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Streptococcus suis: The search for a solution

Mariela Segura
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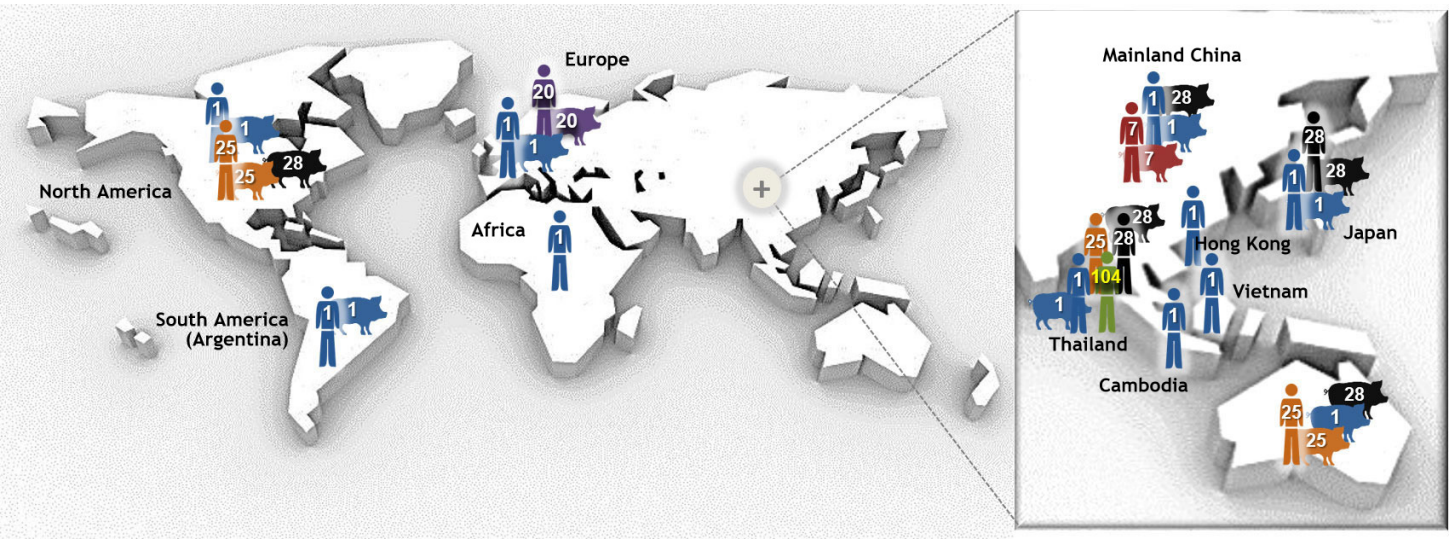
The fight against *Streptococcus suis* has been a long-time struggle for swine producers, veterinarians, and researchers worldwide. *S. suis* is an important disease responsible for meningitis, septicemia and other invasive pathologies mainly in post-weaned piglets. *S. suis* varies in its genetic makeup around the world complicating its diagnostic and epidemiological surveillance. *S. suis* is also an important globally emerging zoonotic disease for swine industry workers (occupational disease). In some regions (most notably certain Asian countries), it is a frequent cause of serious disease outbreaks in humans exposed to diseased animals or contaminated pork products. Currently, the use of antibiotics is being limited all over the world – meaning swine producers are going to have to depend on prevention methods other than prophylactic/metaphylatic use of antibiotics. So why is there no effective commercial vaccine against *Streptococcus suis*? (Segura M., 2015).

All about *Streptococcus suis*

Streptococcus suis is naturally present in the upper respiratory tract of pigs, as well as the digestive and genital tracts. Up to 100% of pigs in a herd are carriers of this bacterium, meaning they are colonized without demonstrating clinical signs. That said, these carrier pigs can still pass on the bacteria to other animals (Gottschalk M, Segura M., 2019).

