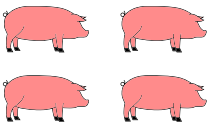
swine etiology
in the Iberian Peninsula:
statistics

exopol

Published in 2021

swine etiology in the Iberian Peninsula: statistics

which animals should be selected?



analyzing **more than one animal** so that the results are representative of the group of affected animals

For example, 4-5 piglets from different litters in case of diarrhea in lactating piglets

selecting **animals with clinical symptomatology at the beginning of the process**: this will allow the evaluation of the primary triggering agents

sending samples **before starting antibiotic treatment**, since it could interfere with the microbiological results

sending samples from **slaughtered animals or, failing that, that have recently died,** since the autolysis of the samples affects the success of the diagnosis

type of sample will be selected based on:

type of process

studied pathogen

requested diagnostic technique

purpose of the analysis: monitoring or diagnosis

sampling: requirements and due dates

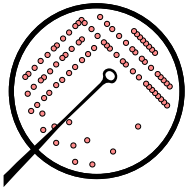
room temperature chilled frozen

		<24h	>24h	comments
EDTA tubes	blood	chilled	frozen	freeze for qPCR studies only
tubes without anticoagulants	serum	chilled	frozen	do not freeze serums with coagulants
sterile tubes	tracheobronquial lavage/scraping	chilled	frozen	freezing will affect microbial culture
	oral fluids	frozen	frozen	freeze and ship with freezer packs
swabs with medium	livestock and necropsies	room temperature	chilled	
air-tight container	skin scraping	chilled	chilled	
	feces	chilled	frozen	freezing will affect microbial culture
	organs	chilled	chilled frozen	take a swab (for microbiology) and freeze the organ (no use to histopathology)
environmental and surface samples	CORIOLIS (air) boot swab (dirty s.) wipes (clean s.)	frozen	frozen	freeze and ship with freezer packs always

microbial culture

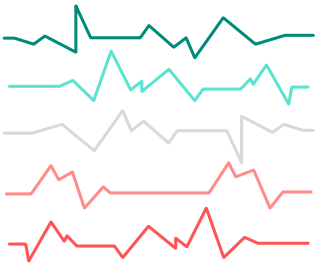
isolating and identify bacteria using MALDI-TOF

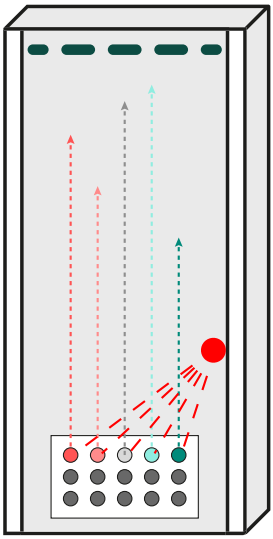
what is it?



clinical samples are sown in the appropriate culture media to obtain colonies of bacterial strains of clinical interest.

the **colonies** that grow are identified through mass spectrometry (MALDI-TOF), which allows for **identification at genus and/or species level** thanks to the "molecular fingerprint" detected through this technique. This molecular fingerprint is specific of each bacterium.





some bacteria are more difficult to grow than others

which bacteria are more difficult to grow?

Brachyspira spp., Clostridium difficile,
Mycoplasma hyopneumoniae, Glaesserella parasuis...

antibiograms

antibiotic susceptibility testing (Kirby Bauer method)

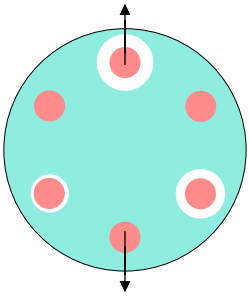
bacterial strains isolated in microbiological culture can be seeded in the right growth media where **discs containing a standardized antibiotic concentration are placed**

depending on the **diameter of the halo** and the it is determined whether the bacteria are sensitive or resistant

how is this interpreted?

sensitive:

antibiotic inhibits bacteria growth:
bacteria cannot grow around the disk because it is sensitive to it



resistant:

antibiotic does not inhibit bacteria growth:
bacteria can grow around the disk because the antibiotic does not take effect

you can check the list of

antibiotics analyzed in swine

in antibiograms and in the different MIC panels of the diagnosis page in our website (www.exopol.com)

minimum inhibitory concentration (MIC)

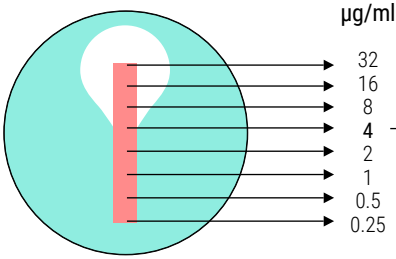
antibiotic susceptibility testing

minimum concentration of antibiotic that inhibits the growth of bacteria

exopol uses two different methods to check it:

MIC in cellulose strip (E-test system)

cellulose strip contains an **antibiotic gradient** that is placed on a culture plate where the tested bacteria was previously inoculated



clinical cut-off points for antibiotic A

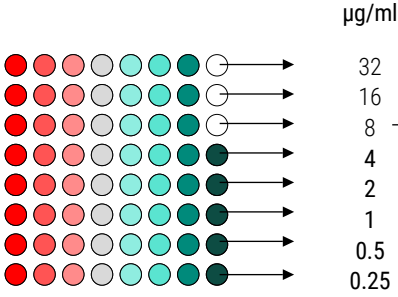
sensitive: ≤0,25

resistant: ≥1

bacteria are **resistant to antibiotic A** because **4 is ≥ 1**

MIC by broth microdilution

testing is performed in 96 well plates where tested bacteria face different concentrations of antibiotic



clinical cut-off points for antibiotic B

sensitive: ≤8

resistant: ≥32

bacteria are **sensitive to antibiotic B** because **8 is ≤ 8**

categorisation of antibiotic classes for veterinary use (EMA)

