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Wild pigs (*Sus scrofa*) population as reservoirs for deleterious mutations in the RYR1 gene associated with Porcine Stress Syndrome

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ABSTRACT

Porcine Stress Syndrome (PSS) is a disorder codified by the ryanodine receptor 1 gene (RYR1) and affects both animal welfare and the quality of the meat product. As a consequence, individuals with this syndrome generate great worldwide economic losses in the porcine industry. In Argentina, the Buenos Aires Province is the most involved on this activity, and productions are to be in open field with a higher frequency of pigs with diverse pathologies. On the other hand, the biggest and oldest wild pigs population is located on the Atlantic coast of Buenos Aires Province, which presents a continuous bidirectional flow of individuals with the productive areas nearby. The aim of this study is to detect the presence of the RYR1 deleterious allele in the wild population from the Atlantic coast of Buenos Aires, in order to evaluate its possible role as a genetic reservoir for said allele. For this purpose, 106 wild pigs from 28 sites were studied, finding a 6.6% of carrier individuals, indicating that the wild population is not free of this allele. This constitutes the first analysis to detect the presence of the RYR1 deleterious allele, associated to the PSS in wild pigs from Argentina, being one of the few studies to report it worldwide and suggesting wild pigs populations to be a possible genetic reservoir for this disease.

1. Introduction

Pork meat is one of the most consumed worldwide, not only for its flavour, but also because it constitutes a great source of protein, energy and micronutrients (Salas & Mingala, 2017). In the last decades, its intake has increased exponentially along with a demand for leaner meat, leading to the attainment of new porcine genetic lines in order to obtain meat in less time, resulting in pigs with a greater muscle-fat ratio (Bonelli & Schifferli, 2001). However, this genetic breeding carried the presence of undesired traits, as is the case of Porcine Stress Syndrome (PSS) or Malignant Hyperthermia (MH).

PSS is a hereditary, monogenic and recessive autosomal syndrome, generated from a mutation in the Ryanodine Receptor 1 gene (RYR1), commonly known as the Halothane gene. Said mutation causes a neuromuscular disorder in the pig, as it is expressed both in the brain Purkinje cells and the skeletal muscle (Bonelli & Schifferli, 2001; Fujii et al., 1991; Smith & Bampton, 1977). Individuals carrying one deleterious allele exhibit a C to T transition in the nucleotide 1843 (c.1843C>*T*), generating a substitution of arginine to a cysteine at position 615 in the aminoacidic sequence (p.Arg615Cys), thus producing

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an abnormal protein Bonelli and Schifferli (2001); Fujii et al. (1991); Smith and Bampton (1977). In skeletal muscle, RYR1 codes for a calcium channel of the sarcoplasmic reticulum which, in recessive homozygosity, induces hyperactivation, leading to several pathologies in the pig, such as muscle rigidity, hyperthermia, cardiac arrhythmias and sudden death, amongst others (Ilie, Băcilă, Cean, Cziszter & Neo, 2014; Salas & Mingala, 2017). PSS can manifest in any condition that provokes physical stress to the pig as are transport, vaccination, castration, giving birth or exposure to high temperatures (Ilie et al., 2014). Additionally, PSS affects not only animal welfare, but also the meat quality, generating a pale, soft and exudative (PSE) product for fresh consumption, as well as dark, dry and tough meat, and precluding even its use as stationed products. This generates a meat of low quality for the standards in the current market (Ghio & Lucero de la Sota, 2014; Ilie et al., 2014). Therefore, the presence of pigs with PSS causes vast economic losses and constitutes a matter of great concern in the porcine industry worldwide.

In Argentina, the increase on the demand for lean meat towards the end of the last century resulted in a massive use of breeds as Pietran and Belgian Landrace or commercial hybrids, which allowed to meet the market's requirements. Coupled to this decision, appeared a wider PSS expression in production systems, where Argentina became one of the most affected countries in Latin-America (Carduza et al., 2009; Ghio & Lucero de la Sota, 2014; Lloveras et al., 2008).

