer[2] and Ferran Jori[3,6]

[1]Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut/Federal Research Institute for Animal Health, Naumburger Str. 96a, 07743 Jena, Germany

[2]Institute of Diagnostic Virology, Friedrich-Loeffler-Institut/Federal Research Institute for Animal Health, Suedufer 10, 17493 Greifswald - Insel Riems, Germany

[3]CIRAD, UMR ASTRE, University Montpellier, INRA, 34398 Montpellier, France.

[4]Research unit for animal husbandry development (LRDE), INRA SAD, Quartier Grossetti, 20250 Corte, Corsica, France

[5]Epidemiology Section, Department of Production Animals Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110, South Africa.

[6]Department of Animal Science and Production, Private Bag 0027 Botswana College of Agriculture, Gaborone, Botswana

Corresponding author:
Dr. Stefanie Barth, stefanie.barth@fli.de;
phone: +49 3641 804 2270, fax: +49 3641 804 2482

Abstract

On the Mediterranean island of Corsica, cohabitation between sympatric domestic pigs and Eurasian wild boar (*Sus scrofa*) is common and widespread and can facilitate the maintenance and dissemination of several pathogens detrimental for the pig industry or human health. In this study, we monitored a population of free ranging domestic pigs reared in extensive conditions within a 800 ha property located in Central Corsica which was frequently visited by a sympatric population of wild boar between 2013 and 2015. We used GPS collars to assess evidence of a spatially shared environment. Subsequently, we analysed by PFGE of *Xba*I-restricted DNA if those populations shared faecal *E. coli* clones that would indicate contact and compared these results with those collected in a distant (separated by at least 50 km) population of wild boar used as control. Results showed that one out of eight wild boar sampled in the study area shed *E. coli Xba*I-clones identical to clones isolated from domestic pig sounders from the farm, while wild boar populations sampled in distant parts of the study area shared no identical clone with the domestic pigs monitored. Interestingly, within the sampled pigs, two identical clones were found in 2013 and in 2015, indicating a long-time persisting colonization type. Although the method of isolation of *E. coli* and PFGE typing of the isolates requires intensive laboratory work, it is applicable under field conditions to monitor potential infectious contacts. It also provides evidence of exchange of microorganisms between sympatric domestic pigs and wild boar populations.

Keywords: *Escherichia coli*, field study, biological contact marker, wild boar, domestic pig, transmission

Introduction

Wild boar and domestic pigs belong to the same species (*Sus scrofa*) and are known to interact when they meet in the open landscape. Such interactions have been observed on different continents (Jori et al., 2017a; Meng et al., 2009) and are known to be responsible for the maintenance and dissemination of several important pig pathogens, including bacteria (Richomme et al., 2013), viruses (Albina et al., 2000; Ruiz-Fons et al., 2008) and parasites (Richomme et al., 2010b). Transmission of pathogens between both pig populations might occur via physical contact (e.g., breeding, fighting) or indirectly by sharing the same contaminated habitat. The occurrence and analysis of these interactions can be assessed through different methods including questionnaires among stakeholders (Kukielka et al., 2016; Trabucco et al., 2014), serology (Wyckoff et al., 2009), molecular methods (Jori et al., 2016), telemetry combined with data loggers (Pepin et al., 2016) or camera traps (Kukielka et al., 2016). *Escherichia coli* has been similarly used in several mammalian species, e.g. to assess interactions between sympatric wild and domestic populations or individuals sharing the same environment (Mercat et al., 2016; Rwego et al., 2008b; Springer et al., 2016; VanderWaal et al., 2014). It is assumed that social interactions can facilitate the exchange of microorganisms that are likely to influence the composition of the gut microbiome within a population of individuals cohabiting the same environment (VanderWaal et al., 2014; Springer et al., 2016). Therefore, genetic similarities in the gut microbiome between different populations can be used to infer direct or indirect interactions that could facilitate exchange of microorganisms, including pathogen spread. In the case of pig species, this method was recently assessed experimentally and it was demonstrated that at least in captivity, indirect contact between wild boar and domestic pigs is traceable by faecal *E. coli* isolates (Barth et al., 2017). However, application of this method in the field has never been tested. In this study, we attempted to assess if faecal *E. coli* could be used as an indicator of infectious

contacts as well as potential pathogen transmission between a population of free ranging domestic pigs reared in traditional extensive Corsican conditions and a population of sympatric, free ranging wild boar.

For this purpose, we selected an extensive traditional pig farm located in Central Corsica where interactions between domestic pigs and natural populations of wild boar were commonly reported (Jori et al., 2017b). To assess the occurrence of potential interactions between the two pig populations, telemetry methods were used. In addition, *E. coli* isolates from faecal samples of the domestic pig populations were analysed and compared with faecal *E. coli* isolates from wild boar individuals collected either in the same farm or from another population of wild boar living in a distant location. The latter were used as a control group for comparison.

Material and Methods

Study area

Corsica is a French Mediterranean island located off the western shores of the Italian peninsula, approximately 11 km north of the Italian island of Sardinia. Pig breeding and production is mainly conducted in traditional free-range farming systems, which are stretched out over large surface areas with a median size of 557 km^2 (Dubost, 2001), encompassing a mosaic of mountainous pastures and plain areas. Traditionally, Corsican pig production takes advantage of outdoor resources (chestnuts, acorns, etc.) in order to produce cured pork quality products (Relun et al., 2015). Pig farming systems are, thus, characterized by more than 100 ha large areas of pasture, with heterogeneous vegetation (i.e., Mediterranean shrubs/bushes, chestnut, and oak areas) as well as a high degree of variation in the landscape (i.e., altitude, sun exposure, vegetation, and slopes).

Localisation of pig groups in this large and diverse territory varies during the course of the year. During the winter months when natural resources are scarcer, they tend to remain close to the farm for supplementary feeding and reproduction. During autumn and early winter (coinciding with the chestnut harvesting period), free ranging sounders are left in the mountain plains. This is a key moment for the animals to achieve the physiological and nutritional condition required to produce high quality cured pork products (quality of the fat, taste of the products). Wild boar are generally present in the forested areas of the farm territory (close to the farm settlement or in the mountains) all year round. Although domestic pig-wild boar interactions are more regularly observed during autumn and early winter (Trabucco et al., 2014; Jori et al., 2017b), the presence of wild boar close to the animals near the farm settlement remains important all year round (Trabucco et al., 2014). Wild boar hunting is a well-established and culturally-rooted activity in Corsica (8 to 10 % of the population practices hunting), with around 30,000 wild boar hunted annually (ONCFS, 2012).

Our study area was located in a typical extensive farm, housing a population of 600 free-ranging pigs from the local "Nustrale" breed, reared to produce high quality cured pork products. It is located in the village of Ucciani, Corse-du-Sud Department, Southern part of Corsica (Figure 1), and representative of the traditional extensive large scale pig production. Vegetation in this area is typical for Mediterranean mountains and mainly composed of oak (*Quercus ilex*) thickets interspersed by chestnut trees (*Castanea sativa*) and beech (*Fagus sylvatica*). The study farm encompassed a territory of 800 ha with altitudes ranging between 270 and 1,650 m above sea level. During the summer period, pig herds are kept around the farm facilities (270 m in altitude), fed by the farmer, whereas during autumn and winter, pig herds are spread out over the entire area. To minimize possible interactions between wild boar and reproductive sows, all reproduction and piglet management handling (e.g., female castration) were performed before autumn (De Sainte-Marie and Casabianca, 1998; Relun et al., 2015).