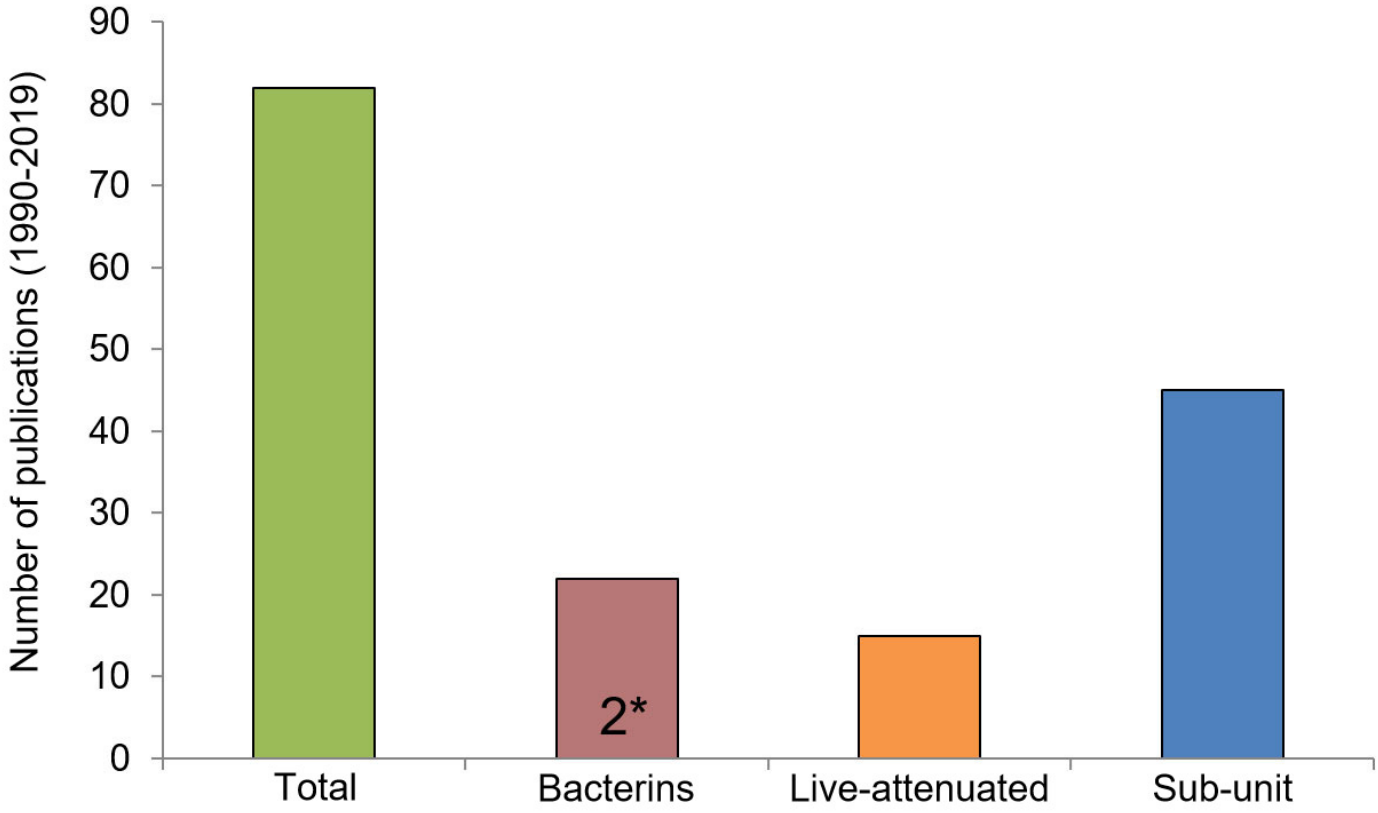


S. suis exists worldwide and differs greatly from region to region (Figure 1). The bacterium was originally classified into 35 serotypes defined by the sugars found in the "capsule" surrounding the bacterial surface (a few of these serotypes are now being debated as whether or not belonging to the *S. suis* species). Yet, the most common serotypes recovered form pig clinical cases are 2 (worldwide), 9 (certain European countries), 3, 1/2, and 7 (mainly North America; and Asia for serotype 3 as well). *S. suis* is also classified in "sequence types", which are based on the bacterium 'DNA fingerprint' (Goyette Desjardins, et al. 2014). Each serotype of *S. suis* therefore contains numerous sequence types (Figure 1). All this diversity means that individual *S. suis* infections have unique characteristics in terms of serotype, sequence type, zoonotic potential and clinical outcomes. This huge amount of variation helps explain why it is so difficult to create one "universal" vaccine that protects against all *S. suis* infections in pigs worldwide (Goyette Desjardins, *et al.* 2014).