Furthermore, the national porcine production is actively growing, with Buenos Aires as one of the main productive Provinces, representing 25% of the national productive activity (Brunori & Juarez, 2008; Figueroa, 2020). There, 75% of the production systems are characterized by being developed in extensive fields at all stages, with few reproductive females, precarious facilities and infrastructure of the establishments, and little or no access to veterinary advice for adequate health plans, as well as reduced possibilities of commercial genetic line acquisition. Most of these productions are for local consumption and, to a lesser degree, for surplus sales (Brunori & Juarez, 2008). One of the main problems with these productive systems is that, given their reduced size, they usually have less than 10 reproductive mothers. As a result, there is an increase in the probability of consanguineous mating, leading to an increment in homozygosis, coupled with the manifestation of deleterious recessive alleles that are otherwise masked; such is the case with the RYR1 gene.

Added to this situation, the large yearly economic fluctuations in Argentina generate an unstable market that not only complicates the productions' success in the productive stratum, but also tends to result in culmination of these systems for lack of economic gain and difficulty in maintaining correct breeding conditions (Figueroa, 2020). Furthermore, these productions rarely acquire modern commercial breeds from international companies, thus their productivities are lower and their insertion in the commercial market almost impossible. However, they do insert genetic variation through local breeds, which hold a greater biodiversity and, therefore, a wider adaptability to the surrounding environment (FAO, 2010; Revidatti, Capellari, Prieto & Delgado, 2005).

The biggest and oldest wild pig population in Argentina is located on the Atlantic coast of the Buenos Aires Province and it descends from domestic Iberic pigs brought by Spanish conquerors (Merino & Carpinetti, 2003). Recently, Acosta, Figueroa, Fernández, Carpinetti and Merino (2019) made use of the mitochondrial marker Control Region and the nuclear Amelogenin gene to confirm this origin, while also proving a strong hybridization with modern breeds, especially in the Mar Chiquita region. This population is constantly growing, facilitated by the omnivorous diet, the ease to adapt to diverse environments and an elevated reproductive rate, in addition to the favourable ecologic conditions of the area such as vegetation, weather, watersheds and the absence of natural predators (Merino & Carpinetti, 2003). Specifically in the Bahía Samborombón area, the number of individuals is estimated at 10,000 wild pigs, with records of a 7.78 individuals/km^2 population density (Merino & Carpinetti, 2003; Pérez Carusi et al., 2015).

Porcine establishments nearby this wild population have the habit of using wild pigs in their productions, with the objective of incorporating desirable characters, as rusticity, maternal abilities and disease resistance, at a low cost (Acosta et al., 2019; Carpinetti, Di Guirolamo, Delgado & Martínez, 2016; Giménez Dixon, 1991). Freeing pigs from these establishments, either at will or accidentally, further contributes to the gene flow between domestic and wild populations (Acosta et al., 2019). It is from this dynamic that the wild population could act as a reservoir for some detrimental genetic combinations, as is the case of the deleterious allele in the RYR1 gene.

In Europe, several studies have focused on searching the deleterious RYR1 allele in wild *Sus scrofa* populations (Ernst, Kuciel & Urban, 2003; Klimas, Klimienė, Mickeikienė & Morkūnienė, 2018; Kuryl, Zurkowski, Urbanski & Wyszynska-Koko, 2004; Müller et al. 2000; Zinovieva et al., 2013), finding it only on wild boars from the Gdańsk forests (Poland) (Borman et al., 2016). In Latin America, similar studies on domestic pigs have identified this allele on a very low frequency of heterozygotes in Colombia (Hernández, Terranova & Muñoz Flórez, 2008; Rodríguez, 2004; Sarabia et al., 2011). In Argentina, studies were only performed on domestic pigs, detecting presence of this detrimental allele, both in homozygote and heterozygote conditions (Figueroa, 2020; Marini et al., 2012).

Given the lack of analysis in wild populations, both of wild pigs and wild boars, this study aims to detect the presence of the RYR1 deleterious allele in wild populations from the Buenos Aires Province coast, Argentina, determining if wild populations are a possible reservoir for this allele.

2. Materials and methods

2.1. Study area and sample collection

The study area is located on the eastern limit of the Pampa Deprimida region (Soriano, León, Sala, Lavado & Deregibus, 1992), including the Bahía Samborombón natural reserve on the central-north part. The area contains a variety of habitats, ranging from intertidal mudflats and creeks, tidal salt marshes, permanent and seasonally flooded freshwater lagoons and marshes, slow-flowing streams, and grasslands to islands of higher ground with trees (mostly *Celtis tala*) and shrubs. The "cangrejales", with very high river crabs (*Chasmagnathus granulata*) densities, are an example of the extremely rich productivity of this area (Soriano et al., 1992). As to the southern area, the dominant landscape is characterized by low beaches, with the presence of coastal dunes ranges. The dunes vegetation is mainly herbaceous with some bushes (Celsi, 2016). The climate throughout the study area is temperate and humid, with an average rainfall of 900 mm per year (Soriano et al., 1992).