Category D: prudence

Aminoglycosides: spectinomycin

Tetracyclines: chlortetracycline, doxycycline, oxytetracycline, tetracycline, minocycline[1]

Group G and M penicillins: cloxacillin, penetamate, benzylpenicillin (G), phenoxymethylpenicillin (V)

Sulfamides, diaminopyrimidines and combinations: sulfadiazine, sulfadimethoxine, sulfadoxine, sulfadimidine, sulfamethoxazole, sulfamethoxypyridazine[1], sulfaquinoxaline, trimethoprim

Aminopenicillins: amoxicillin, metampicillin, ampicillin

Nitroimidazoles: metronidazole

Cyclic polypeptides: bacitracin

Nitrofuran derivatives: nitrofurantoin[1]

Steroidal antibacterials: fusidic acid (only in companion animals)

Category C: caution

Aminoglycosides: neomycin, gentamicin, streptomycin, apramycin, framycetin, kanamycin, paromomycin, amikacin[1]

Aminopenicillins combined with beta-lactamase inhibitors: amoxicillin-clavulanic acid

Macrolides: erythromycin, spiramycin, gamithromycin, tildipirosin, tylmicosin, tylosin, tylosin, tylvalosin, tulathromycin, azithromycin[1], clarithromycin[1]

Pleuromutilins: tiamulin, valnemulin

Lincosamides: lincomycin, clindamycin, pirlimycin

Amphenicols: florfenicol, thiamphenicol, chloramphenicol[2]

Cephalosporins (1st and 2nd generation): cefacetrile, cefadroxil, cephalexin, cephalonium, cephapirin, cephalothin[1], cefazolin[1]

Rifamycins: rifaximin

Category B: restrict

Polymyxins: colistin

Quinolones: enrofloxacin, danofloxacin, difloxacin[1], marbofloxacin, flumequine, pradofloxacin, ciprofloxacin[1]

Cephalosporins (3rd and 4th generation): cefovecin, cefquinome, ceftiofur, cefotaxime[1], ceftazidime[1], cefpodoxime[1]

Category A: avoid

Antibiotics in this category are not authorized as veterinary medicines. They should not be used in food-producing animals. They may be given to companion animals under exceptional circumstances. For example: imipenem, ticarcillin+ clavulanic acid and rifampin.

[1] Not authorized as veterinary medicines in Spain.

[2] Should not be used in food-producing animals for human consumption.

serology

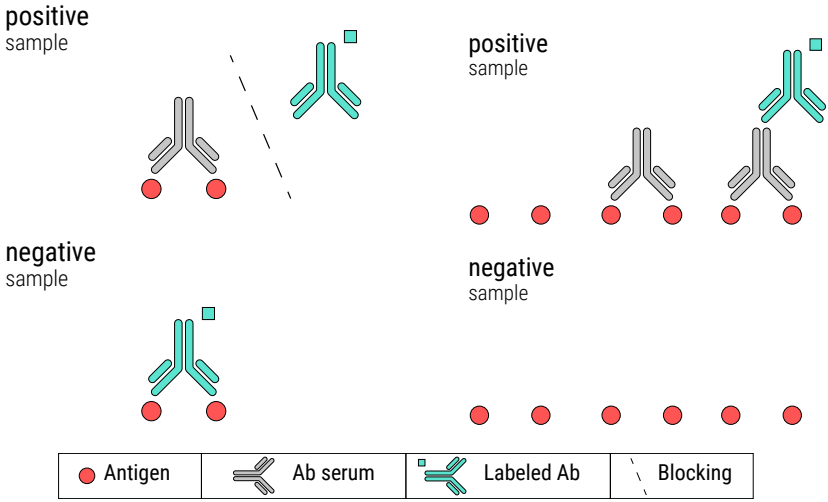
what is a serology?:

detection of developed antibodies vs. pathogens

positive — the animal has been vaccinated or infected

negative — the animal has never been infected and/or has not seroconverted

blocking ELISA	indirect ELISA



- ✓ individual diagnosis
- ✗ not useful for evaluating the rate of antibodies at flock level
- ✓ more specific

- ✓ prevalence at farm level
- ✓ evaluating the rate of antibodies at flock level
- ✗ possibility of false positives if the expected prevalence is low

DIVA ELISA

- ✓ differentiation between antibodies by field infection and vaccinated animals

Real Time PCR (qPCR)

what is a qPCR?:

pathogen detection throughout the amplification process of specific genes

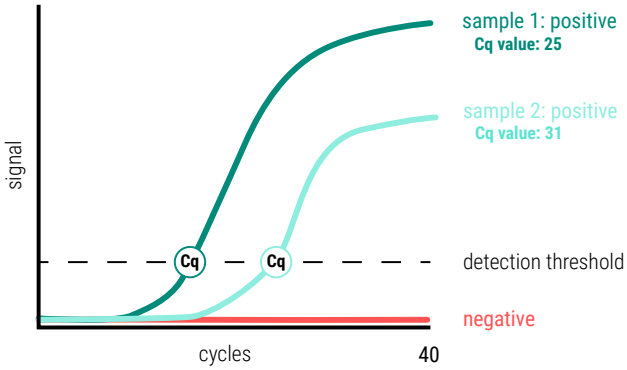
positivo — pathogen is present in sample

negativo — pathogen is not present in sample or is present in small quantities below the detection limit

what are the advantages of a qPCR?