Types of vaccines

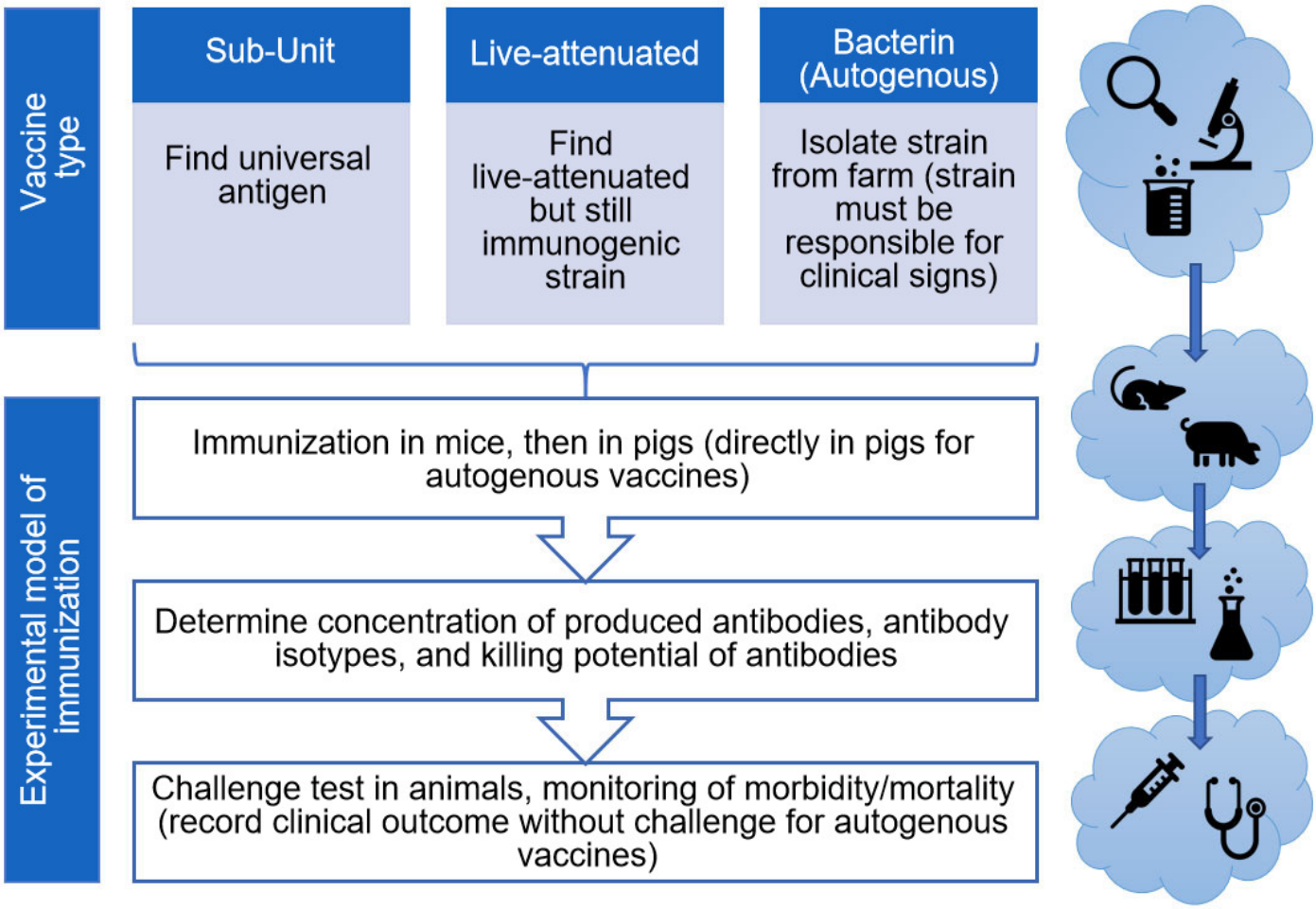
There are numerous types of vaccines, all with their own pros and cons. Animals can be protected by injecting them with either a component (sub-unit) of the bacterium, live-attenuated bacteria or killed (inactivated)-bacteria. Experimental *S. suis* sub-unit vaccines seem promising, but require strong adjuvants (solutions to amplify the immune system response). Additionally, because *S. suis* is so diverse, finding a specific component (Ex. a protein) able to protect against all *S. suis* strains remains a challenge. The combination of different *S. suis* proteins (antigens) in a sub-unit vaccine would likely afford the best chance of a more "universal" and effective protection. On the other hand, while live-attenuated vaccines offer the hypothetical benefits of requiring no boosters or adjuvants, they present a public health risk, given that *S. suis* is zoonotic and that the injected strain could revert to virulence. The second drawback of *S. suis* live-attenuated vaccines is that naturally or experimentally infected animals produce low levels of antibodies, so it is difficult to envisage that an attenuated strain (which can be easily eliminated by the host) will be able to induce a protective response when a virulent strain is not able to do so. Indeed, this fact might explain why a multiple-injection protocol was used in most live-attenuated vaccine studies (Segura M., 2015).

