Assessment of antibodies against three zoonotic bacteria and associated risk factors in pig farms in Colombia

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Summary

The aim of this study was to determine the seroprevalence of *Salmonella* spp., *Mycobacterium bovis* and *Brucella* spp., together with associated risk factors, in pigs from various farms in seven regions of Colombia. A total of 350 blood samples were obtained from pigs at different stages in the production cycle of 23 farms, which were tested using the enzyme-linked immunosorbent assay (ELISA) diagnostic kits Pigtype®-Salmonella Ab (Qiagen®, Hilden, Germany), INgezim TB porcine and INgezim Brucella porcine (Ingenasa®, Madrid, Spain). The overall seroprevalence for *Salmonella* spp. was 42.85% (n = 150) and, for *M. bovis*, it was 5.42% (n = 19). No positive samples were detected for *Brucella* spp. In the farms evaluated, the presence of pests, such as rodents, was found to be the management variable with a statistically significant association with seropositivity for *Salmonella* spp. and *M. bovis.* The results suggest that, at some point in the primary production cycle, pigs came into contact with zoonotic bacteria, resulting in seropositivity, which may pose a risk to public health and national pig production.

Keywords

Brucella spp. – Colombia – *Mycobacterium bovis* – Pig – *Salmonella* spp. – Seroprevalence – Zoonosis.

Introduction

The World Organisation for Animal Health (OIE) stresses the importance of controlling infectious animal diseases in order to protect the health of both animals and the human population, as the OIE reports that around 60% of infectious diseases of humans come from animals (1). These diseases transmissible from animals to humans, or vice versa, are called zoonoses. These types of infection, including food-borne diseases, are a major cause of morbidity and mortality in humans worldwide, as food of animal origin can be a source of pathogen transmission. To minimise the risk of occurrence, prevention and control measures should be established at all stages of the supply

chain, as far as marketing and consumption, in other words, 'from farm to fork' (2).

It is well known that infections from pathogenic bacteria with zoonotic potential can occur in primary pig production, either through occupational transmission, environmental contamination or the consumption of contaminated pork meat or pork by-products (3). In the United States of America (USA), the Centers for Disease Control and Prevention (CDC) in Atlanta reported 9,968 outbreaks and 351 deaths related to pork meat over the period 2015–2017, although there is no indication of the aetiological agent involved (4). Bacteria commonly associated with transmission through the consumption of pork products include: *Salmonella enterica*, *Yersinia enterocolitica*, *Campylobacter* spp., *Listeria monocytogenes* and, less frequently, *Mycobacterium* spp., *Clostridium* spp. and *Brucella* spp. (5, 6).

Bacteria of the genus *Salmonella* belong to the family Enterobacteriaceae and are Gram-negative, facultative anaerobic bacilli that grow optimally at 37 °C. One of the main animal reservoirs is pigs, which become a source of infection for humans when people consume pork products or been exposed to pigs when handling these animals (7), causing salmonellosis. This zoonosis is one of the main causes of gastrointestinal disorders (8) and triggers symptoms such as diarrhoea, fever, arthritis, pneumonia, meningitis and septicaemia. In European Union countries, approximately 56.8% of human salmonellosis cases are thought to be attributable to pigs (9) and/or their by-products. In the USA, reported values are between 3% and 10% (10, 11), the most recent data being from 2015–2017, during which period there were 281 outbreaks associated with the consumption of pork meat and pork by-products (4). This pathogen affects pig health in the form of asymptomatic to acute gastrointestinal, and even septicaemic, conditions and death. Infection and disease can occur at any time during the primary production cycle, although the disease is usually more severe in young animals (3).

