

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

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## Summary

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 *Escherichia 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 of eight wild boars 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

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

## 1 | 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,

Payne et al., 2017; Meng, Lindsay, & Sriranganathan, 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, Segalés, & Gortázar, 2008) and parasites (Richomme, Lacour et al., 2010). 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, Henke, Campbell, Hewitt, & VerCauteren, 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, for example, to assess interactions between sympatric wild and domestic populations or individuals sharing the same environment (Mercat et al., 2016; Rwego, Isabirye-Basuta, Gillespie, & Goldberg, 2008; Springer, Mellmann, Fichtel, & Kappeler, 2016; VanderWaal, Atwill, Isbell, & McCowan, 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 (Springer et al., 2016; VanderWaal et al., 2014). 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 whether 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, Relun et al., 2017). 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.

## 2 | MATERIAL AND METHODS

### 2.1 | 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 are mainly conducted in traditional free-range farming systems, which are stretched out over large surface areas with a median size of 557 km<sup>2</sup> (Dubost, 2001), encompassing a mosaic of mountainous pastures and plain areas. Traditionally, Corsican pig production takes

advantage of outdoor resources (chestnuts, acorns, etc.) 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).

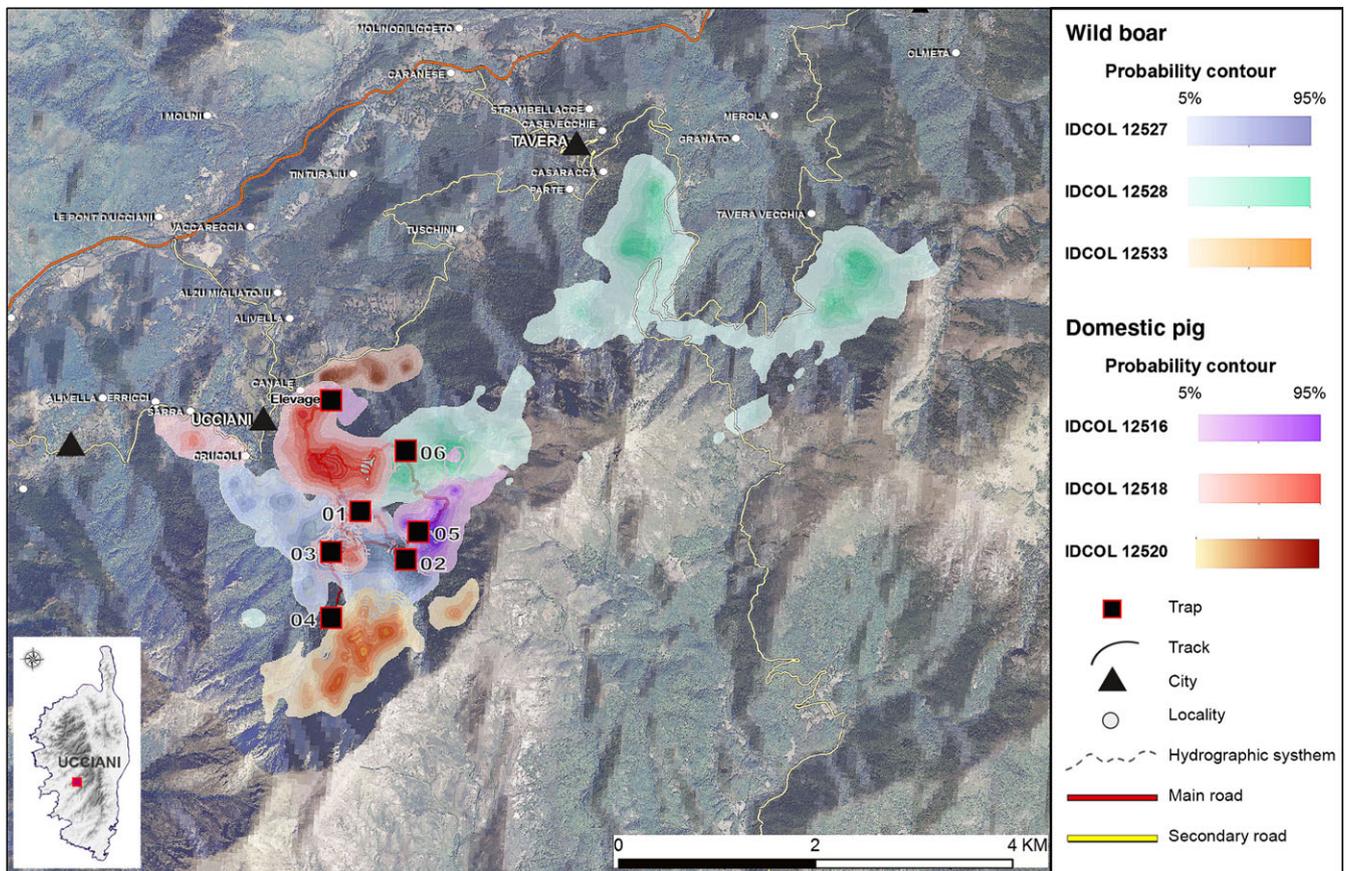
Localization of pig groups in this large and diverse territory varies during the course of the year. During the winter months when natural resources are scarcer, they tend to remain close to the farm for supplementary feeding and reproduction. During autumn and early winter (coinciding with the chestnut 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 (Jori, Relun et al., 2017; Trabucco et al., 2014), 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%–10% of the population practices hunting), with around 30,000 wild boars 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 & Casabianca, 1998; Relun et al., 2015).