- ✓ pool testing of samples - it is an extremely sensitive technique
- ✓ characterizing and typifying pathogens makes designing applicable autovaccines and choosing the vaccine which protects against the identified serotypes possible
- ✓ differentiating between field strains and vaccine strains
- ✓ carrying out epidemiological studies
- ✓ quantifying: detecting the pathogen concentration present in samples due to Cq value*

*Cq value: cycle in which the number of copies exceeds the detection threshold: the lower the Cq value, the higher the concentration of pathogens in the sample



sequencing

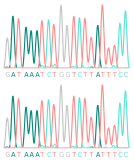
what is sequencing?:

determination of the nucleotide sequence of one or more genes

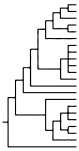
what are its applications?

sequencing *(e.g. PRRS ORF5)*

we compared the sequences obtained with those of vaccine strains or previous sequenced samples

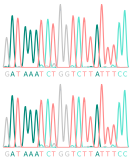


we generate phylogenetic trees

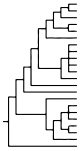


sequencing + genotyping *(e.g. Rotavirus A or Influenza A)*

we compare the sequences obtained with those of vaccine strains or previous sequenced samples



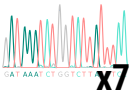
we generate phylogenetic trees



we obtain the strain genotype

MLST technique *(e.g. Brachyspira hyodysenteriae)*

we sequence seven genes



through changes in sequences we determine the alleles of each gene: the combination of the alleles of the 7 genes determines the ST (sequence type)

MLVA technique *(e.g. Mycoplasma hyopneumoniae)*

we sequence several loci with nucleotide tandem repeats

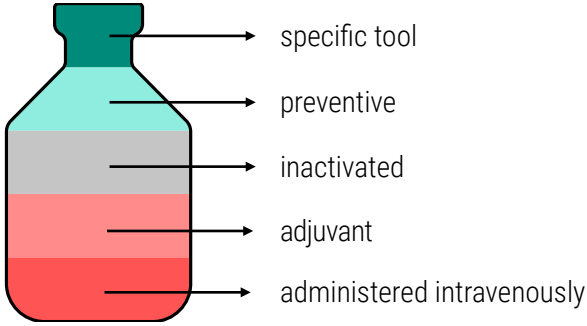
↓

based on the number of repeats we determine if it is the same strain

autogenous vaccines

what are autogenous vaccines?

immunological veterinary medicinal products manufactured by the isolation of the pathogenic agents from an epidemiological unit, inactivated and administered to the same herd



when should autogenous vaccines be administered?

- ✓ when there is no registered standard veterinary vaccine
- ✓ when there is no reasonably effective vaccine
  (e.g. high antigenic variability)

what are the requirements that must be met?

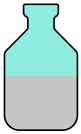
- presence of an infectious disease
- confirmatory laboratory diagnosis
- selecting the involved strains or serotypes
- under veterinary prescription
- produced by an authorized laboratory
  (Nª REG Exopol: 235/50/015-A)

specific to each farm

vaccines are not effective against some pathogens if they do not contain the serotypes or antigenic variants present on the farm.

we identify the **serotypes, virulence factors, toxinotypes or sequences specific genetic characteristics** of the strains isolated in each case in order to incorporate all of them into the autovaccine, thus guaranteeing maximum efficiency

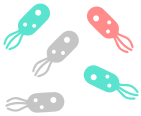
an example:



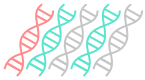
initial autogenous vaccine: two serotypes

→ shipping of new sample to monitor the farm

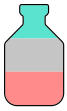
shipping of new **samples**



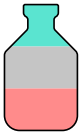
isolation of **bacteria**



tracking of the **new serotypes** via qPCR



incorporating the new serotypes into the **autovaccine**



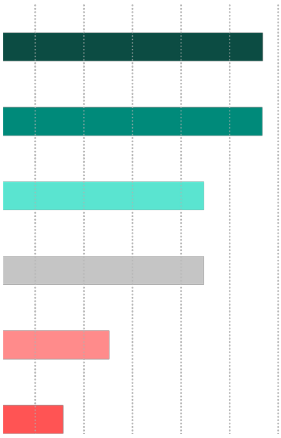
final autogenous vaccine: three serotypes

available autogenous vaccines for swine

Actinobacillus pleuropneumoniae
Brachyspira hyodysenteriae
Brachyspira pilosicoli
Corynebacterium spp.
Clostridiodes difficile
Clostridium perfringens type A
Erysipelothrix rhusiophatiae
Escherichia coli
Glaesserella parasuis
Mycoplasma hyorhinis
Pasteurella multocida
Salmonella sp.
Staphylococcus hyicus
Streptococcus suis
Trueperella pyogenes

We prepare autovaccines specific to farms or epidemiological units based on laboratory diagnosis and in which it is possible to combine different pathogens

primary autogenous vaccines produced in 2021:



Pasteurella multocida type B
Brachyspira hyodysenteriae
Meningitis
Actinobacillus pleuropneumoniae
Neonatal diarrhea
Epidermidis