The family Mycobacteriaceae includes *Mycobacterium bovis*, which belongs to the *Mycobacterium tuberculosis* complex (MTC) (12) and is

an acid-fast, aerobic bacillus that is fastidious and grows slowly at 37 °C. Its main reservoirs are cattle, sheep, goats, pigs, canines, felines, deer and humans (13). With regard to its zoonotic potential, the World Health Organization (WHO), in spite of difficulties and poor epidemiological knowledge due to lack of reliable surveillance data, reported that there were around 145,000 new cases of tuberculosis of zoonotic origin in 2016, with more than 10,000 deaths (14). In Latin America, *M. bovis* has been reported to cause human tuberculosis in Argentina, Brazil, Ecuador, Venezuela and, in Colombia it has been isolated from a patient with tuberculosis (15, 16); however, it is not known whether it was of animal origin. Humans can contract the infection from infected animals via the aerogenous or cutaneous route, occupational exposure or oral route, by consuming unpasteurised dairy products or, less frequently, undercooked pork (17, 18). Owing to pulmonary involvement, respiratory symptoms may develop initially and, later, the disease may manifest as extrapulmonary tuberculosis, in which case it is called military tuberculosis (digestive, lymph node or cutaneous), characteristic of cases of zoonotic origin (13, 19). In pigs, the infection can occur at any age. However, the prevalence tends to increase with age (12), as it is a disease of chronic course that, in the early stages, may be asymptomatic and may not manifest itself until the time of slaughter, when it is detected by the presence of caseified granulomatous lesions in the submandibular and mesenteric ganglia which, as they can spread to the liver, spleen and lungs (20), increase economic losses from carcass seizure.

Brucella spp. belongs to the family Brucellaceae and is a Gram-negative, aerobic coccobacillus that is fastidious and grows slowly at 37 °C (21). Although it is a species-specific microorganism, it can cause disease in different hosts, including humans (22). Porcine brucellosis affects various organs but it has tropism by the reproductive system. Its economic importance is therefore associated with losses related to abortion, repeat oestrus, in some cases endometritis, and the costs of clinical treatment of the disease. Human infection is rare. In the USA, its incidence is less than 0.5 cases per 100,000 people (23) and, while epidemiological data are limited, this disease is characterised as being severe, debilitating, disabling and of chronic course and is usually

transmitted by contact with the secretions and tissues of infected animals (such as aborted foetuses, abortion tissues and genital secretions), which is why it is generally an occupational zoonosis (24). Moreover, when these secretions and tissues are not disposed of properly, they can contaminate flooring, bedding, water streams, canals and wells (22), all of which become a source of environmental infection.

Although microbiological culture is the reference method for determining the presence of these pathogenic bacteria, antibody titre determination by serology can be used as a screening tool, which makes it possible to determine the exposure of pigs to the pathogen and to implement epidemiological surveillance programmes, as well as being quick and economical (12, 25, 26) and, while it may be somewhat lacking in specificity, if performed in conjunction with microbiological methods, it supports the final diagnosis (27). It also makes it possible to determine the degree of increase and/or reduction in prevalence, data that can be used to establish prevention and control measures within production establishments, applying the herd health concept (28). Serology in meat juice samples can also be implemented in slaughter plants, as it is useful for risk-based decision-making during meat inspection (29).

While swine salmonellosis is not a notifiable disease under international and national regulations, its impact on public health is well known. In accordance with Colombian regulations arising from Resolution No. 3714 (30) and Decree No. 1071 of 2015 (31), *Brucella* spp. and *M. bovis* have been included as notifiable pathogens. Despite this, epidemiological statistics are still insufficient. Studies are therefore required to determine the health status of the pig population in relation to these zoonotic pathogens. For this reason, the current study determined the seroprevalence of *Salmonella* spp., *M. bovis* and *Brucella* spp., as well as the risk factors associated with this seroprevalence, in pig farms in several regions of Colombia where the greatest technified and semi-technified pig production is found. In 2019, Antioquia had 1,642 farms, Valle del Cauca had 232, Cundinamarca, 3,664, and Meta, 2,138. The national pig population

included 6,473,525 animals, distributed among 237,380 pig farms (21,044 technified/semi-technified farms and 216,336 backyard farms), it accounted for 1.4% of agricultural gross domestic product (GDP) and was included in 4.8% of GDP (that of the livestock sector) (32).