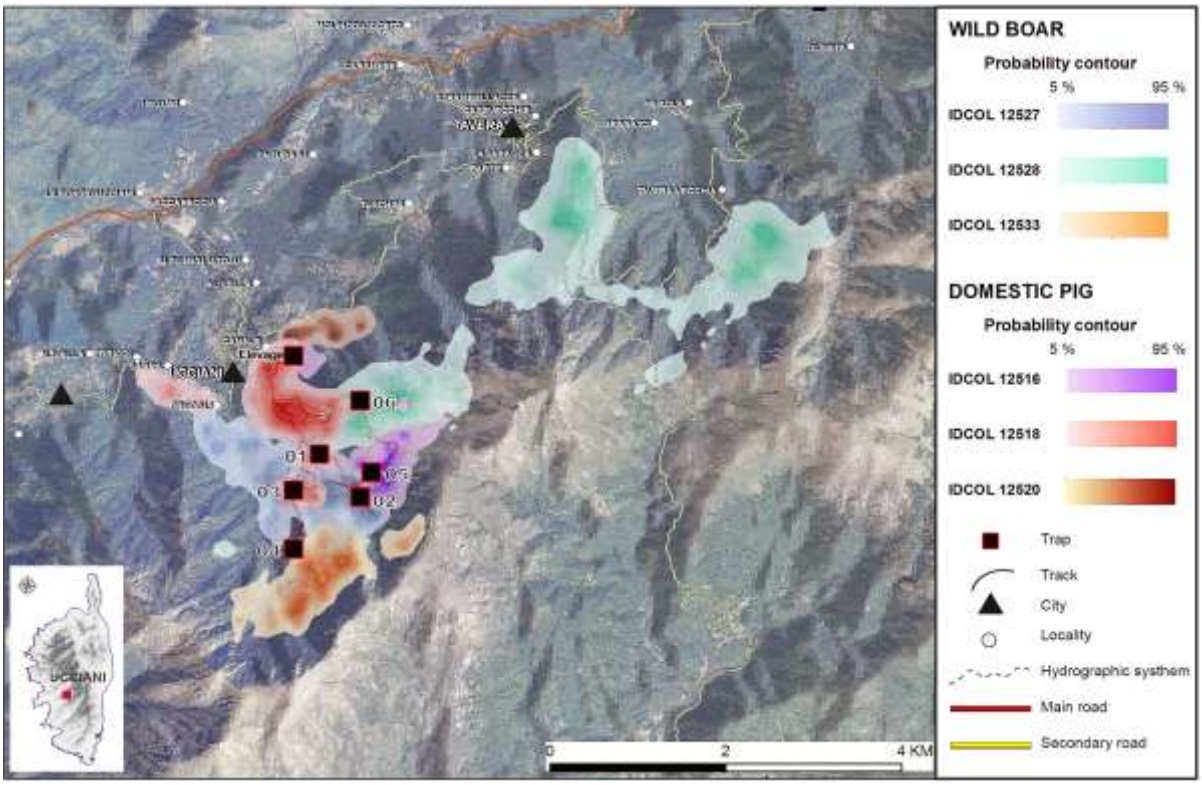


Figure 1. *Map showing the study area, its location in Corsica, the position of the traps where the wild boar were captured and the home range contours on a sample of 3 wild boar and 3 domestic pigs.*

Telemetry protocol

Wild boar

Nine adult wild boar were captured with corral traps and equipped with a GPS-GSM collar between June and August 2013. In the Mediterranean area, summer is a period of food scarcity, thus favourable for baiting and capturing wild boar. Baiting started in June 2013 using maize and automatic feeders hanging over 6 corral traps located in the farm rangeland (Figure 1). Corral traps were adapted from a standard design recommended by the French Office for Hunting and Wildlife (ONCFS, 2012). The attendance of the traps by wild boar was monitored using camera-traps. Pictures taken by cameras were sent in real-time on an e-mail server shared by the capture team. Trapped individuals were tele-anesthetized (Zoletil 100®, Virbac, Carros, France) from a short distance with pistols (Dan-Inject ApS, Børkop, Denmark), blood sampled, ear-tagged, equipped with a GPS-GSM collar (Vectronics

Aerospace GmbH, Berlin, Germany), and released from the corral trap once completely awake. GPS-GSM collars were scheduled to acquire locations at 1 h fix-intervals. The field operations conformed to French legal requirements regarding capture and tracking protocols on large ungulates (Prefectural order N° 2013-200-0006 dated July 2013 authorizing the capture of wild boar for scientific purposes in Corsica). Success rates for collared pigs were calculated as the ratio between the number of successfully acquired GPS locations compared to the total number of locations expected during the focal period.

Domestic pigs

Early October 2013, i.e. just before the chestnut and acorn period, 10 adult domestic sows were fitted with similar GPS-GSM collars also scheduled to acquire locations at 1 hour fix-intervals.

Faecal sampling

Wild boar

Of the 9 wild boar captured in corral traps, 8 were sampled for faecal material directly from the rectum when they were immobilized (sounder WB_{GPS}).

In addition, a total of 47 faecal samples (sounder $WB_{control}$) were collected during the hunting season between November 2014 and February 2015 in a hunting area located 50 km away from the study farm. Those samples served as controls to be compared with samples from the wild boar monitored in our study area.

Domestic pigs

Seven of the 10 domestic sows equipped with collars (group DP_0) were sampled for faecal material during the tracking period. In addition, starting in October 2014, two sounders (adult sows with their piglets) were monitored longitudinally for the presence of faecal *E. coli*. Both sows were sampled when giving birth. Their piglets were monitored four times: at birth (T_0)

and subsequently at T_0+1 month, T_0+3 months, and T_0+4 months. Sow 1 gave birth to 4 piglets on October 17th 2014, resulting in 21 faecal samples (group DP_1). Sow 2 gave birth to 3 piglets on February 9th 2015, resulting in 12 faecal samples (group DP_2). Faecal samples from domestic pigs were collected directly from the ground shortly after defecation and stored at -80 °C until shipping on ice to FLI (Friedrich-Loeffler-Institut) in Germany for subsequent analysis.

Isolation of coliform bacteria

The quantification and isolation of coliform bacteria from the faeces as well as the storage of single colonies was performed as described previously (Barth et al., 2017). Briefly, up to ten putative *E. coli* isolates were isolated from each faecal sample according to the colony morphology on MacConkey, Gassner, and sheep-blood agar (Sifin Diagnostics GmbH, Berlin, Germany). Due to the detection limit of 100 cfu/g faeces, the number of isolates per faecal sample varied from 0 up to 10. Overall, 731 *E. coli* isolates were analysed; 327 and 404 isolates from domestic pigs and from wild boar, respectively.

Analysis of PFGE patterns of restricted DNA from *E. coli* isolates

Contour-clamped homogeneous electric field-pulsed-field gel electrophoresis (CHEF PFGE) and cluster analysis was performed as previously described (Geue et al., 2010; Barth et al., 2017). In addition to restriction with *Xba*I, selected agarose plugs were digested with 15 U *Ava*II or 15 U *Spe*I (New England Biolabs GmbH, Frankfurt/Main, Germany) at 37 °C (*Spe*I 18 h, *Ava*II 5 h). For separation of *Ava*II- or *Spe*I-digested DNA fragments, the pulse times in the CHEF Mapper XA system increased from 6.75 to 35.38 s with a gradient of 6 V/cm and a constant angle of 120° during 19 h. Interpretation of PFGE patterns was performed by visual inspection and computer analysis with Bionumerics (version 6.6, Applied Maths NV, Sint-Martens-Latem, Belgium). Distance matrices were calculated by pairwise comparisons of the

fragment patterns produced by the restriction endonucleases used for the PFGE analysis including DNA fragments between 49 and 630 kb length (Lambda Ladder PFG Marker, New England Biolabs). The cluster analysis of the *Xba*I-fragmented DNA was based on the Dice algorithm with 2.0 % tolerance and 0.5 % optimization and the un-weighted pair group method with arithmetic mean (UPGMA).