### 2.2 | Telemetry protocol

#### 2.2.1 | 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 six corral traps located in the farm rangeland (Figure 1). Corral traps were



**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 three wild boar and three domestic pigs

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<sup>®</sup>, Virbac, Carros, France) from a short distance with pistols (Dan-Inject ApS, Børkop, Denmark), blood sampled, ear-tagged, equipped with a GPS-GSM collar (Vectronic Aerospace GmbH, Berlin, Germany) and released from the corral trap once completely awake. GPS-GSM collars were scheduled to acquire locations at 1-hr fix intervals. The field operations conformed to French legal requirements regarding capture and tracking protocols on large ungulates (Prefectural order No 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.

### 2.2.2 | Domestic pigs

Early October 2013, that is, 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-hr fix intervals.

## 2.3 | Faecal sampling

### 2.3.1 | Wild boar

Of the nine wild boars captured in corral traps, eight were sampled for faecal material directly from the rectum when they were immobilized (sounder WB<sub>GPS</sub>).

In addition, a total of 47 faecal samples (sounder WB<sub>control</sub>) 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.

### 2.3.2 | Domestic pigs

Seven of the 10 domestic sows equipped with collars (group DP<sub>0</sub>) were sampled for faecal material during the tracking period. In addition, starting in October 2014, two groups (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 four piglets on 17 October 2014, resulting in 21 faecal samples (group DP<sub>1</sub>). Sow 2 gave birth to three piglets on 9 February 2015,

resulting in 12 faecal samples (group DP<sub>2</sub>). Faecal samples from domestic pigs were collected directly from the ground shortly after defecation and stored at  $-80^{\circ}\text{C}$  until shipping on ice to FLI (Friedrich-Loeffler-Institut) in Germany for subsequent analysis.

## 2.4 | Isolation of coliform bacteria

The quantification and isolation of coliform bacteria from the faeces as well as the storage of single colonies were 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 were from domestic pigs and from wild boar, respectively.

## 2.5 | 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 were performed as previously described (Barth et al., 2017; Geue et al., 2010). In addition to restriction with *Xba*I, selected agarose plugs were digested with 15 U *Av*all or 15 U *Spe*I (New England Biolabs GmbH, Frankfurt/Main, Germany) at  $37^{\circ}\text{C}$  (*Spe*I 18 hr, *Av*all 5 hr). For separation of *Av*all- 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  $120^{\circ}$  during 19 hr. 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 unweighted pair group method with arithmetic mean (UPGMA).

