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Short Communication

Isolation of *Haemophilus parasuis* from Diagnostic Samples in the South of Brazil

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Abstract

Haemophilus parasuis is one of the first and most prevalent colonizers of piglets, affecting the swine population from 2 weeks to 4 months of age. In this work, the relative incidence of *H. parasuis* isolated from swine in the west of Santa Catarina from 2007 to 2010 was investigated. White, flat and non-hemolytic colonies (satellitism growth with *Staphylococcus aureus*) of approximately 1 mm in length were identified by biochemical tests. Out of 384 samples examined during this period, 32 (8.33%) tested positive to isolation of *H. parasuis.* In 2007 and 2008 only 1 case of *H. parasuis* was isolated per year from a total of 62 and 60 samples, respectively. In 2009, 19 cases from 126 (15.08%) and in 2010, 11 cases of *H. parasuis* were isolated from a total of 136 samples (8.09%). These results suggest a growing incidence of this bacterium in the swine flock in this area.

Key Words: *Haemophilus parasuis*, swine, respiratory system, microbiology

Pig breeding in farms is an activity of high economical importance in Southern Brazil. The pig flock in this region increased from 15,984,115 to 17,798,250 in the last 3 years. In this same period, the flock in the western of Santa Catarina State increased from 5,491,599 to 5,952,862. This area is responsible for 75.87% of the swine flock in the state and 16.17% of the swine flock in the country (3). Therefore, the slightest problem in animal sanitation may significantly impact the economy of both the state and country. This clearly indicates the need of appropriate methods to control major pathogens such as *Haemophilus parasuis* (9).

The bacterium *H. parasuis* is the etiological agent of Glässer's disease, which is characterized by polyserositis and fibrinopurulent polyarthritis (14, 15). *H. parasuis* is one of the earliest and most prevalent colonizers of piglets and this microorganism can affect the swine population from 2 weeks to 4 months of age. However, it is more generally observed in pigs from 5 to 8 weeks (9, 14). This disease is currently considered an economical threat to the swine industry due to the high cost of antibiotic treatments and the discarding of affected animals since it also occurs in farms with high sanitary standards (8).

H. parasuis infections have been traditionally diagnosed through clinical signs, presence of lesions during necropsy, and bacteriologic culture (20). Bacterial isolation is difficult and the best method to achieve this is to collect exudates of certain organs. The most commonly used cultural medium is tryptose blood agar because *H. parasuis* is a fastidious microorganism (14). Alternative techniques for the diagnosis of *H. parasuis* infections have been proposed, like immunohistochemistry, PCR-based assays, restrictive endonuclease fingerprinting, enzyme immunoassays (ELISA), among other (10, 12, 16, 17).

Considering the importance of diagnosing *H. parasuis* in swine and clean sanitation practices in pig breeding, the objective of this work was to study the relative incidence of *H. parasuis* isolated from swine in the west of Santa Catarina from 2007 to 2010.

H. parasuis strains were isolated from samples of clinical cases and sent to Center of Diagnosis of Animal Sanitation from 2007 to 2010. A total of 384 cases were analyzed during this period.

The samples used to isolate the microorganism were lungs, and pericardial and cerebrospinal fluids. Blood agar (5% defibrinated sheep blood) plates with a streak of *Staphylococcus aureus*, which provides the required Factor V, were used. Plates were incubated for 48 h at 37°C in microaerophilic condition (5% CO_2 in air). If satellitism growth was observed, the colonies were analyzed. White, flat and non-hemolytic colonies approximately 1mm in length were identified using biochemical tests and gram staining. These colonies were replicated in Nicolet agar in microaerophilic condition (5% CO_2) at 37°C for 48h. All isolates were subsequently characterized biochemically by fermentation of glucose and sucrose and non-fermentation of lactose, mannitol, xylose, arabinose, threalose, raffinose, esculin (negative hydrolysis), negative for urease production and non decomposition of tryptophan in indole and catalase positive (6). The gram stain showed gram negative coccobacilli. Samples presenting this profile were considered positive for *H. parasuis*.

Between January 2007 and December 2010, 32 *H. parasuis* strains were isolated from 384 samples examined (8.33%). In 2007, only 1 strain of *H. parasuis* was isolated from 62 samples (1.61%). One strain was also isolated in 2008 (1.66%), but an increase in positive cases for *H. parasuis* was observed in 2009, since 19 strains were isolated from 126 samples (15.08%). In 2010, 11 cases of *H. parasuis* were isolated from a total of 136 samples (8.09%) (Fig. 1).