Data analysis

Significant differences in the mean numbers of *E. coli* isolates and identified PFGE clones were calculated using IBM SPSS Statistics (version 19.0.0.2, IBM Deutschland GmbH, Ehningen, Germany).

The relationships between animals based on shared *E. coli* clones were analysed by social network analysis (SNA). According to SNA vocabulary, the wild and domestic suids represent the vertices and each shared *E. coli Xba*I-clone represents an edge of the network. Specific network parameters (density, diameter, k-core) were calculated and graphs were first performed using R (version 3.3.3) with the igraph package (version 0.7.1) (R Core Team, 2016). The k-core is the maximal subgraph in which each vertex is adjacent to at least k other vertices of the subgraph (Fortunato, 2010). For a better reproduction quality of the captions, the graphs were subsequently edited using Microsoft PowerPoint (version 2016) software.

Table 1. *Telemetry protocol summary*

Pigs	Collar ID	Sex	**Age** (months)	**Start tracking**	**End tracking**	**Success rate** (%)	**Distance by 24 h** (km±SD)	**Distance from farm** (km)	**Home range** (km^2)
Wild boar	528	M	24	02/10/13	01/01/14	40	3.5±1.3	3.8±1.9	4.6
	533	F	36	02/10/13	02/01/14	12	1.9±0.1	2.4±0.3	0.8
	534	M	36	02/10/13	01/01/14	50	3.2±1.5	1.2±0.5	3.3
	536	F	36	02/10/13	02/01/14	92	1.8±0.8	1.5±0.2	0.6
Domestic pigs	516	F	60	02/10/13	02/01/14	93	1.6±0.6	1.2±0.6	0.8
	518	F	30	02/10/13	21/12/13	77	2.5±0.9	0.6±0.4	1.9
	519	F	48	16/10/13	14/11/13	37	1.8±0.6	0.8±0.4	1.0
	520	F	60	16/10/13	10/11/13	30	1.5±0.7	0.3±0.3	0.4
	521	F	24	02/10/13	15/12/13	78	1.7±0.8	0.3±0.2	0.6
	522	F	24	02/10/13	10/11/13	41	0.7±0.6	0.1±0.0	0.3

Geospatial analysis focused on a period starting in October 2013 for 3 months (autumn and early winter) using movement data from 6 domestic pigs and 4 wild boar. Beyond this period, sample size decreased drastically due to collar losses, collar failures or individual mortality (due to hunting). During the focal period, GPS collars returned data with success rates of 59.3 ± 26.4 % from domestic pigs and 48.5 ± 33.2 % wild boar (mean ± SD), respectively (Table 1). Spatial behaviour of GPS-tracked individuals was characterised by computing home range area, the distance between the barycentres of the home range and the farm buildings, and the daily distances ranged. Home ranges were computed (up to the 0.95 isopleth) for each collared individual using a movement-based kernel density estimation method (Benhamou and Cornélis, 2010). Daily distances ranged by GPS-tracked individuals were calculated using 24 h time series during which at least 80 % of the expected GPS locations were acquired. We then computed home range overlaps to quantify the extent to which the collared individuals shared space. Home range overlaps were computed using a volume index ranging between 0 (no area shared) and 100 % (identical utilization distributions) (Germain et al., 2008).

Results

Space use and space sharing behaviour

GPS-tracked wild boar displayed home ranges of 2.3 ± 1.9 km^2 (mean ± SD; Table 1) located on average 2.2 ± 0.7 km from the farm buildings (Figure 1). In contrast, domestic pigs displayed home ranges of 0.8 ± 0.6 km^2 located on average 0.5 ± 0.3 km from the farm buildings. Wild boar and domestic pigs covered daily distances of 2.6 ± 0.9 km and 1.6 ± 0.7 km, respectively. Home range overlap estimations show that the highest amounts of space sharing were reached within the domestic pig population (19.7 ± 16.4 %, mean ± SD). Lower amounts of space sharing were observed in the wild boar population of (11.2 ± 14.9 %), and even less between wild and domestic populations (6.9 ± 12.5 %) (Table 2).

Table 2. *Spatial overlaps of domestic pigs and wild boar as determined by GPS collars*

		Home range overlap matrix (%)								
		Wild boar			**Domestic pigs**					
		W533	**W534**	**W536**	**D516**	**D518**	**D519**	**D520**	**D521**	**D522**
Wild boar	**W528**	0	23	6	5	7	4	0	0	0
	W533	-	1	1	1	2	1	0	0	0
	W534	-	-	36	20	11	23	3	1	0
	W536	-	-	-	53	7	27	0	0	0
Domestic pigs	**D516**	-	-	-	-	8	15	7	9	8
	D518	-	-	-	-	-	51	6	48	20
	D519	-	-	-	-	-	-	2	33	11
	D520	-	-	-	-	-	-	-	9	26
	D521	-	-	-	-	-	-	-	-	42

Isolation of *E. coli*

By cultivation of faecal samples on Gassner and MacConkey agar, coliform bacteria were detected in 84 of 95 samples (Table 3). The number of coliform bacteria [cfu/g faeces] ranged

Table 3. *Faecal samples, E. coli isolates, and identified PFGE clones*

Pigs	WB sounder / DP group	Sampling of faeces (per animal, time range)	Coliform bacteria: Number faecal samples [positive/total]	Coliform bacteria: Cfu/g faeces [mean ± SEM (min - max)]		*E. coli* isolates†: Total	*E. coli* isolates†: Number per sample [mean ± SD]	*Xba*I-PFGE clones†: Total	*Xba*I-PFGE clones†: Number per sample [mean ± SD]
Wild boar	**WB_{GPS}** (with GPS collar)	once, Jun 2013 till Aug 2013	7/8	$5.2\times10^7 \pm 3.5\times10^7$ ($0 - 2.6\times10^8$)		64	7.86 ± 2.55	18	2.57 ± 1.90 [e]
	$WB_{control}$ (without GPS collar)	once, Nov 2014 till Feb 2015	42/47	$1.3\times10^7 \pm 1.2\times10^7$ ($0 - 5.6\times10^8$)	[a,b]	410	8.31 ± 2.51	127	3.02 ± 1.81 [f]
Domestic pigs	**DP_0** (young sows)	once, Oct 2013 till Nov 2013	3/7	21.4 ± 10.1 (0 - 50)	[a,c,d]	30	9.33 ± 0.58	12	4.00 ± 2.00
	DP_1 (sow no. 1 with 4 piglets)	four times, Oct 2014 till Feb 2015	21/21	$2.6\times10^7 \pm 1.7\times10^7$ ($500 - 3.2\times10^8$)	[d]	203	9.14 ± 1.46	123	5.86 ± 1.96 [e,f,g]
	DP_2 (sow no. 2 with 3 piglets)	four times, Feb 2015 till Jun 2015	11/12	$3.2\times10^8 \pm 1.3\times10^8$ ($0 - 1.4\times10^9$)	[b,c]	110	9.73 ± 0.47	35	3.18 ± 1.10 [g]
total		--	84/95	$5.7\times10^7 \pm 2.1\times10^7$ ($0 - 1.4\times10^9$)		731	8.70 ± 2.12	315	3.71 ± 2.10

DP domestic pig; **SD**, standard deviation; **SEM**, standard error of the mean; **WB**, wild boar; identical superscript letters, significant differences between groups (Kruskal-Wallis test, $p<0.05$); †mean number of *E. coli* isolates and clones is related only to positive faecal samples.

from 0 to 5.6×10^8 for wild boar and from 0 to 1.4×10^9 for domestic pigs (Table 3). Overall, the number of coliform bacteria from wild boar and domestic pigs did not differ significantly (Mann-Whitney-U test, $p = 0.099$). However, the individual groups and sounders showed significantly different loads of bacteria. The lowest average number was detected in faecal samples from group DP_0, while the samples from pigs of group DP_2 shed the most coliform bacteria (Table 3; Kruskal-Wallis test, $p \leq 0.028$).