## 2.6 | 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.

Geospatial analysis focused on a period starting in October 2013 for 3 months (autumn and early winter) using movement data from six domestic pigs and four wild boars. 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  $59.3 \pm 26.4\%$  from domestic pigs and  $48.5 \pm 33.2\%$  wild boar (mean  $\pm$  SD), respectively (Table 1). Spatial behaviour of GPS-tracked individuals was characterized 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 & Cornélis, 2010). Daily distances ranged by GPS-tracked individuals were calculated using 24-hr 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.

**TABLE 1** Telemetry protocol summary

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



**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		<i>E. coli</i> isolates <sup>†</sup>		<i>Xba</i> I-PFGE clones <sup>†</sup>	
			Number faecal samples [positive/total]	Cfu/g faeces (mean ± SEM [min–max])	Total	Number per sample [mean ± SD]	Total	Number per sample [mean ± SD]
Wild boar	WB <sub>GPS</sub> (with GPS collar)	Once, Jun 2013 till Aug 2013	7/8	$5.2 \times 10^7 \pm 3.5 \times 10^7$ (0– $2.6 \times 10^8$ )	64	$7.86 \pm 2.55$	18	$2.57 \pm 1.90$ <sup>e</sup>
	WB <sub>control</sub> (without GPS collar)	Once, Nov 2014 till Feb 2015	42/47	$1.3 \times 10^7 \pm 1.2 \times 10^7$ <sup>a,b</sup> (0– $5.6 \times 10^8$ )	410	$8.31 \pm 2.51$	127	$3.02 \pm 1.81$ <sup>f</sup>
Domestic pigs	DP <sub>0</sub> (young sows)	Once, Oct 2013 till Nov 2013	3/7	$21.4 \pm 10.1$ <sup>a,c,d</sup> (0–50)	30	$9.33 \pm 0.58$	12	$4.00 \pm 2.00$
	DP <sub>1</sub> (sow no. 1 with 4 piglets)	Four times, Oct 2014 till Feb 2015	21/21	$2.6 \times 10^7 \pm 1.7 \times 10^7$ <sup>d</sup> (500– $3.2 \times 10^8$ )	203	$9.14 \pm 1.46$	123	$5.86 \pm 1.96$ <sup>e,f,g</sup>
	DP <sub>2</sub> (sow no. 2 with 3 piglets)	Four times, Feb 2015 till Jun 2015	11/12	$3.2 \times 10^8 \pm 1.3 \times 10^8$ <sup>b,c</sup> (0– $1.4 \times 10^9$ )	110	$9.73 \pm 0.47$	35	$3.18 \pm 1.10$ <sup>g</sup>
Total	–	–	84/95	$5.7 \times 10^7 \pm 2.1 \times 10^7$ (0– $1.4 \times 10^9$ )	731	$8.70 \pm 2.12$	315	$3.71 \pm 2.10$

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,  $p < .05$ ).

<sup>†</sup>Mean number of *E. coli* isolates; and clones are related only to positive faecal samples.

**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 <sub>GPS</sub> (with GPS collar)	WB <sub>control</sub> (without GPS collar)	DP <sub>0</sub> (young sows)	DP <sub>1</sub> (sow 1 with 4 piglets)	DP <sub>2</sub> (sow 2 with 5 piglets)
WB <sub>GPS</sub>	0	0	0	3	0
WB <sub>control</sub>	–	8	0	0	0
DP <sub>0</sub>	–	–	0	1	0
DP <sub>1</sub>	–	–	–	15	5
DP <sub>2</sub>	–	–	–	–	4

DP, domestic pigs; WB, wild boar.

*Xba*I-restricted DNA, dice, with 2.5% tolerance and unweighted pair group method with arithmetic mean (UPGMA). Numbers listed under the same heading for column and row refer to clusters restricted to the given animal group.

five samples sharing clones with six other samples, respectively. In particular, we found connections between animals within group DP<sub>1</sub> or within group DP<sub>2</sub>, 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, for example, part D of the network included samples from groups DP<sub>1</sub>, DP<sub>2</sub> and WB<sub>GPS</sub>.