Materials and methods

A descriptive cross-sectional study was performed using convenience sampling, by selecting semi-technified farms (10–100 breeding females and 100–600 pigs), with the sample number based on owners' willingness to take part in the study and on logistical and budget capacity at the time of obtaining samples. A total of 350 blood samples were taken from pigs at different stages in the production cycle of 23 farms in the departments of Antioquia ($n = 8$), Quindío ($n = 1$), Risaralda ($n = 1$), Caldas ($n = 2$), Valle del Cauca ($n = 3$), Cundinamarca ($n = 6$) and Meta ($n = 2$) (average number of samples per farm: $n = 15$), these being the regions with the highest concentration of pig production in the country (Table I).

Table I

Number of samples obtained by department and age group

Department	**Age group**								
	Suckling piglets (0–4 weeks)	Weaners (5–7 weeks)	Rearing pigs (8–13 weeks)	Fattening pigs (14–22 weeks)	Lactating females	Pregnant females	Replace-ment females	Breeding males	**Total by depart-ment**
Antioquia	0	20	13	8	21	20	5	1	**88**
Caldas	0	15	0	0	2	0	3	2	**22**
Cundinamarca	15	30	4	2	16	30	11	5	**113**
Meta	0	16	3	13	16	23	0	1	**72**
Quindío	0	10	0	0	0	0	0	1	**11**
Risaralda	0	10	0	0	0	0	0	1	**11**
Valle del Cauca	0	17	8	4	0	0	0	4	**33**
Total by age group	**15**	**118**	**28**	**27**	**55**	**73**	**19**	**15**	**350**

A survey was designed, reviewed and endorsed by veterinarians from PorkColombia's animal health technical department. This included demographic data and around 40 variables related to farm management, including housing cleaning and disinfection processes, biosecurity measures for employees and pen characteristics (such as the type of flooring, drinking troughs and feeders), environmental characteristics and the possible presence and control of pests in the establishment, among other parameters (33, 34, 35).

Blood samples were obtained by the veterinarian responsible for each farm by jugular venipuncture, using a Vacutainer® system and Venoject® tube with separator gel and following all established biosecurity measures (36, 37). The duly identified and preserved samples were transported under refrigeration to the laboratories of Pontifical Xavierian University. Sera were obtained by centrifugation at 1,500 revolutions per minute (rpm) for 15 minutes and were stored at –20 °C until processing, which was performed by indirect enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. For the detection of antibodies against *Salmonella* spp., the ELISA diagnostic kit Pigtype®-*Salmonella* Ab from Qiagen® (Qiagen®, Hilden, Germany) was used, and for the detection of antibodies against *Brucella* spp. and *M. bovis*, the kits INgezim Brucella porcine and INgezim TB porcine from Ingenasa® (Ingenasa®, Madrid, Spain) were used, respectively (Table II).

Table II

Processing conditions and interpretation of the enzyme-linked immunosorbent assay, according to the manufacturer

Microorganism	Absorbance	Calculation	Validation criteria	Interpretation of results	S/Sp%
Salmonella spp.	450 nm	$M/P = \frac{Abs\ sample - Abs\ negative\ control}{Abs\ positive\ control - Abs\ negative\ control}$	Negative control ≤ 0.2 Positive control ≥ 0.7	Positive: M/P ≥ 0.3 Negative: M/P < 0.3	97%
Mycobacterium bovis	450 nm	$M/P = \frac{Abs\ sample - Abs\ negative\ control}{Abs\ positive\ control - Abs\ negative\ control}$	$Avg\ Abs\ control\ (positive) - Avg\ Abs\ control\ (negative) \geq 1.25$ Negative control < 0.2	Positive: M/P ≥ 0.25 Negative: M/P < 0.25	78/ 89.5%
Brucella spp.	450 nm	$M/P = \frac{Abs\ sample}{Abs\ positive\ control}$	Positive control > 1 Negative control < 0.3	Positive: M/P ≥ 0.25 Negative: M/P < 0.25	100%