Altogether, we picked 817 colonies from 84 samples positive for coliform bacteria. Thereof, 731 colonies also displayed the expected coliform colony morphology on sheep blood agar and were therefore assumed to be *E. coli* isolates. The number of *E. coli* isolates obtained per sample did not differ significantly between the groups or sounders (Table 3; Kruskal-Wallis test, $p = 0.231$).

Genetic relatedness of the *E. coli* isolates

Out of 731 *E. coli* isolates, 323 *E. coli* with individual profiles were detected by *Xba*I restriction and PFGE analysis resulting in 315 distinct *E. coli* clones when excluding isolates found more than once per individual faecal sample (> 95 % similarity or ≤ 3 different fragments; Tenover et al. (1995); Supplemental Figure S1). Pigs from group DP_1 possessed the most heterogeneous composition of *E. coli* with 5.86 clones per sample on average (Table 3). This number was significantly different from the mean number of clones in both wild boar sounders and group DP_2 (Kruskal-Wallis test; $p \leq 0.032$).

Comparing the 315 *E. coli* clones, we found 221 clones only once (less than 95 % similarity to other clones), while 94 clones formed 36 clusters (clones that share restriction patterns with more than 95 % similarity) with 2 to 6 members (Supplemental Figure S1). Most clusters (n=27) contained clones from one group or sounder (Table 4). Twenty-five clusters were formed by clones only present in domestic pigs (15 clusters with clones from group DP_1, 4

clusters with clones from DP_2, 5 clusters with clones from DP_1 and DP_2, and 1 cluster with clones from DP_0 and DP_1), whereas 8 clusters contained only clones from the wild boar group $WB_{control}$. Three clusters comprised identical clones from wild boar (WB_{GPS}) and domestic pigs (DP_1). The respective clones were found to be identical after *Xba*I restriction and after additional *Avr*II and *Spe*I restriction (Figure 2).

Table 4. *Detected clusters according to the affiliation of clones to different groups and sounders*.

	Number of cluster with clones from the different groups or sounders				
	WB_{GPS} (with GPS collar)	**$WB_{control}$** (without GPS collar)	**DP_0** (young sows)	**DP_1** (sow 1 with 4 piglets)	**DP_2** (sow 2 with 5 piglets)
WB_{GPS}	0	0	0	3	0
$WB_{control}$	-	8	0	0	0
DP_0	-	-	0	1	0
DP_1	-	-	-	15	5
DP_2	-	-	-	-	4

*Xba*I-restricted DNA, Dice, with 2.5 % tolerance and un-weighted pair group method with arithmetic mean (UPGMA). **DP**, domestic pigs; **WB**, wild boar. Numbers listed under the same heading for column and row refers to clusters restricted to the given animal group.

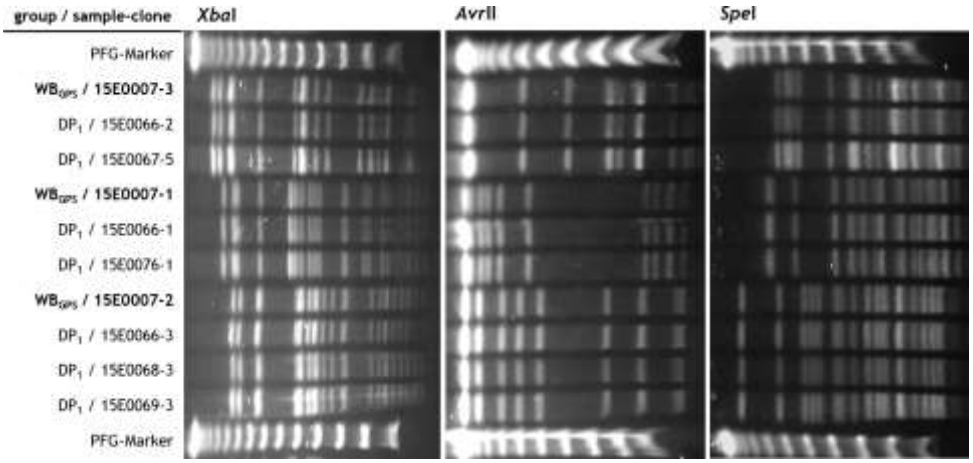


Figure 2. *Confirmation of clonality of identical E. coli XbaI-clones present in faeces from wild boar (sounder WB_{GPS}) and from domestic pigs (group DP_1) by PFGE of DNA digested with AvrII and SpeI*.

DP, domestic pigs; **WB**, wild boar; **Marker**: Lambda Ladder PFG-Marker.

Upon visualizing the results in a network, it became clear that, regardless of the number of clones per sample, some samples (animals) shared identical clones with several other samples (animals) also of other groups or sounders (Figure 3). Overall, in the network we detected 68 interrelations between two individual samples based on the identification of one or up to three shared *E. coli* clones per interrelation. The highest number of interrelations was found in one sample sharing *E. coli* clones with 7 other samples followed by 5 samples sharing clones with 6 other samples, respectively. Especially, we found connections between animals within group DP_1 or within group DP_2, mostly representing one litter at one sampling point (parts A, B or C of the network). Nevertheless, connections between different groups or sounders were also determined, e.g. part D of the network included samples from groups DP_1, DP_2, and WB_{GPS}.

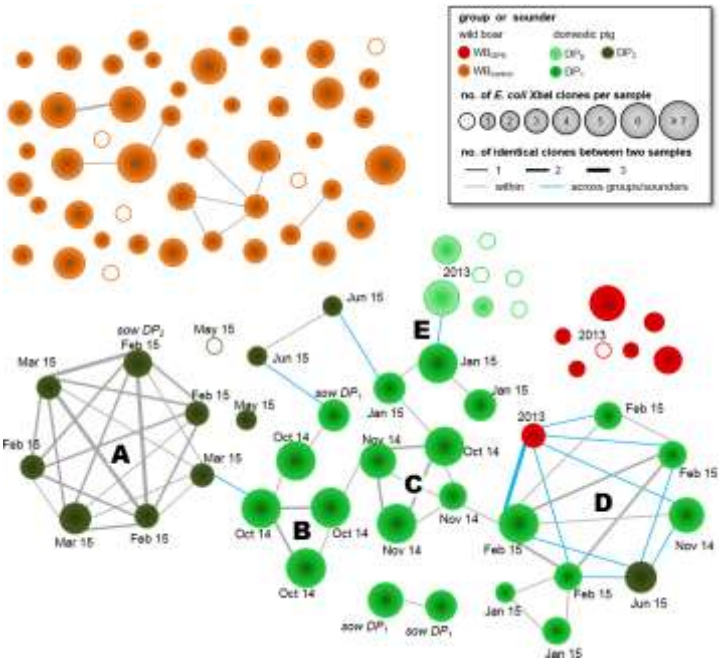


Figure 3. *Occurrence of identical E. coli XbaI-clones in faeces from wild boar and domestic pigs.*

Each circle stands for one faecal sample and represents the number of clones within the sample by the size of the circle. If identical *E. coli Xba*I-clones occurred in different samples, the circles are connected by a line. For some samples, the collection date is given by month and year. Letters A to E refer to parts of the network that were discussed in the text.