A total of $N = 106$ muscle and leather samples were collected from wild pigs (*Sus scrofa*) in 28 localities of the Buenos Aires coast, from Argentina (Fig. 1). These wild specimens comprise a single population, due to the wide home range of the species (these pigs can travel up to 20 km per day in search for food and refuge), and without any geographic barriers for their dispersal (Acosta et al., 2019; Peris Campodarbe, 2019). In addition, each specimen's coat colour was noted, since prior investigations suggest that it could be an indicator of recent hybridization between wild and domestic *Sus scrofa* populations (Canu et al., 2016; Fang, Larson, Soares Ribeiro, Li & Andersson, 2009; Li et al., 2010). The number of pigs to be analysed was decided based on the distribution of the wild population, focusing on the representativeness of animals in all the sites where pig productions are settled. Additionally, we took into consideration accessibility to the collection sites by the provincial agency in charge of its population control.

2.2. Laboratory analysis

Genomic DNA was extracted from all muscle and leather samples by using the phenol chloroform protocol (Sambrook & Russell, 2006) and, depending on the amount of pellet observed, the DNA of each sample

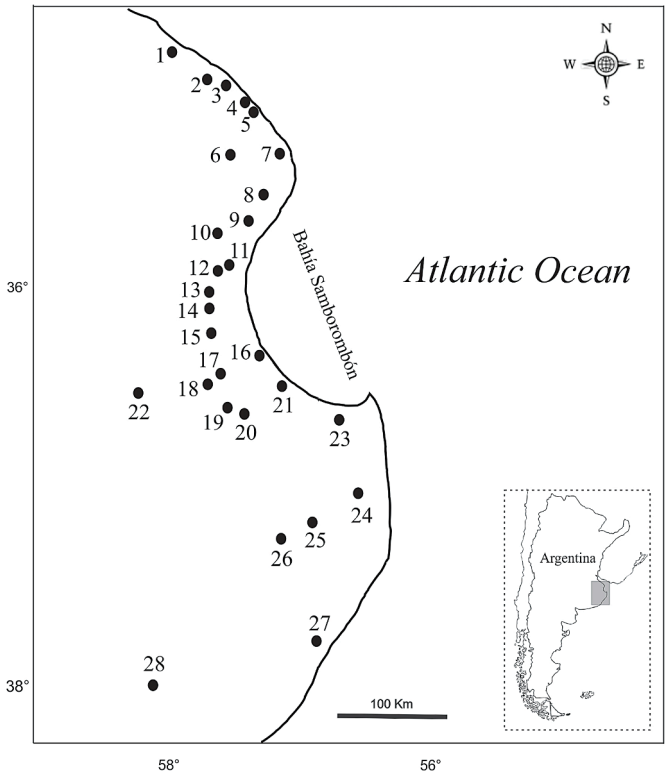

Fig. 1. Sampling localities on the Atlantic coast of the Buenos Aires Province, Argentina. The sampling sites of the $N = 106$ wild pigs used in this study are indicated with a number. 1. Boca Arroyo Espinillo, Atalaya, Magdalena; 2. Ea. El Destino, Magdalena; 3. Ea. San Isidro, Magdalena; 4. Magdalena; 5. Ea. El Bagual, Punta Indio; 6. Verónica, Punta Indio; 7. Ea. Dos de Abril, Punta Indio; 8. Ea. Las Colinas, Bahía Samborombón, Punta Indio; 9. Ea. Barón de Montijo, Bahía Samborombón, Chascomús; 10. Ea. Rincón de López, Bahía Samborombón, Castelli; 11. Canal Aliviador del Salado, Bahía Samborombón, Castelli; 12. Ea. Ensenada de San Martin, Bahía Samborombón, Castelli; 13. Ea. El Principio, Bahía Samborombón, Castelli; 14. Canal San José, Bahía Samborombón, Castelli; 15. Canal 9, Bahía Samborombón, Tordillo; 16. Canal 1, Bahía Samborombón, Tordillo; 17. Ea. Don Miguel, Bahía Samborombón, Tordillo; 18. Ea. El Gato, Bahía Samborombón, Tordillo; 19. Ea. Malele, Bahía Samborombón, Tordillo; 20. Ea. Santa Lucía, Bahía Samborombón, Tordillo; 21. Canal 2, Bahía Samborombón, Tordillo; 22. Canal 9, Bahía Samborombón, Dolores; 23. Campos del Tuyú, Bahía Samborombón, Gral. Lavalle; 24. Paraje Pavón, Gral. Lavalle; 25. Reserva Natural Laguna Salada Grande, Gral. Madariaga; 26. Gral. Madariaga; 27. Mar Chiquita; 28. Balcarce.