The last type of vaccines commonly evaluated for *S. suis* prevention are killed (inactivated)-bacteria or "bacterins", limiting the public health risk – but also their ability to stimulate the immune system, and thus providing controversial results (Segura M., 2015). Actually, **autogenous** bacterins represent the only available option in the field. These vaccines are bacterins prepared for a specific farm by sampling animals from the affected herd. As such, despite the huge variation in *S. suis* infections by region, vaccinated animals are protected from the same strain(s) causing clinical problems within the herd in question. However, the diagnosis of *S. suis* as a primary cause of disease may complicate the choice of the strain(s) to be included in the autogenous vaccine. That said, more investigations of all types of vaccines are necessary before drawing any conclusions on which presents the ultimate solution. As of 2019, the vast majority of publications on *S. suis* immunization studied sub-unit vaccines, then bacterins, and finally live-attenuated vaccines (Figure 2).



Challenges in *S. suis* vaccine development

There is currently no international agreement on how vaccine efficacy should be tested, meaning that it is very difficult to compare the results of different formulations. Not only is there variation among studies with respect to the vaccine formulation, boosters, and immunized animals (sows vs. piglets) but also in what measures are taken to determine that a vaccine is protecting animals after all! Classifying the antibodies into their isotypes (or sub-class of antibodies) is important to allow a prediction of the type of immune response generated by immunization: the ideal response leads to bacterial destruction. In the case of *S. suis*, this effect can be measured by a "killing assay"; which ensures that vaccine-produced antibodies are functional. Functionality depends on the antibody isotype produced: not all of them are able to induce *S. suis* elimination. However, there is currently no standardized protocols for testing *S. suis* vaccine efficacy (e.g. animal model, challenge infection, killing assays, etc.), further contributing to the confusion surrounding interpretation of vaccine trial results (Segura M., 2015). For example, of the 17 studies on *S. suis* vaccines published between 2015 and 2019, the majority tested for the presence of antibodies and performed mortality assessments in mice. Not even half of the aforementioned studies performed killing assays (and of these, the methodology used varied greatly), and/or the analysis of the type(s) of antibodies produced. Even fewer performed a morbidity/mortality assessment or tests in pigs! Of note: while vaccination of mice offers interesting predictive power in the case of negative results, promising vaccine candidates must absolutely be tested in pigs under controlled experimental challenge conditions (Figure 3). Yet, experimental *S. suis* infection of conventional piglets under laboratory conditions gives inconsistent results, provoking another drawback in vaccine development. Indeed, most *S. suis* serotypes are unable to cause any clinical signs under experimental conditions. In the case of autogenous vaccines, reports are almost inexistent (only 2 published papers in the last 30 years, Figure 2) or incomplete and, in most of them, a control (non-vaccinated) group is missing in order to afford scientifically sound conclusions (Segura M., 2015).



Unfortunately, vaccine testing itself is not the only area where knowledge is lacking. More studies on maternal antibody interference are necessary in order to determine conclusively whether it is preferable to vaccinate sows or piglets, and when? It is key to find the optimal window for piglet vaccination; after antibodies passed on by the sow have disappeared, but before the piglet is totally unprotected (and thus vulnerable to infection). Finally,

the chosen vaccine also needs to be practical for large-scale application; minimizing the number of necessary boosters, prioritizing immunization of sows rather than piglets would all prove valuable features to decrease cost and labor for producers. These matters further complicate the development of an ideal vaccine.

The need

With increasing restrictions on antibiotic use around the world, more studies on *S. suis* vaccine development and/or improvement are crucial. Given the vast diversity among *S. suis* infections by region, autogenous vaccines are likely to be the best option for protection against this bacterium that poses a risk to swine and human health. That said, testing protocol for these vaccines urges to be standardized internationally and more studies need to be performed in order to promptly draw coherent conclusions on the subject – before we lose control of *S. suis*.

Diagnosis of the infection caused by App

Marcelo Gottschalk
30-Jan-2017 (4 years 5 months 9 days ago)

App may cause three types of infections:

1. acute (high or intermediate mortality level)
2. chronic (low mortality, few and low specific clinical signs, reduced growth rates and/or lesions at slaughter)
3. subclinical (no clinical signs, no lesions at slaughter).