Abs: absorbance
Avg: average
M/P: ratio of the absorbance of the sample compared to that of the control
Nm: nanometres
S: sensitivity
Sp: specificity

The following formula was applied to determine the seroprevalence (SP) for each of the microorganisms:

$$SP: \frac{Number\ of\ positive\ samples}{Total\ number\ of\ samples} x100$$

The free software R (version 3.2.5, www.r-project.org) was used to determine the relationship between the demographic and management variables and the seropositivity results in the samples analysed, and Pearson's chi-square and Fisher's statistical tests were applied. The positivity data for *Salmonella* spp. ($n = 150$) were analysed using Pearson's chi-square test of association and those for *Mycobacterium bovis* ($n = 19$) were analysed using Fisher's exact test of association (independence) because the number of seropositive samples was not representative enough to use Pearson's chi-square test.

Results

A total of 350 blood samples were obtained from pigs at different stages in the production cycle of 23 semi-technified farms (Table I), located in Antioquia, Caldas, Cundinamarca, Meta, Quindío, Risaralda and Valle del Cauca.

The overall seroprevalence for *Salmonella* spp. was 42.85% ($n = 150$) and, for *M. bovis*, it was 5.42% ($n = 19$), while no positive samples were detected for *Brucella* spp.

With regard to the number of seropositive samples for *Salmonella* spp. by department, the following distribution was determined: Meta = 47, Caldas = 11, Antioquia = 37, Valle del Cauca = 12, Quindío = 4 and Cundinamarca = 39, while Risaralda presented no seropositivity for this microorganism. With respect to *M. bovis*, the distribution was as follows: Meta = 10, Cundinamarca = 8 and Antioquia = 1 (Fig. 1); all seropositive animals in each region belonged to the same farm.

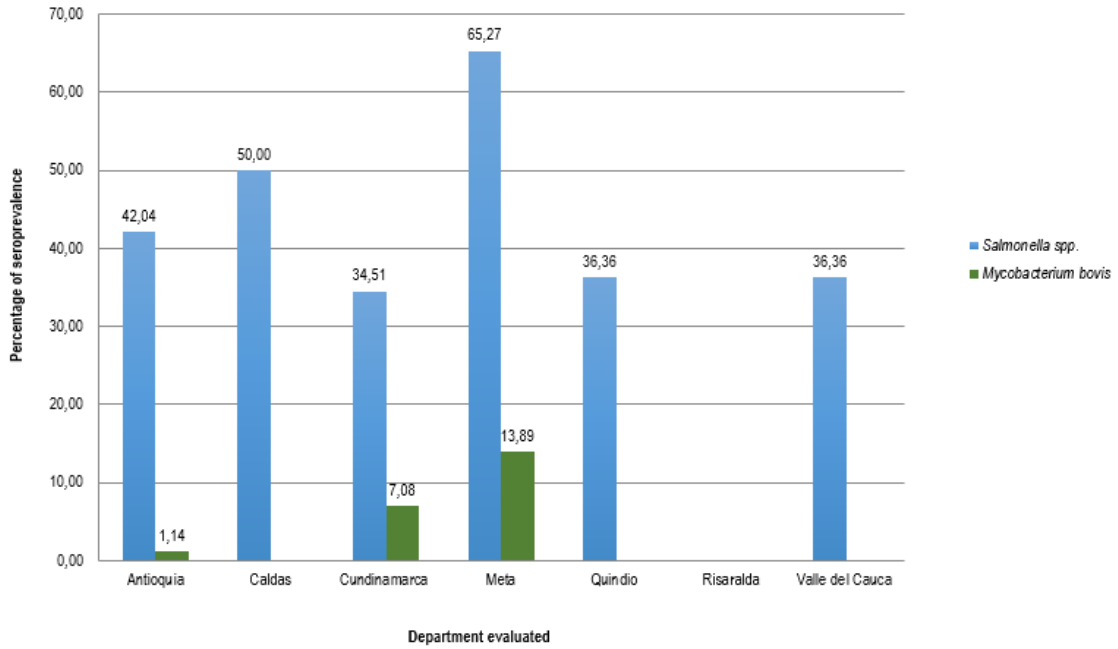

Fig. 1

Percentage of seroprevalence for *Salmonella* spp. and *Mycobacterium bovis* by department