For statistical purposes, we analysed the global network and formed sub-networks of the groups DP_1, DP_2, and $WB_{control}$, respectively. The sub-networks were built by removal of all connections to samples of other groups. While the density of the global network (number of present of all possible interrelations) was 2.1 %, the density of the sub-network DP_1 was 16.2 %, the one formed by DP_2 was 54.5 %, and the one consisting of the $WB_{control}$ group was 0.9 %. The diameter of the global network (shortest path between the two furthest samples) encompassed 9 other samples, the diameter of the sub-network DP_1 involved 8 samples and those of DP_2 and $WB_{control}$ 2 other samples, respectively. These findings imply that *E. coli* clones are most likely shared by direct animal-to-animal contact in the case of DP_2 (with many *E. coli* clones shared) and in $WB_{control}$ (with few *E. coli* clones shared), whereas animals are primarily connected indirectly, i.e., through intervention of many others, in the global network and DP_1. Calculating the k-cores (a maximal subgraph that contains animals having at least k shared clones) and plotting them in the network, all but 3 samples of DP_2 regrouped in the 8-core part of the network (part A) reflecting the intense interconnection of those animals (samples) (Figure 4). The samples belonging to group DP_1 grouped in the 5-, 4-, and 3-core (parts D, C, and B, respectively), with one DP_2 and one WB_{GPS} sample being part of the 5-core.

Additionally, while most of the clones were found only at one sampling time point, other clones were present over longer time periods. For example, one clone was found once in 2013 (DP_0) and again once in 2015 (DP_1; part E of the network) or clones in part D of the network sampled in 2013 (WB_{GPS}), 2014 (DP_1) and 2015 (DP_1, DP_2) (Figure 3, Supplemental Figure S1).

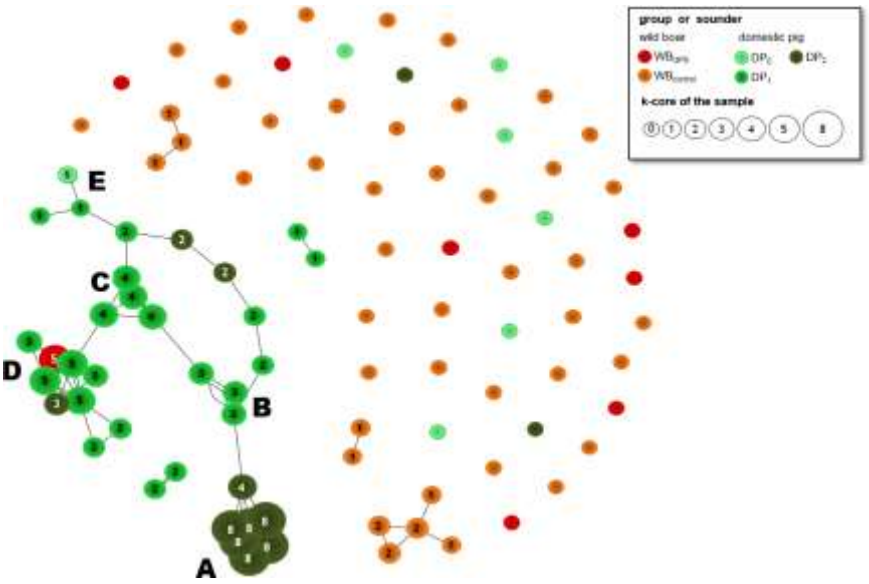


Figure 4. *K-core-based network of shared E. coli XbaI-clones in faecal samples from wild boar and domestic pigs*

Each circle stands for one faecal sample. The size of the circle represents the k-core (maximal subgraph containing nodes with at least k-degrees). Letters A to E refer to parts of the network that were discussed in the text.

Discussion

Historically, pig farming in Corsica is based on a traditional pastoral system with extensive outdoor free-ranging livestock. The number of pigs reared on the island is estimated to be approximately 26,360 animals (Richomme et al., 2010b). In recent years, the development of tourism and the commercial success of high quality cured meat products from pigs reared in free-ranging conditions has boosted and subsequently consolidated an outdoor pig production industry (Relun et al., 2015). Concurrently, the agricultural decline and abandonment of agricultural land in recent decades has led to a notable increase in wild boar populations as evidenced by the approximately 30,000 wild boar hunted annually (ONCFS, 2012). Similar to other Mediterranean locations, these conditions provide an ideal environment for the interaction between wild and domestic pig populations (Jori et al., 2017b) and the subsequent

maintenance and transmission of pathogens detrimental for both the pig industry (Albina et al., 2000; Mur et al., 2016) and human health (Pavio et al., 2016; Richomme et al., 2010a; Charrier et al., 2017). Previous work developed to collect information on interactions between wild and domestic pigs among farmers and hunters indicated a high incidence of direct contacts in extensive pig farms. These mostly resulted from sexual attraction of wild boar by domestic sows in the autumn months, while feeding interactions occurred all year-round depending on fruit availability (Trabucco et al., 2014; Jori et al., 2017b). Therefore, Corsican pig farming estates provide a well-characterized environment to validate shared carriage of *E. coli* strains as biological indicator of infectious contacts between wild boar and domestic pigs under field conditions. Our study farm had reported sexual interactions between domestic sows and wild boar, and fights between wild and domestic boar had been observed at least twice during the year preceding the study (Trabucco et al., 2014). Monitoring of spatial interactions was conducted during 3 months in autumn, which is considered a highly favourable period for sexual driven interactions between wild boar and domestic sows (Jori et al., 2017b). Indeed, spatial analysis confirmed an overlap of home ranges between both populations under study, even though low GPS success rates kept us from unveiling if those interactions were through direct physical contact or by sharing the same environment. The comparative analysis of *E. coli* microbiota as a measure to determine potential contacts between populations of different species or within groups of the same species has been used for several mammalian species, particularly wild and domestic bovids (Mercat et al., 2016), mustelids (Pesapane et al., 2013), primates (Rwego et al., 2008b), and humans (Rwego et al., 2008a). Our study provides evidence for the first time that this method can also be applied successfully to domestic and wild free ranging suid populations interacting under field conditions. These interactions can be the result of direct contact between individuals, the fact of sharing the same environment contaminated with faeces (water holes or feeding sites), or

also through the consumption of infected carcasses or offal remaining from infected animals (Jori et al., 2017b).

E. coli is an ideal candidate to monitor potential contacts between wild boar and domestic pigs, since it represents a dominant part of the aerobe microbiota in the intestine of several vertebrates, is shed in high quantities in the faeces, is genetically heterogenous, and can survive in the environment, depending on temperature and moisture, for more than 1 year (Fremaux et al., 2008; Schierack et al., 2007; Gordon and Cowling, 2003). By using PFGE analysis, several authors tracked single *E. coli* clones for several months in different species, including mallard ducks (at least 3 years (Rödiger et al., 2015)), cattle herds (at least 15 months (Geue et al., 2009; Liebana et al., 2005)) or sheep flocks (at least 11 months (Sánchez et al., 2009)). The persistence of *E. coli* clones in suids reported to date is based on shorter observational periods and on animals reared in intensive conditions only. For instance, the probiotic strain *E. coli* Nissle 1917 was shown to persist in pigs after experimental oral inoculation for at least 5 weeks, some *E. coli* pathotypes (STEC, EPEC, ETEC) for at least 2 months and some *E. coli* clones (based on biochemical profiling) over periods of 3-4 months (Barth et al., 2009; Booher et al., 2002; Katouli et al., 1995). In the current study, out of the 315 different identified *E. coli Xba*I clones, one clone was detected with a 7 month interval in one pig of group DP_1 and DP_2, respectively. One other clone was found with a gap of more than 1 year: once in one domestic pig in autumn 2013 during the sampling of the GPS collar wearing pigs of group DP_0 and again in one piglet of sow DP_1 in January 2015. This data indicates that a single *E. coli* clone can circulate among and persist in domestic free-range pig herds over several months or even years and confirms the suitability of this method as biological indicator during long-term monitoring studies.