The diagnosis of the acute form is usually not complicated

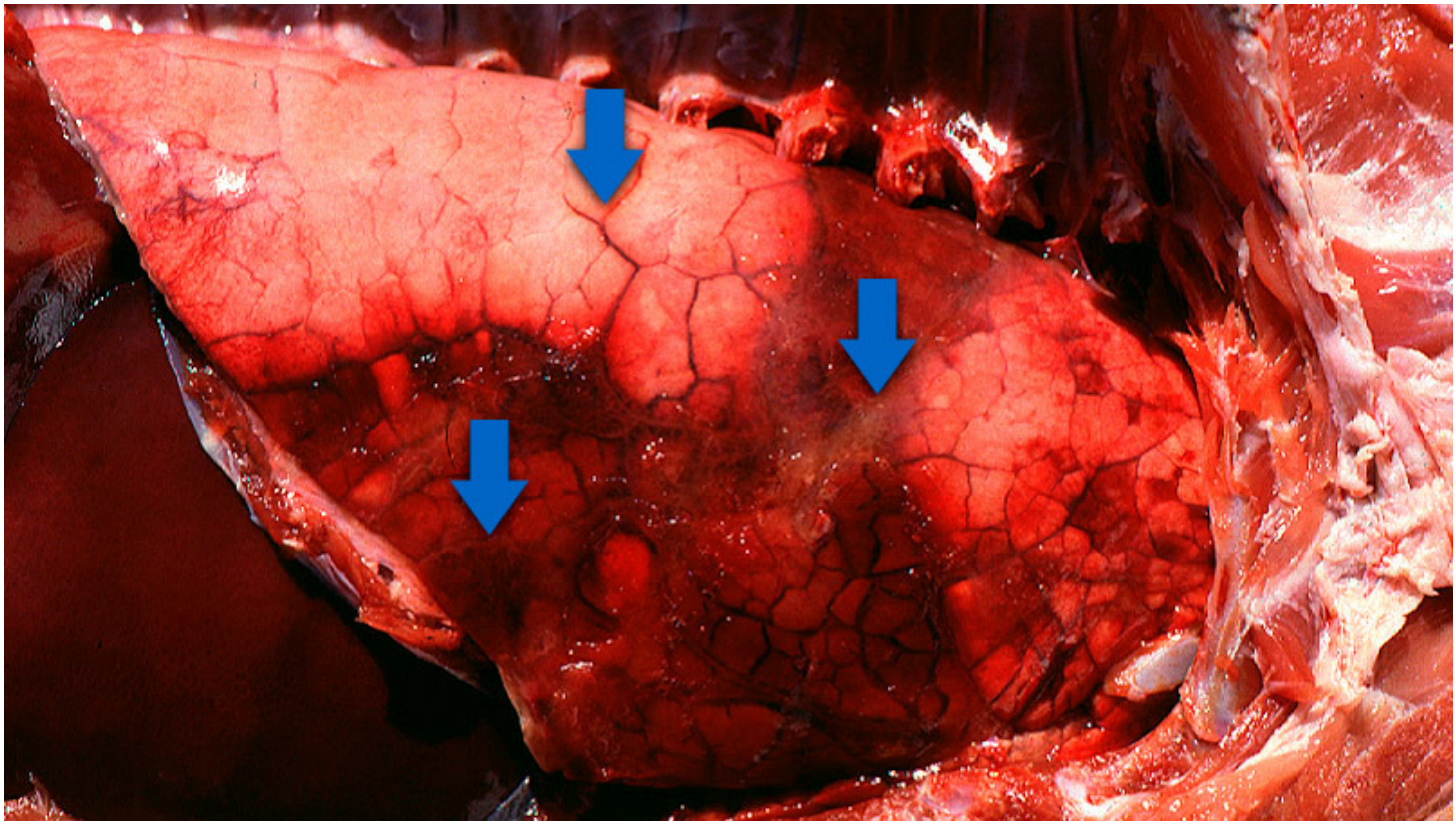
- Affected animals usually present lung lesions which are quite pathognomonic of App-affected animals. Samples should be taken from affected lungs and sent to the laboratory.
- Any diagnostic laboratory with experience on swine bacterial isolation would be able to correctly isolate and identify App. However, in certain European countries (for ex. Spain, Belgium, France), atypical App strains (called biotype II isolates) are present and this may be challenging for some laboratories.
- Identification of the serotype may be important: in an affected herd, this information will indicate the level of risk. High virulent serotypes are primary pathogens; intermediate virulent serotypes usually cause mortality in high-health status herds, or in the presence of co-infections and/or other predisposing factors. Knowing the serotype is also useful to identify which commercial bacterin may be used in a given herd. Finally, there is a need to know the distribution of virulent serotypes in a given area to evaluate the risk of sub-clinically infected herds (see below). Serotyping is now done by PCR and may be available for any diagnostic laboratory.