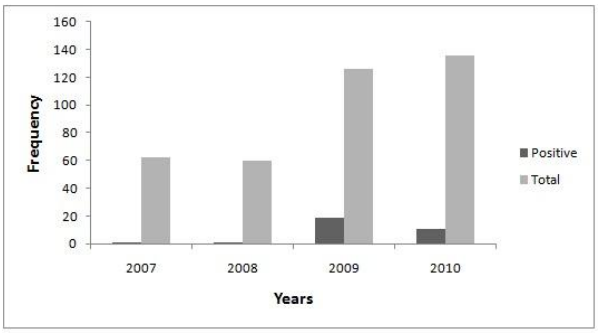

Figure 1. Frequency of the cases of *Haemophilus parasuis* in the period from January 2007 to December 2010, in the South of Brazil.

A total of 103 of *H. parasuis* were isolated from diseased pigs in association with routine diagnostics in Denmark, and most isolates were from cases of animals with bronchopneumonia without systemic disease (1). In the period of June 2003 to December 2004, 828 cases of clinically affected pigs in China were investigated for *H. parasuis*, which was isolated from 183 cases (2). For those cases, co-infection with other species of swine bacterial pathogens was also observed.

The number of positive cases of *H. parasuis* was classified according to the exudate type (Table 1). In the years of 2007 and 2008, strains were isolated from the lung. Although in 2009 and 2010 some strains were also obtained from pericardial and cerebrospinal fluids, most samples positive for *H. parasuis* were also from lungs. The percentage of *H. parasuis* strains isolated from lungs in 2009 and 2010 corresponded to 78.95% and 72.73%, respectively.

Table 1. Number of positive cases for *Haemophilus parasuis* according to the exudate source in the period from 2007 to 2010 in the South of Brazil.

Exudate source	**Years**			
	2007	**2008**	**2009**	**2010**
Lung	1	1	15	8
Pericardial fluid	0	0	3	3
Cerebrospinal fluid	0	0	1	1
Total of cases	1	1	19	11

The results of this work indicate an important increase in isolation of *H. parasuis* in this area of Brazil. This increase was nearly 2,000% (from 1 annual case to 19 cases from 2008 to 2009). The number of exams also increased but not in the same proportion, growing from an average of 61 annual tests (2007-2008) to 126 exams in the year of 2009, and 136 exams in 2010. These numbers could be underestimated since small farms possess limited financial resources to maintain efficient sanitary control. In other words, the number of cases of *H. parasuis* affected piglets may be higher and this etiological agent could be contaminating more animals causing an increased number of deaths. A fact that can be associated with the increased number of *H. parasuis* isolation in the period of 2009 is that the laboratory observed a larger demand of samples, which were positive in the diagnosis of viral pneumonia. This clinical picture might have contributed to a larger spread of this pathogen, because the immunodepression caused by the pneumonia facilitates the installation of this bacterium, resulting in a secondary infection. In 2010, this situation repeated but in a smaller number of diagnosed cases.

The number of *H. parasuis* cases has been increasing worldwide, mainly in the countries that export pork meat because the fattening process is done as fast as possible and in some case without sufficient

sanitary standards. In a study done in Australia, 19 of 20 researched farms showed the presence of *H. parasuis*, even in 2 areas that were considered free from this pathogen for many years (19). In another study, it was confirmed that several genotypes of *H. parasuis* are thoroughly distributed in the southeast of China (4). In this context, the feeding is optimized for the production of fast weight gain and the use of antibiotics is thoroughly diffused. The consequences are the production of weak animals and the development of antibiotic resistance. Another important factor is the presence of *H. parasuis* with other pathogens related to the respiratory system, such as *Pasteurella multocida*, *Streptococcus suis*, *Actinobacillus pleuropneumoniae*, *Actinobacillus suis* and *Bordetella bronchiseptica*. The association of more than one of these microorganisms complicates the function of the immune system of the animal making them easy targets of respiratory diseases. Consequently, the mortality rate increases considerably (5, 7, 18).

Based on these results, a larger sanitary control would be desirable to protect the flock from the spread of *H. parasuis*. Therefore, the adoption of safety regulations to avoid the spread of this pathogen could be: (a) better handling and maintenance of the maternity and daycare (followed by a sanitary emptiness for around six days), (b) the improvement of hygiene in the facilities, (c) frequent disinfections and avoiding infected animals from entering the facilities, (d) to eliminate the factors of predisposition that can act negatively on the pig increasing its sensibility to infection (such as the cut on a tooth/ teeth, allowing lesions in the gum and/or tongue) (10). A more severe sanitary control should be implemented, since other diseases can reduce the immunity of the animal and may facilitate the proliferation of *H. parasuis*, which belongs to the natural microbiota of the upper respiratory tract of healthy swine (11). Finally, the use of prophylactic treatment, such as vaccines, a tool utilized in countries like USA and Spain (13), could be used to control the disease in a certain area.

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