was eluted in 50–100 μl of Tris-EDTA Buffer and stored at −20 °C.

The complete set of samples underwent PCR amplification of a 659 bp fragment comprising the RYR1 gene, using the primers described by Otsu, Phillips, Khanna, De Leon & MacLennan, 1992: Forward: 5′-TCCAGTTTGCCACAGGTCCTACCA-3′ and Reverse: 5′-ATTCACCGGAGTGGAGTCTCTGAG-3′. The reaction was set to a final volume of 20μL, containing 25–100 ng of template DNA, 1.5 mM $MgCl_2$, 0.2 μM of each primer, 0.2 mM of each dNTP, 1X reaction buffer, 0.5 U of Taq T-Plus DNA polymerase and ultrapure sterile water to reach final volume. Thermocycling conditions were set at 94 °C for 10 min, followed by 35 cycles of 45 s at 94 °C, 61 °C for 45 s, and 45 s at 74 °C, with a final extension period at 74 °C for 10 min. All amplifications were performed in conjunction with a negative control (distilled water). DNA fragment amplification was confirmed by electrophoresis on 1% m/v agarose gel, stained with ethidium bromide (10 mg/ml) and visualized under UV light.

Enzymatic digestion was performed in a final volume of 30 μL, including 10 μL (~ 0.1–0.5 μg of DNA) of the PCR product, 1 μL of Alw21l endonuclease (BsiHKAI, Thermo Fisher Scientific) and 2 μL Buffer O 10X, together with nuclease-free water to reach final volume. Samples were incubated at 37 °C for 20 min and 65 °C for 10 min to stop the reaction.

Pigs with the recessive allele (h) have a c.1843C>*T* mutation which eliminates a HinPI endonuclease-recognition site and, simultaneously, generates a recognition site for the BsiHKAI enzyme, enabling recessive allele recognition in an agarose gel. In the case of the dominant genotype (HH), two bands can be observed, one of 524 and another of 135 bp (the latter band is a positive control cleavage site). Meanwhile, the recessive genotype (hh) is manifested with three bands of 358, 166 and 135 bp, due to the combination of the BsiHKAI enzyme restriction site and the positive control. Finally, the heterozygous genotype (Hh) manifests with four bands of 524, 358, 166 and 135 bp. The enzymatic reaction products were observed on a 2% m/v agarose gel, stained with ethidium bromide (10 mg/ml), and visualized under UV light.

From the results obtained through PCR/RFLP, allelic and genotypic frequencies of the RYR1 *locus* were calculated for the wild pig population.

In order to determine if there is a possible association between coat colour and presence or absence of the RYR1 deleterious allele, we employed the statistical tool Chi-squared test (χ^2) with a significance level of α=0.05 through the RStudio software (https://rstudio.com/), under the null hypothesis that there is no association between the variables of interest.

3. Results

In the complete dataset ($N = 106$) of specimens belonging to the population of wild pigs from the coast of Buenos Aires, 7 heterozygotes (Hh; 6.6%) and 99 dominant homozygotes (HH; 93.4%) specimens were identified. None of the samples belonged to recessive homozygote (hh) individuals. Allelic frequencies were $p_{(H)}$=0.965 and $q_{(h)}$=0.035.

Heterozygote individuals found for the RYR1 gene were from the farms Barón de Montijo (ID: 9), Ensenada de San Martín (ID: 12) and Don Miguel (ID: 17) and the localities of Gral. Madariaga (ID: 26) and Mar Chiquita (ID: 27). In both Ensenada de San Martín (ID: 12) and Mar Chiquita (ID: 27) we found two carrier individuals, while amongst the rest of the localities and farms, only one heterozygote specimen was identified (Table 1).