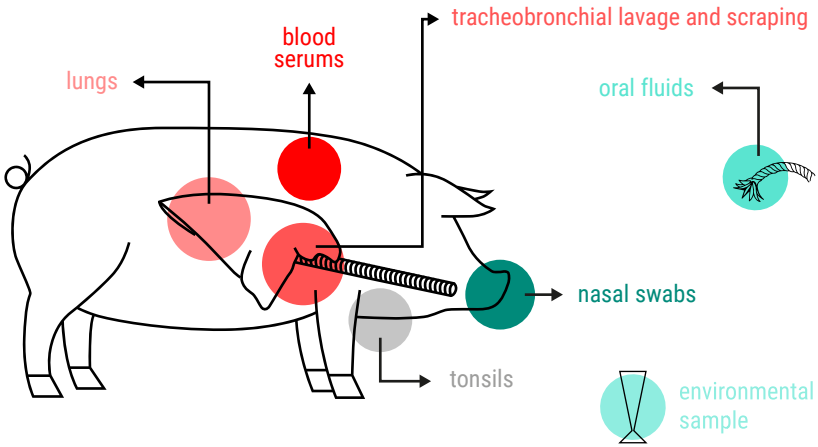
statistical results:

we compiled our laboratory data obtained in the last 5 years to provide you with statistics about the presence and incidence of pathogens in different processes, the evolution of antibiotic sensitivity, what serotypes are present, etc. in Spanish and Portuguese farms

- respiratory processes
- nervous processes, polyserositis, joint processes
- digestive processes
- reproductive processes
- other processes: septicemia and dermatitis

• respiratory processes

• sampling



• diagnostic panels

Respiratory:

qPCR: PRRS (EU/NA), Influenza A, Circovirus type 2, Circovirus type 3, Mycoplasma hyopneumoniae, Mycoplasma hyorhinis, Actinobacillus pleuropneumoniae, Glaesserella parasuis, Streptococcus suis, Pasteurella multocida, Bordetella bronchiseptica, Actinobacillus suis

Atrophic rhinitis:

qPCR: Bordetella bronchiseptica, Toxinogenic Pasteurella multocida

Respiratory - Serology:

Serology: PRRS (EU/NA), Influenza A, Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Circovirus type 2 (IgG and IgM)

Influenza A - Subtyping

qPCR: subtypes H1av, H1hu, H1pan, H3, N1, N2, N1pan

Influenza A - Sequencing (H gene: haemagglutinin)

Influenza A - Sequencing (N gene: neuraminidase)

Actinobacillus pleuropneumoniae - Serotyping

qPCR: Actinobacillus pleuropneumoniae, serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9/11, 10, 12, 13, 14, 15, 16, 17, 18, 19

Mycoplasma hyopneumoniae - Description (MLVA)

PCV2 - Sequencing (ORF2)

PCV3 - Sequencing (capside)

PRRS - Sequencing (ORF5)

One of the basic pillars for the **control of PRRS** in a pig holding is **monitoring** PRRS.

A regular inspection can help us **prevent new outbreaks or know how to control them more effectively.**

· PRRS diagnosis

via Real Time PCR:

health monitoring with: blood, serum, semen, tongues, processing fluids and oral fluids

sampling and analysis of **air** with equipment **CORIOLIS**

sampling and analysis of **surfaces, mammary glands and snouts**

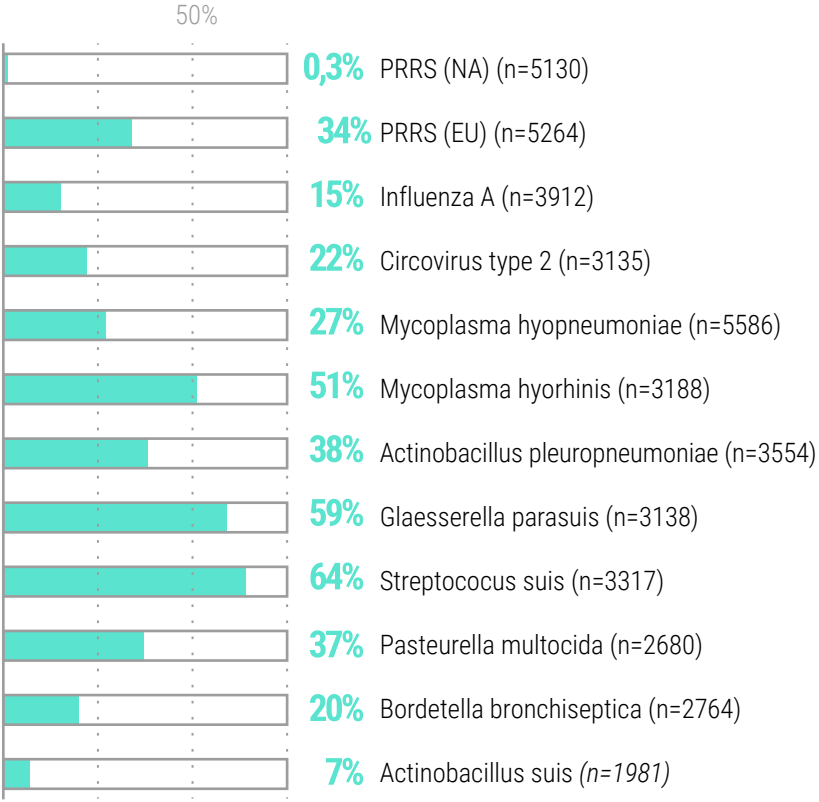
serology:

serology control in serums

• statistical results: diagnosis

pathogens analyzed in respiratory panel

% positives in the last 5 years



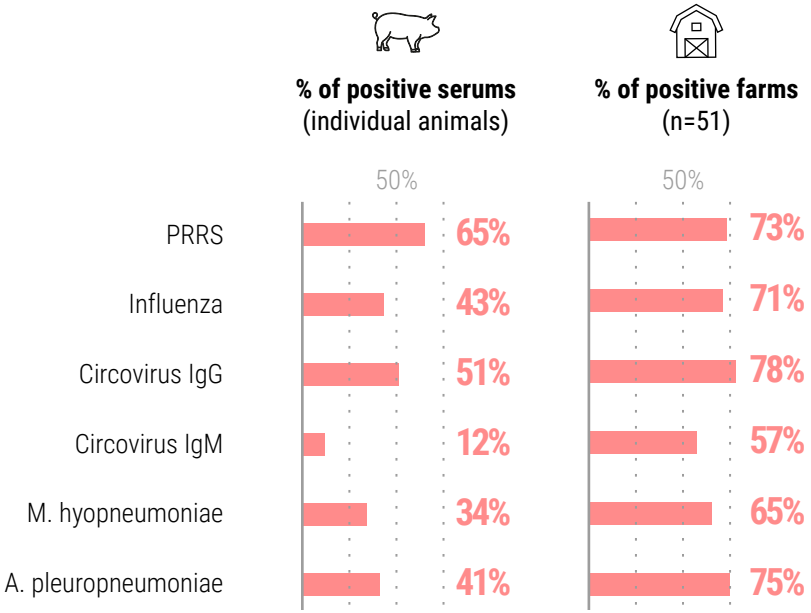
Percentage of positive cases for each of the agents tested via qPCR in the last five years.

It is important to differentiate between **primary agents** that cause respiratory processes, such as **PRRS** or **Actinobacillus pleuropneumoniae** and secondary or opportunistic agents (such as Streptococcus suis).

It is important to note that most clinical cases are respiratory complexes with more than one agent involved.

pathogens analyzed in respiratory-serological panel

seropositivity study at the individual and farm level using ELISA techniques



These results **should not be taken as prevalence data since they are biased samples of animals with respiratory symptoms**.

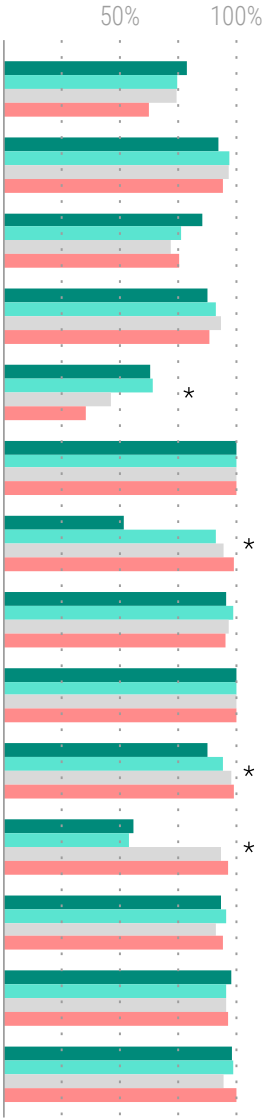
Likewise, bear in mind that **vaccinated animals will have positive serological results** (except in the case of Actinobacillus pleuropneumoniae since a DIVA test is performed).

Lastly, **a positive result is indicative of contact with the agent; and it cannot be assessed whether it is an acute process or not**, except in the case of Circovirus, where the presence of both IgM (acute response) and IgG (adaptive response) is detected.

Positive serums: the percentage of positive serums with respect to the total of tested serums. Positive farms: those that have obtained at least one positive serum.

antibiotic susceptibility testing (Kirby Bauer) of Pasteurella multocida

comparison of the sensitivity percentage of 330 antibiograms performed in the following semesters:



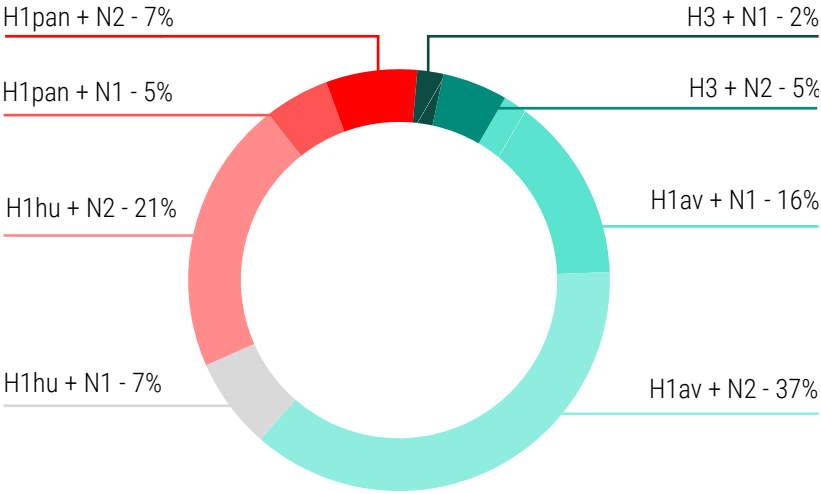
	oct. 18 mar. 19	apr. 19 sep. 19	oct. 19 mar. 20	apr. 20 sep. 20
Gentamicin	79%	75%	74%	62%
Enrofloxacin	92%	97%	97%	94%
Tetraciclin	85%	76%	72%	75%
Trimethoprim /sulfamethoxazole	88%	91%	93%	88%
Penicillin	63%	64%	46%	35%
Ceftiofur	100%	100%	100%	100%
Ampicillin	52%	91%	94%	99%
Tianfenicol	96%	99%	97%	95%
Amoxicillin + clavulanic acid	100%	100%	100%	100%
Gamithromycin	88%	94%	98%	99%
Amoxicillin	56%	54%	93%	97%
Florfenicol	93%	96%	91%	94%
Tilmicosin	98%	96%	96%	97%
Tiamulin	98%	99%	94%	100%

We evaluated the evolution of the sensitivity for each antibiotic over time using the Chi-square test. We considered that time and sensitivity variables are dependent, i.e., there are significant differences between the % of sensitive ones for the different time periods, if p-value < 0.01 (*).