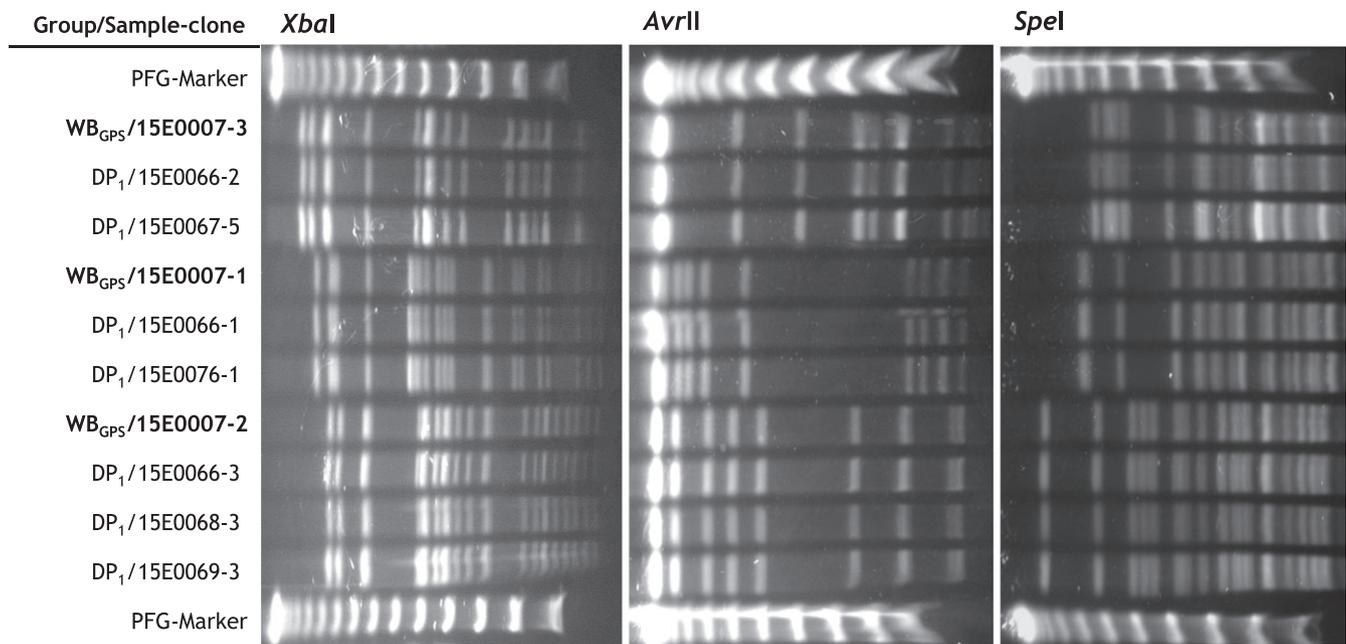
For statistical purposes, we analysed the global network and formed subnetworks of the groups DP<sub>1</sub>, DP<sub>2</sub> and WB<sub>control</sub>, respectively. The subnetworks were built by removal of all connections to samples of other groups. While the density of the global network (number of present of all possible interrelations) was 2.1%, the

density of the subnetwork DP<sub>1</sub> was 16.2%, the one formed by DP<sub>2</sub> was 54.5%, and the one consisting of the WB<sub>control</sub> group was 0.9%. The diameter of the global network (shortest path between the two furthest samples) encompassed nine other samples, the diameter of the subnetwork DP<sub>1</sub> involved eight samples and those of DP<sub>2</sub> and WB<sub>control</sub> two 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<sub>2</sub> (with many *E. coli* clones shared) and in WB<sub>control</sub> (with few *E. coli* clones shared), whereas animals are primarily connected indirectly, that is, through intervention of many others, in the global network and DP<sub>1</sub>. Calculating the k-cores (a maximal subgraph that contains animals having at least k shared clones) and plotting them in the network, all but three samples of DP<sub>2</sub> 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<sub>1</sub> grouped in the 5-, 4- and 3-core (parts D, C and B, respectively), with one DP<sub>2</sub> and one WB<sub>GPS</sub> 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<sub>0</sub>) and again once in 2015 (DP<sub>1</sub>; part E of the network) or clones in part D of the network sampled in 2013 (WB<sub>GPS</sub>), 2014 (DP<sub>1</sub>) and 2015 (DP<sub>1</sub>, DP<sub>2</sub>) (Figure 3, Figure S1).