Despite the small sample size, the recovery rate of *E. coli* clones from wild boar was high. *E. coli* were isolated in 87.5% of the wild boar sampled (7/8) in the WB_{GPS} group. With 1 to 6

E. coli clones per sample (mean 2.57), this number is slightly lower than in our previous study in captive animals (mean 3.00 to 3.63 *E. coli* clones per sample; (Barth et al., 2017)). From the animals of the WB_{GPS} group, one wild boar excreted three individual *E. coli Xba*I clones; each of these clones was also found in at least two different domestic pigs of group DP_1, directly connecting this wild boar with 5 different samples from DP_1 pigs. None of the clones from the remaining WB_{GPS} faecal samples were found in any other sample. Although a direct link to the domestic pigs tested was found in only 1 of 8 tested WB_{GPS} (12.5 %), this interrelation was very intense as it encompassed the entire pig group tested (at least 4 different animals of group DP_1), rather than only one animal. The clonality of the transmitted clones was confirmed by using additional restriction enzymes targeting different recognition sites in the DNA sequence. A laboratory contamination between the samples was excluded as the samples were processed on different days. Similarly, in our previous experimental study, one clone present in the faeces of one donor wild boar was spread to different recipient domestic pigs, whereas other domestic pigs housed together in one pen did not acquire one of the wild boar clones (Barth et al., 2017). The likelihood of transmission and colonization may be influenced by diverse individual factors related to host behaviour (e.g., individual faecal shedding quantity of *E. coli*, or snuffling, wallowing and rooting behaviour) and bacterial properties (e.g., the capacity of the respective *E. coli* strain to survive in the environment [number of bacteria shed and their viability in faeces, environment or stomach during ingestion] or its endowment with genes affecting persistence).

In an experimental setting where frequent direct and indirect transmission of O157 clones was shown between piglets, the pigs were inoculated with bacterial doses (5×10^8 cfu/dose) that presumably exceeded infectious doses that can be expected to occur under field conditions by far (Cornick and VuKhac, 2008). In the current study, an intense transmission of *E. coli Xba*I clones between sow DP_2 and its offspring was obvious in the first two months after birth and

supported by demonstration of the highest density level in the sub-network DP_2 with more than half of all possible interrelations. Conversely, only one link between the sow and one of her piglets directly after birth was shown in group DP_1 by detection of an identical *E. coli Xba*I clone. Similar observations were made when PFGE *Xba*I clones of CTX-M-producing *E. coli* were monitored in 5 sows and 2 of their respective piglets in an intensive pig production systems (Hansen et al., 2014). To further support the method applied, none of the 127 *E. coli Xba*I clones from the wild boar control group ($WB_{control}$) clustered with one of the clones from the other groups, neither the GPS tracked wild boar nor the domestic pigs. The low density level of the $WB_{control}$ sub-network may be based on the fact that those animals belonged to several independent sounders. Overall, the heterogeneity of the selected *E. coli* clones allowed a clear discrimination of animal populations in different geographic regions. Taking into account the number of to-be-tested samples and clones and the tedious laboratory work, the method of identifying clones by PFGE still offers some advantages. It is reproducible, can be performed by different persons even in different laboratories, and many laboratories are capable of performing PFGE, as it has been used for 20 years to detect food borne outbreaks, involving e.g. non-typhoidal *Salmonella* sp., *E. coli* O157:H7 or *Listeria monocytogenes* (Swaminathan et al., 2001). We conclude that the method applied was robust and sensitive enough for the current task of detecting possible contacts between wild boar and domestic pigs. In our study, *E. coli* is likely to be a good indicator of pathogen sharing between wild and domestic pigs in Mediterranean habitats, if they are transmitted via the faecal-oral route or if they are able to contaminate a shared environment, and infect wild and domestic animals and humans beings. These include, for instance, pathogens such as *Salmonella* sp. (Chiari et al., 2013), *Leptospira* sp. (Vale-Goncalves et al., 2015), *Mycobacterium bovis* (Naranjo et al., 2008) or the Hepatitis E virus (Jori et al., 2016). In fact, a recent study in Corsica provided molecular evidence of transmission of Hepatitis E virus

strains between wild boar and domestic pig populations (Jori et al., 2016). In that case, the study required the collection of organs and tissues obtained during hunting and slaughtering activities, which was logistically constraining. The *E. coli* method offers the possibility of using fresh faecal samples, which allows for identifying and characterizing locations prone to potential pathogen exchange between wild and domestic pigs without having to sample hunted or immobilised animals. Further studies should be applied to assess the potential of this non-invasive method in other epidemiological settings and with different pig-like species (Jori et al., 2017a; Kukielka et al., 2016).

Conflict of interest

The authors declare that no conflicts of interest exist.

Acknowledgments

The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 311931 (ASFORCE).

References

Albina, E., A. Mesplède, G. Chenut, M. F. Le Potier, G. Bourbao, S. Le Gal and Y. Leforban, 2000: A serological survey on classical swine fever (CSF), Aujeszky's disease (AD) and porcine reproductive and respiratory syndrome (PRRS) virus infections in French wild boars from 1991 to 1998. *Vet. Microbiol.,* 77, 43-57. *doi:* 10.1016/S0378-1135(00)00255-8.

Barth, S., S. Duncker, J. Hempe, G. Breves, G. Baljer and R. Bauerfeind, 2009: *Escherichia coli* Nissle 1917 for probiotic use in piglets: evidence for intestinal colonization. *J. Appl. Microbiol.,* 107, 1697-1710. *doi:* 10.1111/j.1365-2672.2009.04361.x.

Barth, S., L. Geue, A. Hinsching, M. Jenckel, J. Schlosser, M. Eiden, J. Pietschmann, C. Menge, M. Beer, M. Groschup, F. Jori, E. Etter and S. Blome, 2017: Experimental Evaluation of Faecal *Escherichia coli* and Hepatitis E Virus as Biological Indicators of Contacts Between Domestic Pigs and Eurasian Wild Boar. *Transbound. Emerg. Dis.,* 64, 487-494. *doi:* 10.1111/tbed.12389.

Benhamou, S. and D. Cornélis, 2010: Incorporating Movement Behavior and Barriers to Improve Kernel Home Range Space Use Estimates. *J. Wildlife Manage.,* 74, 1353-1360. *doi:* 10.2193/2009-441.

Booher, S. L., N. A. Cornick and H. W. Moon, 2002: Persistence of *Escherichia coli* O157:H7 in experimentally infected swine. *Vet. Microbiol.,* 89, 69-81. *doi:* 10.1016/S0378-1135(02)00176-1.

Charrier, F., S. Rossi, F. Jori, O. Maestrini, C. Richomme, F. Casabianca, C. Ducrot, N. Pavio and M. Le Potier, 2017: Aujesrzky Disease and Hepatitis E viruses transmission between domestic pigs ansd wild boars in Corsica: Evaluating the importance of wild/domestic interface and the efficacy of management measures. *Front. Vet. Sci.*, in press.