The diagnosis of the chronic form is somehow more difficult

- Pleurisy lesions observed at slaughter are somehow characteristic but not necessarily pathognomonic.
- Detection of App in such lesions, especially when they are relatively old, is very difficult and false negative results are usually obtained.
- Very often co-infections (such as *Mycoplasma hyopneumoniae*) are also present and complicate the diagnosis.
- If some clinical signs are present, a sero-profile using ApxIV toxin-ELISA (see below) may be done, first at the presence of clinical signs and then 21-28 days after, to confirm a clear sero-conversion.
- In the absence of clinical signs, lesions at slaughter along with clear and high ApxIV toxin-ELISA positive results ***might*** be an indication of App infection: between 25 and 30 serum samples should be tested. If positive, cross-sectional serum samples may be taken to confirm: 10 samples once a week from

10 to 20 week of age. A herd susceptible to be affected by App would be those with clear positive serological results that increased at a given time.

- In the absence of clinical signs, lesions at slaughter with the presence of LPS-based ELISA positive results (see below) for a virulent serotype (in a given country) ***is*** an indication of App infection. Serum samples at slaughter may be taken (between 25 and 30 serum samples).



The diagnosis of sub-clinically infected herds must be done through serology

Due to the absence of clinical signs and lesions it is not easy to diagnose sub-clinical infections due to App. Usually, animals are not affected (feed conversion, growth rate, etc.). This becomes an important issue for breeding herds: subclinical infected animals may mean "carrier animals" that may transmit App from tonsils to other more susceptible animals. A virulent strain may not induce disease in one herd but may cause high mortality in another more susceptible herd. Even within one herd, changes on herd management, environmental conditions or the arrival of co-infections may transform sub-clinical infection with a virulent strain into an outbreak of pleuropneumonia. Serology is an important tool to diagnose sub-clinically infected herds. Two questions arise:

a) Which test should be used?

Two types of ELISA tests are commercially available: App-specific Apxiv test (detecting all strains of App without distinction of serotype) and LPS-based serotype specific test (see Table 1).

Table 1: Use of commercially available ELISA test to detect antibodies against App

	ApxIV App-specific ELISA	LPS serotype/serogroup-specific ELISA
Commercial herds	Not useful (most herds give positive results)	Useful only if virulent serotypes are tested
High health status herds (confirmed as App-free)	Useful	Not useful (testing all individual serotypes is too expensive)

Sensitivity (after experimental infections)	+++	++++
Specificity (experimental infections with other bacterial species)	++++	+++
Routine use for the evaluation of breeders in several countries	No	Yes

The Apxiv test will be positive in any herd infected by App (virulent or non-virulent strains). More than 70% of commercial herds will be positive with this test in most countries. LPS-based tests should be oriented to the most important and virulent serotypes, which will give you the level of risk of a given herd. *There is a need to know the distribution of virulent serotypes in a given country: strains isolated from diseased animals must be serotyped to make this information available.*

b) How many samples and which category of animals should be taken to diagnose an infected herd? How often a herd should be tested to be classified as “non-infected herd”?

We recommend around 30 serum samples at the end of the finishing period; in batch farrowing system different batches should be tested to avoid false negative results. To certify a herd as "free", there is no universal rule, but it is recommended to repeat the testing at least 2-3 times a year.

Finally, for breeding herds, inconclusive serological results may complicate the diagnosis. In such cases, App detection from tonsils through the use of PCR of sero-positive animals may be done. If the herd is positive for different virulent and non-virulent serotypes, a serotype-specific (for the virulent serotype detected by serology) PCR must be used. For herds supposed to be App-free, an App-specific PCR (for all serotypes) can also be used. These tests must be done by experienced laboratories; however, false negative or positive results are sometimes observed.

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Prevention of the disease caused by *Actinobacillus pleuropneumoniae* (App)

Marcelo Gottschalk
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Once clinical disease of pleuropneumonia has been confirmed through laboratory examination, prevention measures must be applied.

Antimicrobials: If the appearance of clinical signs is predictable, antimicrobial treatments before specific high risk periods can be established. The reinforcement of political restrictions on the use of antimicrobials in order to reduce antimicrobial resistance in veterinary medicine makes this practice difficult/impossible in some European countries. However, the prophylactic/metaphylactic prevention of swine pleuropneumonia is still commonly used in other European as well as most American and Asian countries. There is not a clear recommendation about which specific antimicrobial drugs must be used: it is important to isolate the App involved in the case and to perform an antibiogram to correctly choose the antimicrobial. It has been reported that the massive use of very strong bactericidal antimicrobials may prevent the development of immunity in treated animals: once the antimicrobial treatment is stopped, clinical cases come back (bacteria remain hidden in the tonsils). The next question is: how much time the treatment must be kept? Usually, treatments should be kept for 2-3 weeks, but sometimes longer periods of time are needed to prevent new clinical cases. When disease appears in finisher animals, antimicrobials with shorter withdrawal periods must be chosen. This kind of prevention measures should be temporary. The use of vaccination is recommended to reduce antimicrobial use. However, under extreme conditions (highly virulent strains), antimicrobial support might be necessary to complete protection given by vaccines. Antimicrobial treatments do not eliminate App from tonsils of carrier animals.