In general, these strains show low antibiotic resistance, presenting **more resistance to gentamicin, tetracycline, and penicillin. A significant increase in strains sensitive to ampicillin, gamithromycin, and amoxicillin has been observed**. However, the appearance of penicillin-resistant strains has increased in recent years.

Influenza A subtypes identified in the last 5 years

84 clinic cases analyzed in 80 farms



Influenza A subtyping based on hemagglutinin (HA) and neuraminidase (NA) proteins. **The most prevalent subtypes are H1avN2 and H1huN2.**

In 15 % of cases, a co-infection caused by two different subtypes of Influenza A was detected.

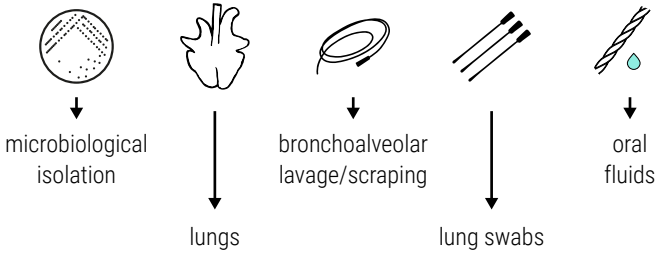
Porcine pleuropneumonia is a leading health problem due to the high economic impact it causes.

Control measures for this disease undergo Actinobacillus pleuropneumoniae serotype characterization. Since immunity is serotype-specific, it is essential to design the best strategy to combat this disease, i.e. the application of autovaccines containing such serotypes detected.

· serotyping of Actinobacillus pleuropneumoniae

accurate diagnosis = serotype identification via qPCR
(1, 2, 3, 4, 5, 6, 7, 8, 9/11, 10, 12, 13, 14, 15, 16, 17, 18, 19)

where are APP serotypes detected?



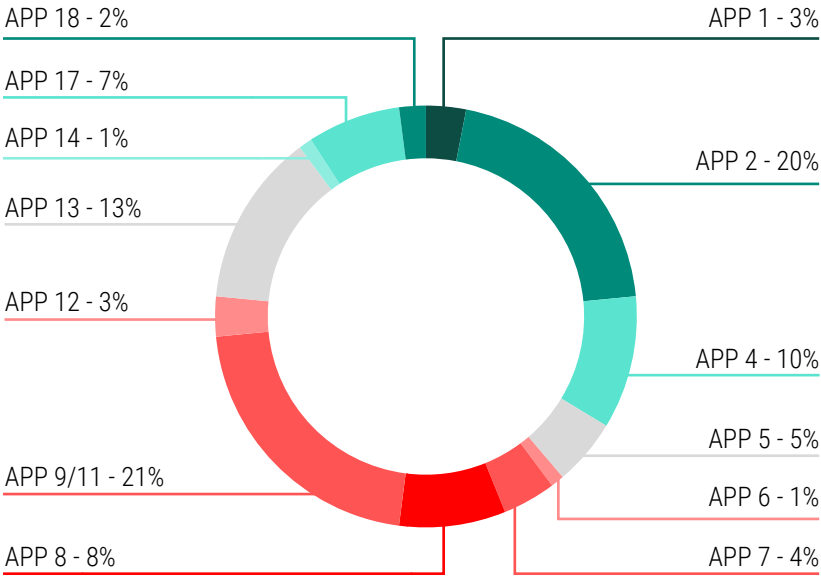
complete diagnosis with **serotype detection**

knowing the **epidemiological status** of a holding

designing a **control strategy** for this disease

APP serotypes identified in the last 5 years

468 clinic cases analyzed in 381 farms



Serotyping of isolates using the CPS gene, responsible for capsular polysaccharide biosynthesis. Thus far, 18* serotypes have been analyzed, 9/11, 2 and 13 being the most prevalent in Spain.

Typing is of great epidemiological interest in order to make preventive decisions, i.e. autovaccines.

**This graph does not include the study of serotype 19 since this serotype was recently described.*

In the last 2 years, there has been a **significant increase in strains resistant to gentamicin, tetracycline, and penicillin. By contrast, sensitivity to ampicillin and florfenicol has increased.**

antibiotic susceptibility testing (Kirby Bauer) of Actinobacillus pleuropneumoniae

comparison of the sensitivity percentage of 396 antibiograms performed in the following semesters:

	oct. 18 mar. 19	apr. 19 sep. 19	oct. 19 mar. 20	apr. 20 sep. 20
Gentamicin	88%	86%	56%	46%
Enrofloxacin	92%	95%	93%	100%
Tetraciclin	70%	70%	34%	60%
Trimethoprim /sulfamethoxazole	88%	88%	86%	90%
Penicillin	45%	50%	13%	21%
Clindamycin	13%	10%	9%	4%
Ceftiofur	100%	100%	100%	100%
Ampicillin	56%	73%	72%	79%
Tianfenicol	97%	95%	96%	98%
Amoxicillin + clavulanic acid	100%	100%	100%	100%
Gamithromycin	95%	100%	95%	100%
Amoxicillin	67%	74%	68%	79%
Florfenicol	94%	96%	82%	98%
Tilmicosin	97%	99%	96%	100%
Tiamulin	99%	98%	98%	98%

We evaluated the evolution of the sensitivity for each antibiotic over time using the Chi-square test. We considered that time and sensitivity variables are dependent, i.e., there are significant differences between the % of sensitive ones for the different time periods, if p-value < 0.01 (*).

antibiotic susceptibility testing (Minimum Inhibitory Concentration) of Actinobacillus pleuropneumoniae