Regarding coloration coats, most sampled individuals were black as expected for wild pigs ($N = 98$); while on a lower proportion we found red-brown ($N = 5$), mottled ($N = 2$) and bay ($N = 1$), suggesting a possible recent hybridization with domestic pigs from the area (Table 1). From these, only the mottled hog from the Ensenada de San Martín farm (ID: 12) presented the RYR1 deleterious allele. The six remaining heterozygotes were of black coating. The statistical association analysis, performed through Chi-squared test ($\chi^2_{\alpha=0,05}$) for the coat colour and RYR1 allele variables was of $p = 0.087$; hence, we did not reject the null hypothesis and therefore, we did not find an association between colour coating and the presence of this detrimental allele.

4. Discussion

This study constitutes the first report on the presence of the deleterious allele for the RYR1 gene in a wild pig population (*Sus scrofa*) from Argentina, associated to the Porcine Stress Syndrome (PSS).

PSS arose as a consequence of the paradigm shift in porcine production during the XX century, when characters associated to leaner meat began to be positively selected (Bonelli & Schifferli, 2001). Consequently, the frequency of carrier (Hh) and affected individuals (hh) escalated, reaching extreme values in cases like the Pietran and Belgian Landrace breeds, where 80% of their population specimens were found to possess the deleterious allele in a homozygous (hh) condition (Fujii et al., 1991; Ghio & Lucero de la Sota, 2014). Likewise, other breeds of productive interest like Poland China, Duroc and Yorkshire, as well as commercial hybrids of these, acquired the detrimental allele, but on a lower frequency, further contributing to its dispersion (Fujii et al.,

1991). Given the economic losses caused by this syndrome, porcine breeding companies tried to eradicate it, promoting the use of other breeds like the Large White, which has similar meat quality and composition characteristics, but does not express PSS (Ghio & Lucero de la Sota, 2014). Nonetheless, up to date there are still domestic and wild pigs worldwide carrying this detrimental allele (Borman et al., 2016; Feng et al., 2012; Figueroa, 2020; Hernández et al., 2008; Marini et al., 2012; Rui & Shuiming, 2020; Sánchez et al., 2006; Soma, van Marle-Köster & Frylinck, 2014).

Argentina is not an exception to this paradigm; presence of the RYR1 deleterious allele has been reported on the main productive provinces in the country: Buenos Aires, Córdoba, Santa Fe, Chaco and Tucumán, both on intensive confinement systems and on extensive farming productions with low monetary investment (Figueroa, 2020; Marini et al., 2012).

In the Buenos Aires Atlantic coast, porcine productions are mainly extensive, with precarious infrastructure and without economic possibilities to acquire bred genetic lines. For this reason, the use of individuals from wild populations is imbedded in the productive culture, as it is an abundant genetic resource close by the production sites and without cost (Acosta et al., 2019; Carpinetti et al., 2016; Giménez Dixon, 1991). Wild females are used as reproductive mothers with domestic males, because they have great maternal abilities and disease resistance, added to their diminished aggressiveness in comparison to wild males (Acosta et al., 2019; Carpinetti et al., 2016). Instead, the capture of wild males has the sole purpose of elaborating charcuterie or obtaining fat and it is limited to the winter season (Giménez-Dixon, 1991). In addition to this, individuals from production systems, both wild and domestic, are occasionally freed willingly or accidentally, which produces a greater gene flow between these population types. This close relationship amongst different morphotypes of the *Sus scrofa* species in our study zone, enables the transmission of characteristic alleles from domestic productions to wild populations and vice versa.

Wild specimens of the species, both wild pigs and wild boars, are expected to not present the RYR1 deleterious allele, given their wild condition and the low frequency in which it is found in domestic populations. Several investigations have been carried out in Europe in search of the deleterious allele RYR1 in wild populations of *Sus scrofa*, and in none of them has the allele been found (Ernst et al., 2003; Klimas et al., 2018; Kuryl et al., 2004; Müller et al. 2000; Zinovieva et al., 2013), except for the research of Borman et al., 2016. They studied wild boar populations in the forests of Gdańsk (Poland), identifying an unexpectedly high frequency for this allele, on a proportion of 65% and 28% for sick (hh) and carrier (Hh) individuals, respectively, where the cross-breeding of wild population with domestic pigs in neighbouring areas is identified as the most probable cause. Moreover, due to the Iberic origin of the wild population from the Buenos Aires Atlantic coast, which do not contain the deleterious allele for the gene under study, along with the absence of modern improved breeds (Acosta et al., 2019), it would be reasonable to expect a low or nil proportion of individuals carrying the allele. However, in this study we found 6.6% of the individuals were carriers of the deleterious variant, indicating that the wild population is not free from it and that it might be acting as a genetic reservoir for this detrimental allele. In contrast, the percentage found in the wild population of the coast of Buenos Aires is low, even when comparing our results with those obtained in the central productive regions of Argentina, where values for the Buenos Aires Province were found to reach 13% heterozygous (Hh) and 3% recessive homozygous (hh), while for the provinces of Córdoba, Santa Fe, Chaco and Tucumán, 31% heterozygous (Hh) and 4.2% homozygous have been reported. This difference could be due to the sources used by local pig productions in the study area to enrich their gene pools, with low investment in modern breeds (Figueroa, 2020; Marini et al., 2012).