Chiari, M., M. Zanoni, S. Tagliabue, A. Lavazza and L. G. Alborali, 2013: *Salmonella* serotypes in wild boars (*Sus scrofa*) hunted in northern Italy. *Acta Vet. Scand.*, 55, 42. *doi:* 10.1186/1751-0147-55-42.

Cornick, N. A. and H. VuKhac, 2008: Indirect transmission of *Escherichia coli* O157:H7 occurs readily among swine but not among sheep. *Appl. Environ. Microbiol.,* 74, 2488-2491. *doi:* 10.1128/AEM.02897-07.

De Sainte-Marie, C. and F. Casabianca, 1998: Entre logique individuelle et intégration : « La fruitière », comme modèle d'organisation pour des producteurs ferliers de charcuterie Corse. *Études et Recherches sur les Systèmes Agraires et le Développement, INRA Editions*, pp. 297-315.

Dubost, M., 2001: Corse: une montagne authentique et vivante. Le recensement 1999 des unités pastorales en Corse. *Pastum*, pp. 21-24.

Fortunato, S., 2010: Community detection in graphs. *Phys. Rep.,* 486, 75-174. *doi:* 10.1016/j.physrep.2009.11.002.

Fremaux, B., C. Prigent-Combaret, M. L. Delignette-Muller, B. Mallen, M. Dothal, A. Gleizal and C. Vernozy-Rozand, 2008: Persistence of Shiga toxin-producing Escherichia coli O26 in various manure-amended soil types. *J. Appl. Microbiol.,* 104, 296-304. *doi:* 11.1111/j.1365-2672.2007.03532.x.

Germain, E., S. Benhamou and M. L. Poulle, 2008: Spatio-temporal sharing between the European wildcat, the domestic cat and their hybrids. *J. Zool.,* 276, 195-203. *doi:* 10.1111/j.1469-7998.2008.00479.x.

Geue, L., S. Klare, C. Schnick, B. Mintel, K. Meyer and F. J. Conraths, 2009: Analysis of the clonal relationship of serotype O26:H11 enterohemorrhagic *Escherichia coli* isolates from cattle. *Appl. Environ. Microbiol.,* 75, 6947-6953. *doi:* 10.1128/AEM.00605-09.

Geue, L., S. Schares, B. Mintel, F. J. Conraths, E. Müller and R. Ehricht, 2010: Rapid microarray-based genotyping of enterohemorrhagic *Escherichia coli* serotype O156:H25/H-/Hnt isolates from cattle and clonal relationship analysis. *Appl. Environ. Microbiol.,* 76, 5510-5519. *doi:* 10.1128/AEM.00743-10.

Gordon, D. M. and A. Cowling, 2003: The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. *Microbiology,* 149, 3575-3586. *doi:* 10.1099/mic.0.26486-0.

Hansen, K. H., V. Bortolaia, P. Damborg and L. Guardabassi, 2014: Strain diversity of CTX-M-producing Enterobacteriaceae in individual pigs: insights into the dynamics of shedding during the production cycle. *Appl. Environ. Microbiol.,* 80, 6620-6626. *doi:* 10.1128/AEM.01730-14.

Jori, F., M. Laval, O. Maestrini, F. Casabianca, F. Charrier and N. Pavio, 2016: Assessment of Domestic Pigs, Wild Boars and Feral Hybrid Pigs as Reservoirs of Hepatitis E Virus in Corsica, France. *Viruses,* 8, 236. *doi:* 10.3390/v8080236.

Jori, F., A. Payne, R. Kock, A. Nava, K. Ståhl and S. Rossi, 2017a: Wild and feral pigs: disease transmission at the interface between wild and domestic Suiform species in the Old and the New World. In: M. Melletti and E. Meijaard (eds), *Ecology, Evolution and Management of Wild Pigs and Peccaries*. Cambridge University Press, Cambridge, England.

Jori, F., A. Relun, B. Trabucco, F. Charrier, O. Maestrini, D. Chavernac, D. Cornelis, F. Casabianca and E. M. C. Etter, 2017b: Questionnaire-Based Assessment of Wild Boar/Domestic Pig Interactions and Implications for Disease Risk Management in Corsica. *Front. Vet. Sci.,* 4, 198. *doi:* 10.3389/fvets.2017.00198.

Katouli, M., A. Lund, P. Wallgren, I. Kühn, O. Söderlind and R. Möllby, 1995: Phenotypic characterization of intestinal *Escherichia coli* of pigs during suckling, postweaning, and fattening periods. *Appl. Environ. Microbiol.,* 61, 778-783.

Kukielka, E. A., F. Jori, B. Martinez-Lopez, E. Chenais, C. Masembe, D. Chavernac and K. Stahl, 2016: Wild and Domestic Pig Interactions at the Wildlife-Livestock Interface of Murchison Falls National Park, Uganda, and the Potential Association with African Swine Fever Outbreaks. *Front. Vet. Sci.,* 3, 31. *doi:* 10.3389/fvets.2016.00031.

Liebana, E., R. P. Smith, M. Batchelor, I. McLaren, C. Cassar, F. A. Clifton-Hadley and G. A. Paiba, 2005: Persistence of *Escherichia coli* O157 isolates on bovine farms in England and Wales. *J. Clin. Microbiol.,* 43, 898-902. *doi:* 10.1128/JCM.43.2.898-902.2005.

Meng, X. J., D. S. Lindsay and N. Sriranganathan, 2009: Wild boars as sources for infectious diseases in livestock and humans. *Philos. Trans. R. Soc. Lond. B Biol. Sci.,* 364, 2697-2707. *doi:* 10.1098/rstb.2009.0086.

Mercat, M., O. Clermont, M. Massot, E. Ruppe, M. de Garine-Wichatitsky, E. Miguel, H. Valls Fox, D. Cornelis, A. Andremont, E. Denamur and A. Caron, 2016: Escherichia coli Population Structure and Antibiotic Resistance at a Buffalo/Cattle Interface in Southern Africa. *Appl. Environ. Microbiol.,* 82, 1459-1467. *doi:* 10.1128/AEM.03771-15.

Mur, L., M. Atzeni, B. Martinez-Lopez, F. Feliziani, S. Rolesu and J. M. Sanchez-Vizcaino, 2016: Thirty-Five-Year Presence of African Swine Fever in Sardinia: History, Evolution and Risk Factors for Disease Maintenance. *Transbound. Emerg. Dis.,* 63, E165-E177. *doi:* 10.1111/tbed.12264.

Naranjo, V., C. Gortazar, J. Vicente and J. de la Fuente, 2008: Evidence of the role of European wild boar as a reservoir of *Mycobacterium tuberculosis* complex. *Vet. Microbiol.,* 127, 1-9. *doi:* 10.1016/j.vetmic.2007.10.002.

ONCFS, 2012: Tableaux de Chasse Ongulés Sauvages saison 2011-2012. *Supplément Faunae Sauvage*, pp. 1-8.

Pavio, N., M. Laval, O. Maestrini, F. Casabianca, F. Charrier and F. Jori, 2016: Possible Foodborne Transmission of Hepatitis E Virus from Domestic Pigs and Wild Boars from Corsica. *Emerg. Infect. Dis.*, 22, 2197-2199. *doi:* 10.3201/eid2212.160612.

Pepin, K. M., A. J. Davis, J. Beasley, R. Boughton, T. Campbell, S. M. Cooper, W. Gaston, S. Hartley, J. C. Kilgo, S. M. Wisely, C. Wyckoff and K. C. VerCauteren, 2016: Contact heterogeneities in feral swine: implications for disease management and future research. *Ecosphere*, 7, e01230. *doi:* 10.1002/ecs2.1230.