The *Salmonella* spp. positive samples by age group were distributed as follows: breeding males = 13, replacement females = 11, lactating females = 30, pregnant females = 37, weaners = 27, suckling piglets = 1, rearing pigs = 15 and fattening pigs = 16 (Fig. 2). Based on the above and taking into account maternal antibody dynamics, this might suggest passive immunity in the early stages of production (suckling piglets/weaners), which would result in seropositivity of 8% (n = 28), while in the other stages it would result in seroprevalence of 34.85% (n = 122).

The *M. bovis* positive samples were obtained according to the following distribution: lactating females = 9, replacement females = 3, pregnant females = 5, breeding males = 1 and weaners = 1 (Fig. 2). In this case, only one weaner showed antibodies against the pathogen, which corresponds to 0.29% seropositivity, possibly also due to passive immunity. The remaining 5.14% was distributed among the other age groups.

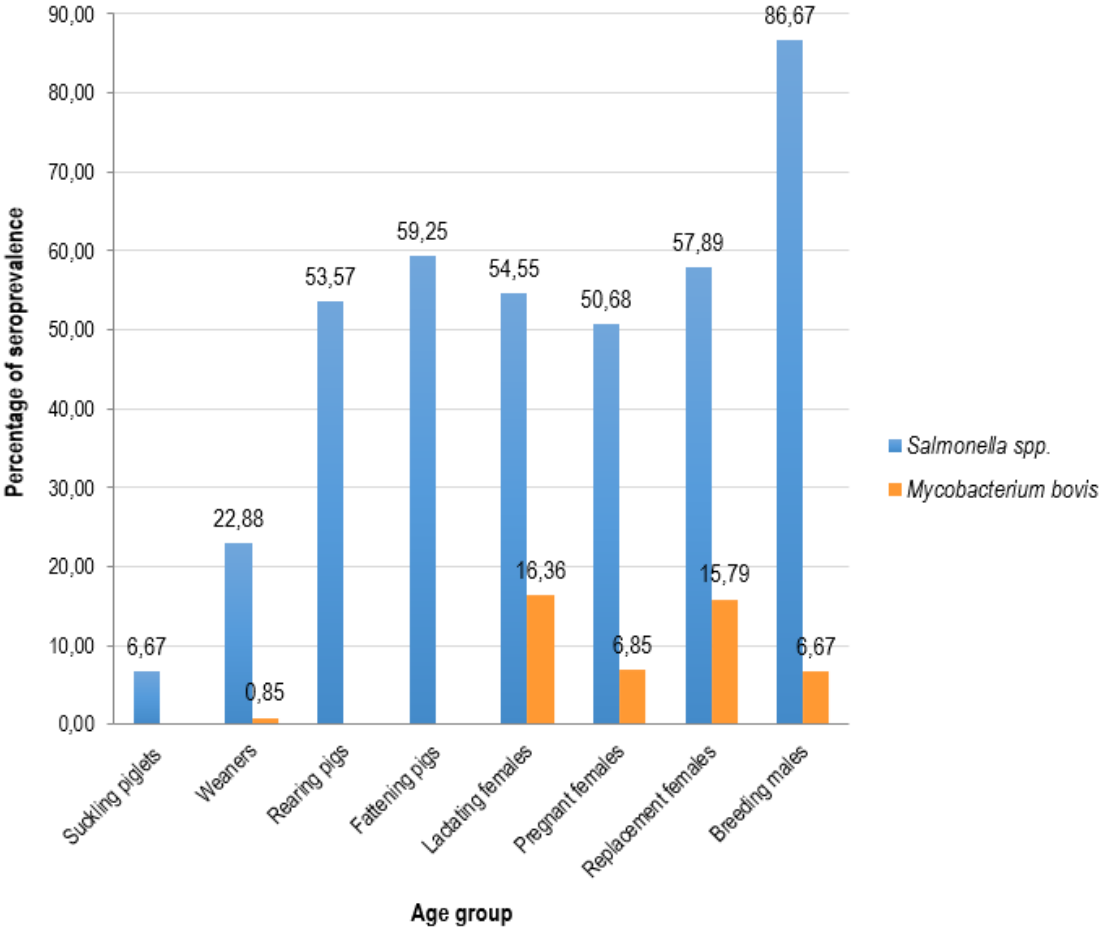

Fig. 2

Percentage of seroprevalence for *Salmonella* spp. and *Mycobacterium bovis* by age group

The associations between seropositivity to the pathogens tested and the demographic and management variables on the farms surveyed were checked for statistical significance ($p < 0.05$). In the case of *Salmonella* spp., one of the variables that showed this association was the type of pen enclosure (in particular, a concrete wall) ($n = 21$ [91.30%], $p = 0.003$), as well as daily manure removal ($n = 21$ [91.30%], $p = 0.008$), the presence of a dung heap as a place to dump faeces ($n = 12$ [52.17%], $p = 0.008$), the presence of rodents (this being the commonest type of pest on the farms) ($n = 19$ [82.61%], $p = 0.004$), the presence of other species on the farms (dogs being the commonest species) ($n = 13$ [56.53%] $p = 0.004$) and, finally, composting for the disposal of dead animals and other biological waste ($n = 13$ [56.53%],

$p = 0.002$). In the case of *M. bovis*, the variables with a positive association were: the presence of another type of livestock farm close to the farm (cattle farms being the most predominant type) ($n = 12$ [70.58%], $p = 0.000$), the presence of a disinfection arch ($n = 7$ [30.34%], $p = 0.000$) and the presence of rodents (which was the commonest type of pest on the farms) ($n = 19$ [82.61%], $p = 0.001$).