Regarding the *Sus scrofa* species coloration, genetic and morphological studies indicated that the dark coating has been traditionally

Table 1
Results obtained for wild pigs by sampling site. IDs are indicated making reference to the map. Additionally, sampling localities, geographic coordinates, number of samples per site (N), RYR1 gene genotypes and coat colours can be observed.

ID Site	Sampling localities (Department)	Geographic Coordinates		N	RYR1 gene		Coat Colour			
		Latitude	Longitude		HH	Hh	Black	Red-brown	Mottled	Bay
1	Boca Arroyo Espinillo, Atalaya, (Magdalena)	34° 59′ 2.17″ S	57° 36′ 34.489″ O	2	2	–	2	–	–	–
2	Ea. El Destino, (Magdalena)	35° 8′ 2.69″ S	57° 23′ 13.919″ O	1	1	–	1	–	–	–
3	Ea. San Isidro, (Magdalena)	35° 8′ 49.47″ S	57° 22′ 29.38″ O	1	1	–	1	–	–	–
4	Magdalena, (Magdalena)	35° 10′ 45.8″ S	57° 18′ 17.56″ O	1	1	–	1	–	–	–
5	Ea. El Bagual, (Punta Indio)	35° 13′ 11.741″ S	57° 17′ 16.248″ O	2	2	–	2	–	–	–
6	Verónica, (Punta Indio)	35° 25′ 29.685″ S	57° 17′ 31.723″ O	1	1	–	1	–	–	–
7	Ea. Dos de Abril, (Punta Indio)	35° 34′ 55″ S	57° 15′ 21″ O	2	2	–	2	–	–	–
8	Ea. Las Colinas, Bahía Samborombón, (Punta Indio)	35° 30′ 60,3″ S	57° 11′ 22,4″ O	2	2	–	2	–	–	–
9	Ea. Barón de Montijo, Bahía Samborombón, (Chascomús)	35° 44′ 34″ S	57° 22′ 41″ O	4	3	1	4	–	–	–
10	Ea. Rincón de López, Bahía Samborombón, (Castelli)	35° 47′ 02,5″ S	57° 25′ 0,219″ O	2	2	–	2	–	–	–
11	Canal Aliviador del Salado, Bahía Samborombón, (Castelli)	35° 50′ 24″ S	57° 24′ 33.263″ O	3	3	–	3	–	–	–
12	Ea. Ensenada de San Martin, Bahía Samborombón, (Castelli)	35° 5687.3″ S	57° 26′ 92.9″ O	6	4	2	5	–	1	–
13	Ea. El Principio, Bahía Samborombón, (Castelli)	35° 56′ 4″ S	57° 26′ 44″ O	1	1	–	1	–	–	–
14	Canal San José, Bahía Samborombón, (Castelli)	36° 1′ 48″ S	57° 24′ 47.296″ O	2	2	–	2	–	–	–
15	Canal 9, Bahía Samborombón, (Tordillo)	36° 0805″ S	57° 17′ 41″ O	3	3	–	–	2	1	–
16	Canal 1, Bahía Samborombón, (Tordillo)	36° 1656.791″ S	57° 6′ 58.978″ O	35	35	–	35	–	–	–
17	Ea. Don Miguel, Bahía Samborombón, (Tordillo)	36° 13′ 0,57″ S	57° 24′ 22,3″ O	1	–	1	1	–	–	–
18	Ea. El Gato, Bahía Samborombón, (Tordillo)	36° 13′ 28″ S	57° 23′ 18.999″ O	5	5	–	5	–	–	–
19	Ea. Malele, Bahía Samborombón, (Tordillo)	36° 17′ 08″ S	57° 20′ 45″ O	6	6	–	6	–	–	–
20	Ea. Santa Lucía, Bahía Samborombón, (Tordillo)	36° 16′ 40,6″ S	57° 23′ 08,4″ O	1	1	–	1	–	–	–
21	Canal 2, Bahía Samborombón, (Tordillo)	36° 11′ 25″ S	57° 22′ 27.999″ O	4	4	–	1	3	–	–
22	Canal 9, Bahía Samborombón, (Dolores)	36° 15′ 0″ S	57° 34′ 4.155″ O	3	3	–	2	–	–	1
23	Campos del Tuyú, Bahía Samborombón, (Gral. Lavalle)	36° 22′ 16.81″ S	56° 54′ 26.539″ O	3	3	–	3	–	–	–
24	Paraje Pavón, (Gral. Lavalle)	36° 42′ 50.04″ S	56° 44′ 20.04″ O	3	3	–	3	–	–	–
25	Reserva Natural Laguna Salada Grande, (Gral. Madariaga)	36° 57′ 20″ S	56° 58′ 04″ O	2	2	–	2	–	–	–
26	Gral. Madariaga, (Gral. Madariaga)	37° 1′ 0.001″ S	57° 7′ 59.998″ O	4	3	1	4	–	–	–
27	Mar Chiquita, (Mar Chiquita)	37° 42′ 47.999″ S	57° 24′ 44.672″ O	5	3	2	5	–	–	–
28	Balcarce, (Balcarce)	37° 52′ 39.58″ S	58° 6′ 52.789″ O	1	1	–	1	–	–	–
	Total			**106**	**99**	**7**	**98**	**5**	**2**	**1**