Vaccination: There are different types of commercial vaccines available. These vaccines can be classified as:

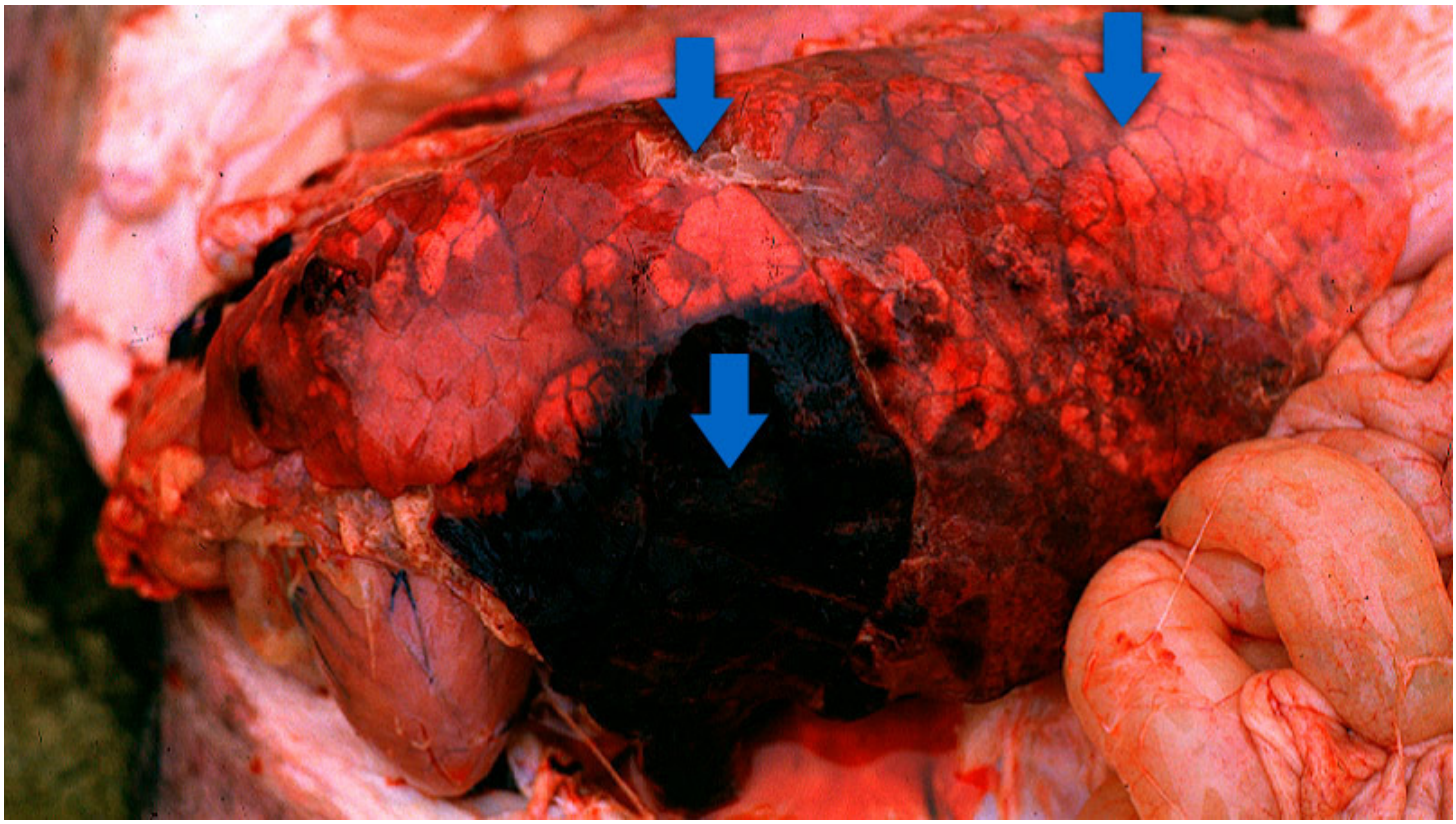
1. Bacterins (washed and killed whole bacteria): Protection given by this type of vaccine is **serotype-specific**: you need to know which serotype is involved in the farm. It is unusual (not impossible when different origins are mixed) to have more than one serotype causing disease in a single farm: a good diagnosis is needed. Antibodies raised against this vaccine are directed to "bacterial body antigens": capsule, surface proteins, bacterial cell wall, etc. When these antibodies are present in the animal lung, they "attach" to bacteria and alveolar macrophages and other cells will ingest and destroy App. In the absence of these antibodies, phagocytic cells cannot “capture” App bacteria, which reproduce and produce, among other products, the toxins and cause lesions.