## 4 | 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

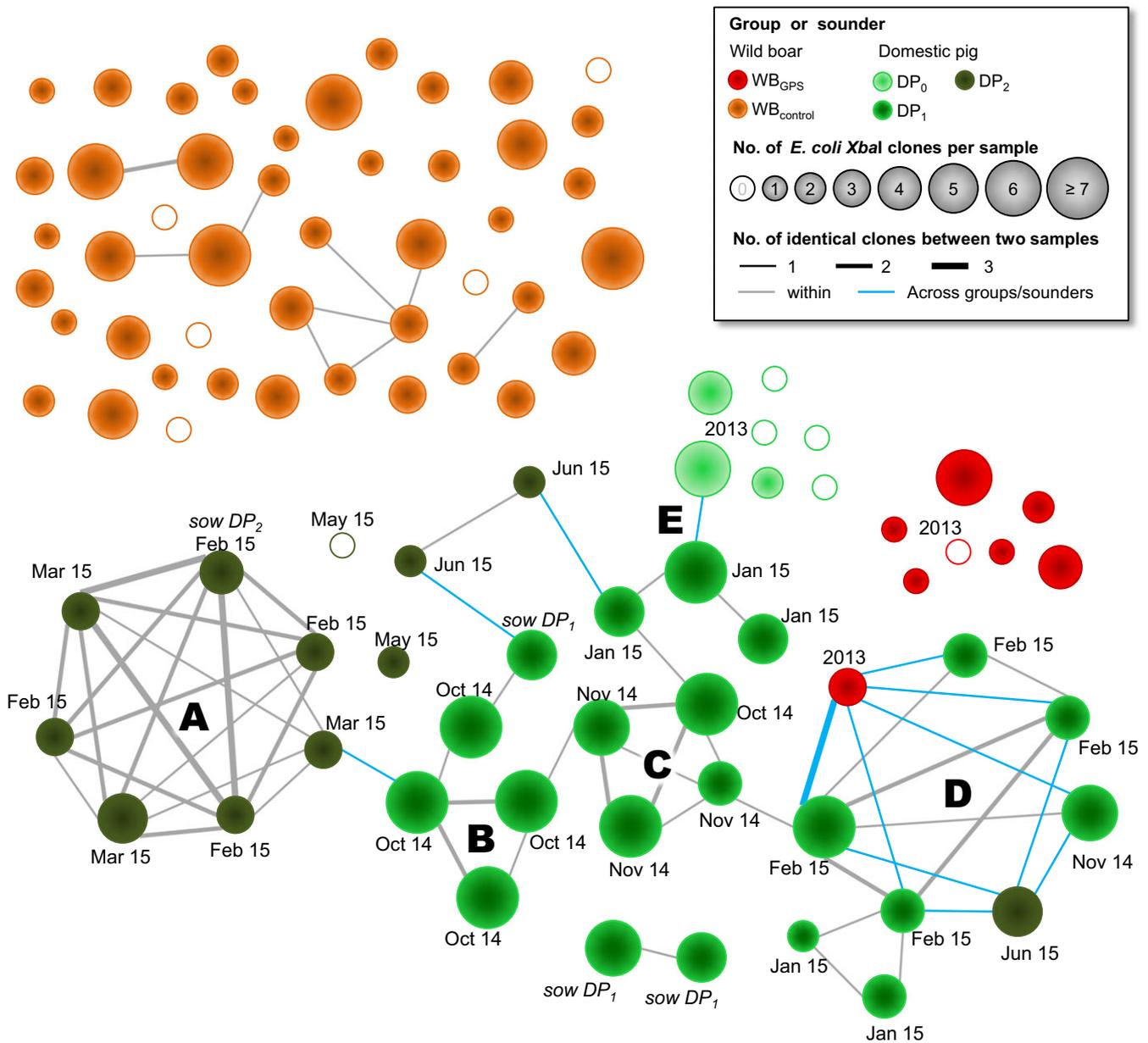


**FIGURE 2** Confirmation of clonality of identical *E. coli* *Xba*I clones present in faeces from wild boar (sounder WB<sub>GPS</sub>) and from domestic pigs (group DP<sub>1</sub>) by PFGE of DNA digested with *Avr*II and *Spe*I. DP, domestic pigs; WB, wild boar; Marker: Lambda Ladder PFG-Marker