MICs carried out in the last three years

Antibiotic	MIC50 (µg/mL)	MIC90 (µg/mL)	sensitive if: (µg/mL)	analyzed samples
Gentamicin	8	>8	≤2	54
Enrofloxacin	≤0,12	>1	≤0,5	55
Tetracycline	8	16	≤0,5	19
Trimethoprim/Sulfamethoxazole	>1	>1	≤2	53
Penicillin	1	>4	≤0,25	54
Clindamycin	4	>8	≤0,5	24
Ceftiofur	≤0,25	>4	≤2	54
Ampicillin	1	>8	≤0,5	54
Gamithromycin	1	>4	≤4	19
Amoxicillin	0,25	>256	≤4	50
Florfenicol	0,5	>4	≤4	53
Tilmicosin	4	>32	≤16	54
Tiamulin	>16	>16	≤16	54
Tulathromycin	≤4	>32	≤64	54
Tylosin tartrate	>16	>16	≤8	53
Marbofloxacin	0,016	0,125	≤1	54
Tildipirosin	1	>8	≤16	19
Danofloxacin	≤0,12	>0,5	≤0,25	54
Chlortetracycline	4	>4	≤0,5	35
Oxytetracycline	4	>4	≤0,5	35

This table shows the MIC50 and MIC90 values of the different antibiotics for APP. MIC50 and MIC90 values are the minimum concentration of antibiotic (in µg/mL) capable of inhibiting the growth of 50% and 90% of the strains analyzed, respectively.

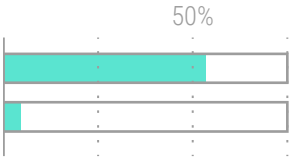
Strains are considered sensitive or resistant based on the clinical cut-off points established by governmental institutions (CLSI or Vetcast).

There is a significant difference between MIC50 and MIC90 of amoxicillin. This could be due to the presence of two populations of APP that show different sensitivity to this antibiotic. 16% of the strains analyzed have resulted in a MIC >256 µg/mL, which leads to the potential presence of a more resistant subpopulation.

These results may be biased, since MIC study is usually performed in the harshest environments, for example when the established antibiotic treatment is not working.

pathogens analyzed in atrophic rhinitis panel

% of positives in the last 5 years



53% Bordetella bronchiseptica (n=122)

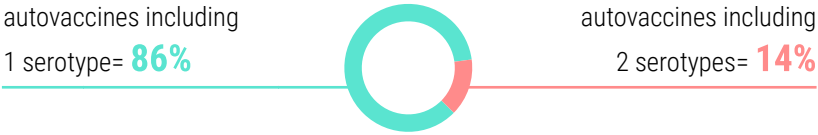
6% Toxigenic Pasteurella multocida (n=122)

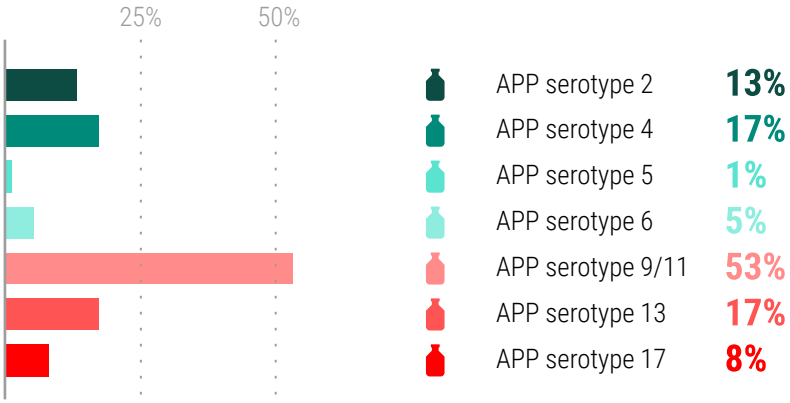
Bordetella bronchiseptica, which is the cause of non-progressive atrophic rhinitis, is the most prevalent agent with 53 % of positive cases, while toxigenic Pasteurella multocida has only been detected in 6 % of the cases analyzed.

• statistical results: autovaccines

autovaccines produced for Actinobacillus pleuropneumoniae in the last 3 years

Autovaccines include strains of each epidemiological unit isolated from clinical cases. Detecting more than one serotype is quite common when analyzing outbreaks that happened on different dates or locations. In this case, autovaccines must include all the serotypes detected.

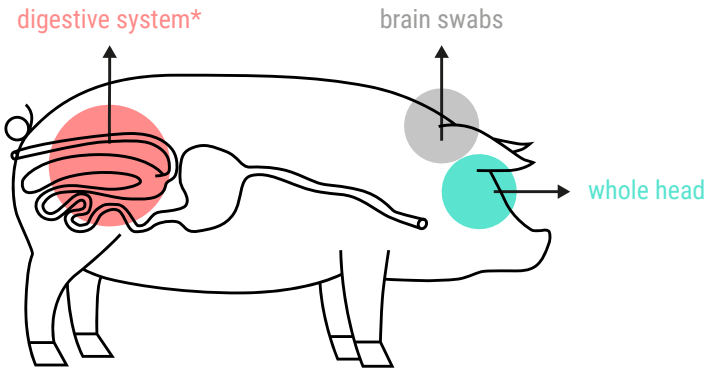




The percentages of autovaccines that include the different serotypes are displayed. Please note that the percentages add up to more than 100% because part of these produced autovaccines include two serotypes.

nervous processes

sampling



* samples for edema disease (ED) diagnosis

diagnostic panels

Nervous disease:

qPCR: E. coli F18, E. coli STX2e, Glaesserella parasuis, Streptococcus suis - Serotyping

Glaesserella parasuis - Serotyping:

qPCR: 1/2/11, 2, 3, 4, 5/12, 6, 7, 8, 9, 10, 11, 13, 14, virulent strain, non virulent strain

Streptococcus suis - Serotyping and virulence factors:

qPCR: S. suis, 1-14, 2-1/2, 1-1/2, 2-14, 3, 4, 5, 7, 8, 9, 14, suilysin (SLY), muramidase-released protein (MRP), extracellular protein factor (EPF)

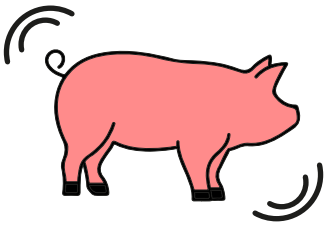
Atypical Porcine Pestivirus (APPV) is the causal agent of Congenital Tremor All, disease that leads to the death of piglets before weaning and causes coarse tremors of the head and the rest of the body.