2. Purified toxoid-based vaccines (sometimes enriched with surface proteins): ApxI, ApxII and ApxIII toxoids are present. Different strains of App produce one or two of these toxins. Protection given by this type of vaccine is usually against all serotypes, since all App strains produce one or two of the toxins included. Antibodies raised against this vaccine "neutralize" the toxins: just prevent them to cause lesions. Antibodies do not react with App bacteria: toxins are secreted. So, App can still reproduce in the lungs, although toxic effect of toxins is neutralized.

3. "Mixed": Bacterin (for specific serotypes) + purified toxoid-based vaccine: it is a combination of "1" and "2". Antibodies produced are anti-toxins and anti-bacterial components (only for serotypes included in the

vaccine).

Please note that autogenous vaccines are usually "bacterins". Its use should be restricted only to cases when the decision of using a bacterin has been taken and the serotype involved is either unknown or absent from commercial vaccines. Strains belonging to a given serotype are antigenically very similar around the world: a commercial vaccine produced with a serotype 2 isolated in Europe will protect against a serotype 2 isolated in Asia.



Vaccination of piglets

- Strong interference with maternal antibodies: usually, first dose should not be applied before 7-8 weeks of age.
- Never use a single dose: a booster reaction is needed.
- Avoid vaccination when high level of transmission of PRRS virus occurs at the same time among piglets.
- Adjust age of vaccination if needed, depending on the age when clinical signs appear, especially those that occur at the end of the finishing period: some commercial vaccines have the information of late protection. Otherwise, the vaccination can be moved later to keep high protection at the moment of the presence of clinical signs. Please allow at least 5-6 weeks after the first dose to reach good protection.

Vaccination of sows/gilts

- Two doses (second dose 3 weeks before farrowing) the first time; repeat one dose 3 weeks before farrowing each time.
- Increase the level of maternal antibodies when clinical cases are present in young animals (not very usual).

- Increase the level of maternal antibodies that reduce/delay colonization of piglets: in multi-site production system, these maternal antibodies would reduce the prevalence of carrier animals at weaning (around 3 weeks of age). Reduction of prevalence may reduce/eliminate the presence of clinical signs in grower-finisher animals. Only bacterins (with antibodies against the whole bacteria) can induce such reduction of colonization: antibodies "attached" to the whole bacteria will prevent/reduce App colonization of tonsils. Antibodies against secreted toxins (toxoid-based vaccines) do not have such effect.

- Vaccination of replacement gilts during quarantine can be done. If the objective is to protect App free highly susceptible animals (prevent disease), vaccination with either a bacterin or a toxoid-based vaccine can be done.

- High level of antibodies (after infection or vaccination) do not eliminate App from tonsils of carrier animals.

How to evaluate antibody response in vaccinated animals?

- If a bacterin is used: None of the commercial kits available (LPS-based ELISA tests) are able to accurately evaluate response to vaccination. These tests have been standardized to detect infection, not vaccination.

- Antibodies induced against vaccination are mainly those directed to the bacterial capsule, proteins and some of them against the LPS. The antigen used in those ELISA test is a purified LPS: it is possible that most antibodies produced after vaccination do not react with this antigen: A NEGATIVE ELISA TEST AFTER VACCINATION DOES NOT IMPLY LACK OF ANTIBODY RESPONSE AGAINST THE VACCINE.

- Even if antibodies against the LPS may be produced after vaccination, the commercial kits available have been standardized to detect levels of antibodies after infection, not vaccination. So, dilutions of sera needed to be done to carry out the test may induce negative results when evaluating antibody response against vaccination.

- Only customised ELISA tests, using whole bacteria as antigen (as that used in the vaccine) can be used to measure antibody response. Antibodies titers before and after vaccination can then be compared.

- If a toxin-based vaccine is used: there is no commercial test to measure antibodies against ApxI, II and III. These tests can only be done by vaccine-producing companies since purified antigens are needed. The ApxIV ELISA test cannot be used, since this toxin is never produced after vaccination (infection only).

Other measures to increase prevetion of App disease

- Strict use of "all in-all out" systems

- Use of batch farrowing

- Control of the environment (temperature, ventilation, etc.)

- Control of predisposal infections: mainly SIV and *Mycoplasma hyopneumoniae*.