Further management parameters were determined that showed no statistically significant association with the seropositivity found for the different microorganisms. However, it is worth mentioning that 69.56% ($n = 16$) of the farms used water from natural sources (rivers, lakes and wells), 91.30% ($n = 21$) had sanitary facilities (toilets and washbasins) and 100% ($n = 23$) had footbaths for disinfecting boots. Among other parameters, the presence of wild species, such as reptiles, was detected in 8.70% of farms ($n = 16$).

Discussion

The overall seroprevalence for *Salmonella* spp. determined by this study was 42.85%, similar to the 47% value reported in the Netherlands in 2001 (38). However, it differs from that reported in Mexico, Italy, Germany, the USA and Spain, where seroprevalence was lower (28.7%, 19.3%, 7.9%, 5% and 4%, respectively) (39, 40, 41, 42, 43). These dissimilarities may stem from differences between the biosecurity programmes in place on individual countries' pig farms. Nonetheless, in Spain, the reported seroprevalence in Iberian pigs (73.42%) is higher than that found in this study, which may be attributable to management conditions (44). In the department of Cundinamarca, this study detected seroprevalence of 34.51%, which is close to the 40% found in 2019 (45). Although this study did not evaluate samples from the department of Tolima, in 2014, Rondón-Barragán *et al.* determined seroprevalence of 36.09% in that department, finding a higher presence of antibodies in the gestation and fattening stages and lower seropositivity in breeding males (46). Their data coincide partially with the current analysis, which found that the highest positivity was in rearing pigs, fattening pigs, replacement females, lactating and pregnant females and breeding males. While none of the animals

sampled showed clinical symptoms, the presence of antibodies in the different age groups could indicate the possibility of infection at any age (3), even as early as lactation, by contact with a potentially seropositive dam, and may also suggest that seropositivity in the lactation and weaning stages could be due to the transfer of maternal antibodies.