animals (Richomme, Lacour et al., 2010). 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 have led to a notable increase in wild boar populations as evidenced by the approximately 30,000 wild boars 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, Relun et al., 2017) 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 (Charrier et al., 2018; Pavio et al., 2016; Richomme, Boschioli, Hars, Casabianca, & Ducrot, 2010). 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 (Jori, Relun et al., 2017; Trabucco et al., 2014). 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, Relun et al., 2017). 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, Ponder, & Alexander, 2013), primates (Rwego, Isabirye-Basuta et al., 2008) and humans (Rwego, Gillespie, Isabirye-Basuta, & Goldberg, 2008). 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, Relun et al., 2017).

*Escherichia coli* is an ideal candidate to monitor potential contacts between wild boar and domestic pigs, because 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 heterogeneous and can survive in the environment, depending on temperature and moisture, for more than 1 year (Fremaux et al., 2008; Gordon & Cowling, 2003; Schierack, Walk, Reiter, Weyrauch, & Wieler, 2007). 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

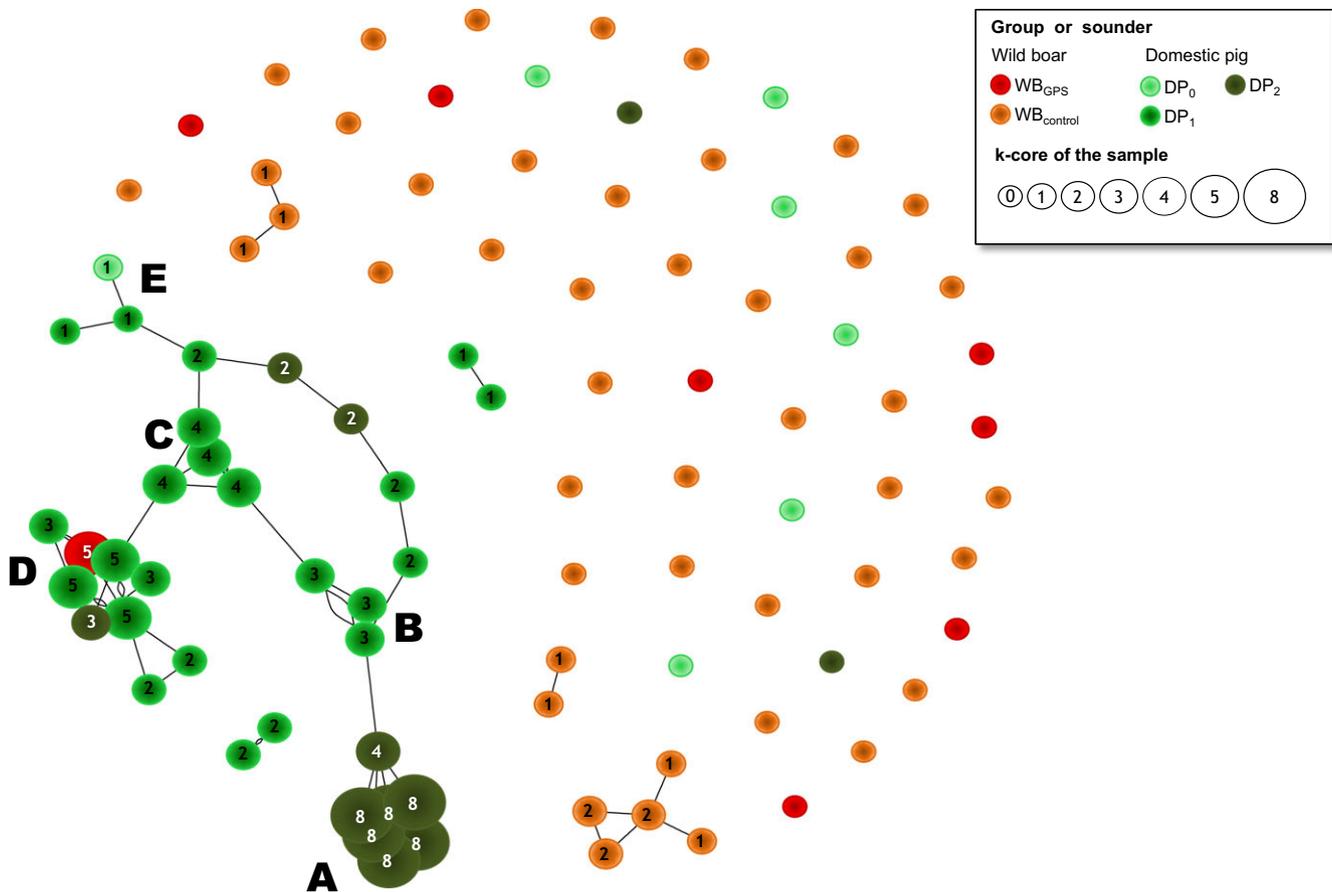


**FIGURE 3** Occurrence of identical *E. coli* *Xba*I clones in faeces from wild boar and domestic pigs. Each circle stands for one faecal sample and represents the number of clones within the sample by the size of the circle. If identical *E. coli* *Xba*I clones occurred in different samples, the circles 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

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, Cornick, & Moon, 2002; Katouli et al., 1995). In the current study, 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<sub>1</sub> and DP<sub>2</sub>, 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<sub>0</sub> and again in one piglet of sow DP<sub>1</sub>

in January 2015. These data indicate 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<sub>GPS</sub> group. With one to six *E. coli* clones per sample (mean 2.57), this number is slightly lower than in our previous study in captive animals (mean 3.00–3.63 *E. coli* clones per sample; [Barth et al., 2017]). From the animals of the WB<sub>GPS</sub> group, one wild boar excreted three individual *E. coli* *Xba*I clones; each of these clones was also found in at least two different