associated to wild individuals, both wild pigs and wild boars, while a dissimilar coating might be a sign of recent hybridization with domestic pigs (Canu et al., 2016; Giménez Dixon, 1991; Koutsogiannouli, Moutou, Sarafidou, Stamatis & Mamuris, 2010). In *Sus scrofa*, a striking difference in coat colour patterns exists between the wild and the domestic form, due to purifying selection acts in favour camouflage coat colour in natural environments and strong human selection in domestic lineages, where they take on the wild boar specimens dark colorations compared to the domestic type (Canu et al., 2016; Fang et al., 2009; Li et al., 2010). In the case of the wild pig population on the Buenos Aires coast, having more than 500 years of adaptation and growth in the environment, all its specimens have acquired the wild characteristics of the species, black coloration being the most frequent (Giménez Dixon, 1991). Despite this, some specimens have been found with different colours such as red-brown, mottled, bay, amongst others. In addition, hybridization between wild and domestic pigs has been recently reported in the Buenos Aires Province (Acosta et al., 2019). For these reasons and given the large number of wild pigs with coats different to the dark colour that inhabit the coast of Buenos Aires, we wanted to evaluate whether the difference in colour in wild pigs could have an association with the presence of the deleterious allele, comprising the first time this association has been evaluated.

In our study, we did not find a statistically significant association between the colour coating pattern and the presence of the PSS-causing allele, thus we do not have significant evidence to ascertain that the coat colour difference in wild pigs is an indicator of the deleterious RYR1 allele presence.

In conclusion, this is the first analysis detecting the presence of the deleterious allele for the RYR1 gene, related to PSS in wild pigs (*Sus scrofa*) from Argentina, constituting one of the few researches worldwide to report it. Furthermore, we corroborate the existence of a possible gene flow between the wild population and domestic pigs in the area, due to the high frequency for the deleterious allele that is only found in specific domestic breeds. Our results suggest that the wild population might be acting as a reservoir for the detrimental RYR1 allele; thus, its use as a source of breeding would be advised to domestic productions, exclusively with a detection analysis prior to its introduction to the production system, in order to avoid the addition of reproductive individuals that might propagate the PSS in this species.

Ethical statement

The samples of wild pigs were obtained from tissue banks supplied by Provincial Organism for Sustainable Development of the province of Buenos Aires (OPDS), the highest organism for the conservation and management of fauna at the provincial level from Argentina. For the capture and sacrifice, them were followed the guidelines established by the American Society of Mammalogists for the use of wild animals in research (Sikes & Animal Care & Use Committee of the American Society of Mammalogists, 2016).

Declaration Competing of Interests

The authors declare no conflict of interest.

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