Historically, one factor that may have contributed to animals' exposure to the bacterium and, hence, to high seroprevalence, is the presence of *Salmonella* spp. in the water used on farms and, while this study's analysis found no statistically significant association between this variable and seropositivity, prior studies by the research group had indeed demonstrated that the main risk factor for contracting *Salmonella* spp. infection was the use of untreated water from natural sources (47). In addition, one of the management variables associated with seropositivity was the frequency of manure removal (daily removal being the most common, in accordance with good husbandry practices for pigs), which should be reflected in a decrease in *Salmonella* spp. infection/seroprevalence in herds. On the contrary, seroprevalence rates in excess of 50% were determined in females in the different groups, as well as in breeding males, a situation that could be contributing to the pathogen's persistence on farms. As the presence of antibodies does not necessarily mean that there is active infection, it is important not only to determine seroprevalence but also to perform stool cultures to ascertain the prevalence of infection in the herd and the degree of excretion of the microorganism, taking into account its faecal-oral transmission, which is the main source of contamination. In fact, this microorganism has a rapid growth curve and its minimum infective dose per oral intake is $10^4 - 10^7$ colony-forming units (CFUs) per gram, meaning that poor farm practices would have a direct relationship with both persistence of the bacterium in the environment and an increased number of infected animals (3, 48). On most of the farms analysed, the place for dumping manure was a dung heap. It is important to bear in mind that dung heaps tend to attract flies, which act as mechanical vectors of the bacterium, either from their body surface, by regurgitation of intestinal contents containing the microorganism

and/or by defecation, contaminating surfaces, water, food and other objects (49).

In addition, the presence of rodents on farms, which act as reservoirs of the pathogen and cohabit with pigs (50), favours the transmission/perpetuation of the pathogen on farms. Similarly, the transit of other animal species on farms favours the spread of microorganisms; in this case, dogs were the most reported species, these hosts being susceptible to the bacterium (7). Moreover, as it is common to find *Salmonella* spp. in the microbiota of reptiles, the presence of these animals can be considered a source of spread of the microorganism to pigs (51, 52); for example, the presence of iguanas was reported on some farms in Cundinamarca. The presence of the bacterium in reptiles should be ascertained in order to confirm their role as potential transmitters of *Salmonella* spp. to pigs. Finally, it is important to bear in mind that workers could contribute to the spread of the bacterium on farms through their work implements or clothing, as the presence of this bacterium has been described in samples obtained from workers' boots (47, 53, 54). Another important aspect to control is therefore boot cleaning and disinfection (55). Even so, this study revealed no statistically significant relationship between the two parameters.

Despite the small number of epidemiological studies of *M. bovis* in pig farming, intradermal tests have been used as a diagnostic method, although they lack objective patterns of interpretation that are independent of the antigen used (27). With regard to serological tests, while suppliers claim good sensitivity and specificity, some studies report low sensitivity, especially in young pigs (12), which could explain why this study detected positivity only in adult pigs (females and males). In Great Britain in 2004–2010, the prevalence of *M. bovis* infection determined by microbiological culture was 11.2% (56), which confirms the presence of this pathogen in pigs and the fact that it poses a public health risk. In the same country, it was reported that *M. bovis* infection occurs in pigs in the finishing stage (fattening), that is to say, in pigs of approximately five months of age (57). While the methodology used in this study does not allow the time of contact with

the bacterium or the presence of an active infection to be defined, it did in fact detect antibodies in the older age groups (in lactating, replacement and pregnant dams, as well as in males). The largest number of seropositive animals came from a single farm in the department of Meta. The Colombian Agriculture and Livestock Institute (ICA) reported in its animal health bulletin an outbreak of the infection in cattle in the same department in 2014 (58), one of whose demographic and management variables with a statistically significant association with positive serology for *M. bovis* was the proximity of other livestock farms to the farm studied, mostly commonly cattle farms, which might suggest that proximity to this species is a source of contamination. Other studies also point to evidence of *M. bovis* transmission between pigs and cattle in France, Australia and New Zealand (59, 60, 61).