Pesapane, R., M. Ponder and K. A. Alexander, 2013: Tracking pathogen transmission at the human-wildlife interface: banded mongoose and *Escherichia coli*. *EcoHealth*, 10, 115-128. *doi:* 10.1007/s10393-013-0838-2.

R Core Team, 2016: *R: A Language and Environment for Statistical Computing (R.app GUI 1.69)*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Relun, A., F. Charrier, B. Trabucco, O. Maestrini, S. Molia, D. Chavernac, V. Grosbois, F. Casabianca, E. Etter and F. Jori, 2015: Multivariate analysis of traditional pig management practices and their potential impact on the spread of infectious diseases in Corsica. *Prev. Vet. Med.*, 121, 246-256. *doi:* 10.1016/j.prevetmed.2015.07.004.

Richomme, C., M. Boadella, A. Courcoul, B. Durand, A. Drapeau, Y. Corde, J. Hars, A. Payne, A. Fediaevsky and M. L. Boschiroli, 2013: Exposure of Wild Boar to *Mycobacterium tuberculosis* Complex in France since 2000 Is Consistent with the Distribution of Bovine Tuberculosis Outbreaks in Cattle. *Plos One*, 8. *doi:* 10.1371/journal.pone.0077842.

Richomme, C., M. L. Boschiroli, J. Hars, F. Casabianca and C. Ducrot, 2010a: Bovine tuberculosis in livestock and wild boar on the Mediterranean island, Corsica. *J. Wildl. Dis.*, 46, 627-631. *doi:* 10.7589/0090-3558-46.2.627.

Richomme, C., S. A. Lacour, C. Ducrot, E. Gilot-Fromont, F. Casabianca, O. Maestrini, I. Vallée, A. Grasset, J. van der Giessen and P. Boireau, 2010b: Epidemiological survey of trichinellosis in wild boar (*Sus scrofa*) and fox (*Vulpes vulpes*) in a French insular region, Corsica. *Vet. Parasitol.*, 172, 150-154. *doi:* 10.1016/j.vetpar.2010.04.026.

Rödiger, S., T. Kramer, U. Frömmel, J. Weinreich, D. Roggenbuck, S. Guenther, K. Schaufler, C. Schröder and P. Schierack, 2015: Intestinal *Escherichia coli* colonization in a mallard duck population over four consecutive winter seasons. *Environ. Microbiol.*, 17, 3352-3361. *doi:* 10.1111/1462-2920.12807.

Ruiz-Fons, F., J. Segalés and C. Gortázar, 2008: A review of viral diseases of the European wild boar: effects of population dynamics and reservoir role. *Vet. J.*, 176, 158-169. *doi:* 10.1016/j.tvjl.2007.02.017.

Rwego, I. B., T. R. Gillespie, G. Isabirye-Basuta and T. L. Goldberg, 2008a: High rates of *Escherichia coli* transmission between livestock and humans in rural Uganda. *J. Clin. Microbiol.*, 46, 3187-3191. *doi:* 10.1128/JCM.00285-08.

Rwego, I. B., G. Isabirye-Basuta, T. R. Gillespie and T. L. Goldberg, 2008b: Gastrointestinal bacterial transmission among humans, mountain gorillas, and livestock in Bwindi Impenetrable National Park, Uganda. *Conserv. Biol.,* 22, 1600-1607. *doi:* 10.1111/j.1523-1739.2008.01018.x.

Sánchez, S., R. Martinez, A. Garcia, J. Blanco, J. E. Blanco, M. Blanco, G. Dahbi, C. López, A. Mora, J. Rey and J. M. Alonso, 2009: Longitudinal study of Shiga toxin-producing *Escherichia coli* shedding in sheep feces: persistence of specific clones in sheep flocks. *Appl. Environ. Microbiol.,* 75, 1769-1773. *doi:* 10.1128/AEM.02043-08.

Schierack, P., N. Walk, K. Reiter, K. D. Weyrauch and L. H. Wieler, 2007: Composition of intestinal Enterobacteriaceae populations of healthy domestic pigs. *Microbiology,* 153, 3830-3837. *doi:* 10.1099/mic.0.2007/010173-0.

Springer, A., A. Mellmann, C. Fichtel and P. M. Kappeler, 2016: Social structure and *Escherichia coli* sharing in a group-living wild primate, Verreaux's sifaka. *BMC Ecol.,* 16, 6. *doi:* 10.1186/s12898-016-0059-y.

Swaminathan, B., T. J. Barrett, S. B. Hunter, R. V. Tauxe and C. D. C. PulseNet Task Force, 2001: PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerg. Infect. Dis.,* 7, 382-389. *doi:* 10.3201/eid0703.010303.

Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing and B. Swaminathan, 1995: Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.,* 33, 2233-2239. *doi*.

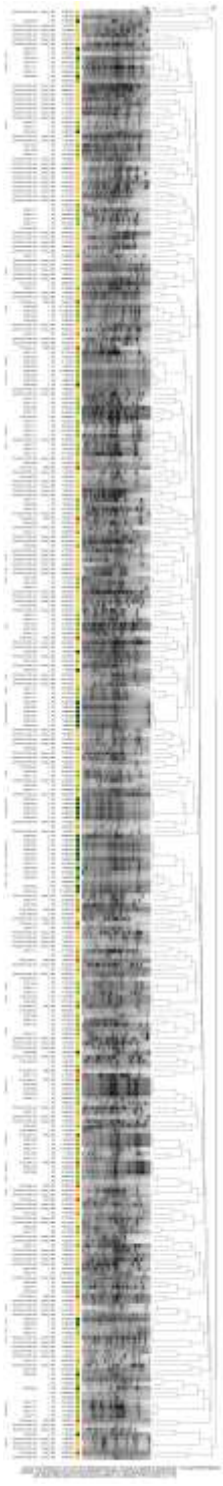
Trabucco, B., F. Charrier, F. Jori, O. Maestrini, D. Cornelis, E. Etter, S. Molia, A. Relun and F. Casabianca, 2014: Stakeholder's practices and representations of contact between domestic and wild pigs: a new approach for disease risk assessment? *Acta Agric. Slov.,* Suppl. 4, 117-122. *doi*.

Vale-Goncalves, H. M., J. A. Cabral, M. C. Faria, M. Nunes-Pereira, A. S. Faria, O. Veloso, M. L. Vieira and M. N. Paiva-Cardoso, 2015: Prevalence of *Leptospira* antibodies in wild boars (*Sus scrofa*) from Northern Portugal: risk factor analysis. *Epidemiol. Infect.,* 143, 2126-2130. *doi:* 10.1017/S0950268814003331.

VanderWaal, K. L., E. R. Atwill, L. A. Isbell and B. McCowan, 2014: Linking social and pathogen transmission networks using microbial genetics in giraffe (*Giraffa camelopardalis*). *J. Anim. Ecol.,* 83, 406-414. *doi:* 10.1111/1365-2656.12137.

Wyckoff, A. C., S. E. Henke, T. A. Campbell, D. G. Hewitt and K. C. VerCauteren, 2009: Feral swine contact with domestic swine: a serologic survey and assessment of potential for disease transmission. *J. Wildl. Dis.,* 45, 422-429. *doi:* 10.7589/0090-3558-45.2.422.

Supplemental material



Supplemental Figure S1*: Dendrogram of analysis of genomic XbaI-restricted DNA of Escherichia coli isolated from porcine faecal samples.*

Dendrogram [Dice coefficient, UPGMA] embracing 315 individual clones. Cluster of *E. coli* clones (> 95 % similarity or ≤ 3 different fragments) are marked with a black line.