**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

domestic pigs of group DP<sub>1</sub>, directly connecting this wild boar with five different samples from DP<sub>1</sub> pigs. None of the clones from the remaining WB<sub>GPS</sub> faecal samples were found in any other sample. Although a direct link to the domestic pigs tested was found in only one of eight tested WB<sub>GPS</sub> (12.5%), this interrelation was very intense as it encompassed the entire pig group tested (at least four different animals of group DP<sub>1</sub>), rather than only one animal. The clonality of the transmitted clones was confirmed 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 snuffing, 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 ( $5 \times 10^8$  cfu/dose) that presumably exceeded infectious doses that can be expected to occur under field conditions by far (Cornick & VuKhac, 2008). In the current study, an intense transmission of *E. coli XbaI* clones between sow DP<sub>2</sub> and its offspring was obvious in the first 2 months after birth and supported by demonstration of the highest density level in the subnetwork DP<sub>2</sub> 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<sub>1</sub> by detection of an identical *E. coli XbaI* clone. Similar observations were made when PFGE *XbaI* clones of CTX-M-producing *E. coli* were monitored in five sows and two of their respective piglets in an intensive pig production systems (Hansen, Bortolaia, Damborg, & Guardabassi, 2014). To further support the method applied, none of the 127 *E. coli XbaI* clones from the wild boar control group (WB<sub>control</sub>) 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<sub>control</sub> subnetwork 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 and 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 foodborne outbreaks, involving, for example, non-typhoidal *Salmonella* sp., *E. coli* O157:H7 or *Listeria monocytogenes* (Swaminathan, Barrett, Hunter, Tauxe, & PulseNet Task Force, 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, Zanoni, Tagliabue, Lavazza, & Alborali, 2013), *Leptospira* sp. (Vale-Goncalves et al., 2015), *Mycobacterium bovis* (Naranjo, Gortazar, Vicente, & de la Fuente, 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 immobilized 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, Payne et al., 2017; Kukielka et al., 2016).

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## CONFLICT OF INTEREST

The authors declare that no conflicts of interest exist.

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## SUPPORTING INFORMATION

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