In a study by Bailey *et al.* in 2013, *M. bovis* was detected in 5.95% of 874 slaughtered pigs with lesions compatible with the presence of the pathogen, and *M. avium* was detected in 7.09%, suggesting a predisposition to contracting the disease when pigs are reared outdoors and/or without strict biosecurity conditions (57). However, according to the observations of this study, they are not determining factors, as the animals in the serologically positive farms were housed in pens with adequate biosecurity measures. Even though the health inspection at the slaughter plant revealed no lesions or nodules compatible with the presence of tuberculosis, it is important to establish surveillance, prevention and control programmes for this disease.

Of note is the fact that the presence of a disinfection arch, together with the use of substances for washing and disinfection, have a statistically significant association with the presence of antibodies against *M. bovis*, especially considering that these biosecurity measures are in place in 100% of the farms evaluated. It might be that improper use of disinfectants (either low application volume, incorrect contact time and/or low efficacy) is the reason why the microorganism can exist in the environment and even travel around the farm, where it becomes a source of infection for animals. Furthermore, many of the disinfectants

used on farms are formulated to control viruses and do not have a broad effect on bacteria (62).

In Colombia, in 2014, porcine brucellosis was reported in 16 farms in the departments of Meta (75%), Cundinamarca (33%) and Valle del Cauca (20%). This study did not detect the presence of antibodies against *Brucella* spp., nor were they reported in the department of Bolívar after applying the Rose Bengal test (63). This absence of antibodies may be related to the implementation of Resolution No. 7231 of 2017 establishing sanitary measures for the prevention, control and eradication of brucellosis in bovine, buffalo, ovine, caprine, porcine and equine species in Colombia (64) and to the good husbandry strategies (65) applied in pig production establishments. In addition, brucellosis is a notifiable disease in Colombia, either in sick animals or when detected at the slaughter plant (31), as it is a disease with a negative impact on public health, being highly contagious for both humans and other animals (22).

In other countries, seroprevalence for *Brucella* spp. has been low. For example, in untechnified farms in Brazil the presence of antibodies was detected in only 0.29% of the samples analysed by complement fixation, a situation that reflects an improvement in the country's animal health system (66). However, using ELISA to evaluate Iberian pig populations, 3.8% seroprevalence (44) was determined in Spain and 14.3% in Mexico (43). In Peru, the techniques Rose Bengal (screening test) and complement fixation (confirmatory test) demonstrated 2.75% seroprevalence in samples from untechnified farms, while no seropositive animals were detected in technified farms (67).

With this report, academia aims to contribute to knowledge about zoonotic diseases in pig farming because knowledge gaps should be addressed by all government sectors through diagnostic and surveillance programmes extending from primary production to slaughter, as stated by WHO, the OIE and the Food and Agriculture Organization of the United Nations (FAO) in 2017 (14). Collaboration and interaction between research institutions and private industry make it possible to design and implement modern production practices, which

means joining forces to reduce the risk of zoonotic pathogen transmission, through awareness-raising, commitment and inter-agency collaboration (68).

Conclusions

- An overall seroprevalence of 42.85% ($n = 150$) for *Salmonella* spp. and 5.42% ($n = 19$) for *Mycobacterium bovis* was determined in tested pigs from different regions of Colombia.
- The presence of antibodies against *Brucella* spp. was not detected in the animals tested.
- The presence of pests, such as rodents, was the management variable with a statistically significant association with seropositivity for *Salmonella* spp. and *M. bovis.*
- In Colombia, pigs come into contact with *Salmonella* spp. and *M. bovis* at some point in the primary production cycle, which may pose a risk to public health and national pig production.

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