· APPV identification via qPCR

causal agent

congenital tremor

- death of piglets before weaning
- coarse tremors of the head and the rest of the

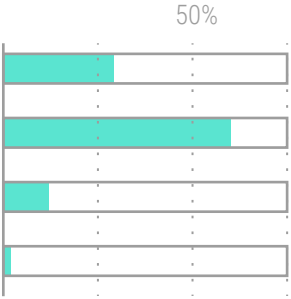


APPV is becoming increasingly important...

- present worldwide
- potential economic loss
- can cause chronic disease and persistent viral infections (PI)

pathogens analyzed in nervous panel

% of positives in the last 5 years



29% Glaesserella parasuis (n=753)

60% Streptococcus suis (n=851)

12% Escherichia coli F18+Stx2e (n=227)

2% Escherichia coli Stx2e (n=227)

The presence of **Streptococcus suis has been diagnosed in 60% of meningitis cases being studied**. Streptococcus suis has been found to be the most prevalent agent followed by **Glaesserella parasuis (29% of cases)**.

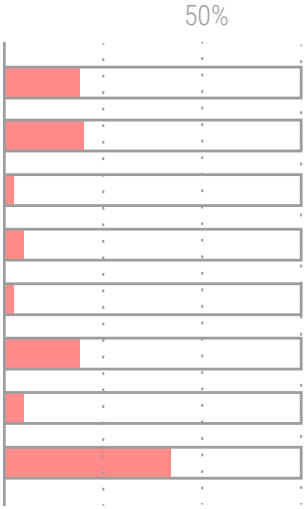
In **12%** of cases, the presence of genes encoding **F18 and Stx2e** was detected in the digestive sample, which is compatible with the presence of strains of enterotoxigenic E. coli that cause **edema disease***.

The presence of **shiga toxin-producing E. coli was detected in 2%** of cases. It was not possible to confirm a case of edema disease since it was not associated with F18 fimbriae.

**Results are obtained by comparing the concentration (inferred from Cq value) of the genes encoding fimbriae and toxins with each other and with the total concentration of E. coli in the sample.*

Streptococcus suis serotypes identified

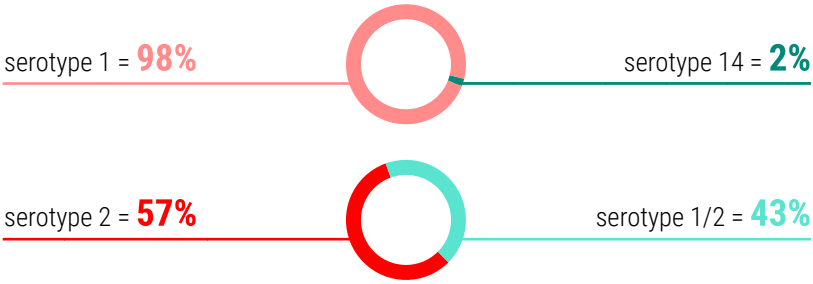
225 clinic cases analyzed in 135 farms in the last 5 years



19% Streptococcus suis serotype 1-14
20% Streptococcus suis serotype 2-1/2
2% Streptococcus suis serotype 3
4% Streptococcus suis serotype 4
2% Streptococcus suis serotype 5
19% Streptococcus suis serotype 7
4% Streptococcus suis serotype 8
42% Streptococcus suis serotype 9

Streptococcus suis serotype frequency - detected directly in clinical samples. **In 16% of cases, we found a co infection with more than one serotype** and in 9% of cases it has not been possible to determine the serotype. Most prevalent serotypes (in descending order):

- meningitis: 9 > 2-1/2 > 1-14 > 7
- arthritis: 9 > 1-14 > 7 > 2-1/2



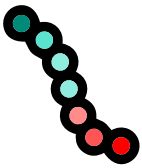
We recently incorporated **specific differentiation between serotypes 1 and 14 and serotypes 2 and 1/2**. We carried out a retrospective study of a total of 45 serotype 1-14 strains and 54 serotype 2-1/2 strains isolated from **clinical cases of meningitis in the last 3 years.**

Antibiotic use reduction programs led to an **increase in clinical cases of meningitis, polyserositis and arthritis in weaning pigs** caused by Streptococcus suis.

Multiple serotypes and virulence factors **whose detection is key to interpreting its role in clinical processes have been stated**. In addition, detection of **serotypes and virulence factors offers detailed information on the epidemiological situation of the production unit**.

· typification of Streptococcus suis

primary pathogen in cases of meningitis / artritis in weaning pigs



primary serotypes
1, 2, 1/2, 3, 4, 5, 8, 9, 14

main virulence factors
suilysin (sly), extra cellular protein factor (epf) y muramidase-released protein (mrp)

typifying Streptococcis suis via qPCR both in microbiological isolation and in clinical samples provides key information to take and develop the most effective control measures in our holdings

antibiotic susceptibility testing (Kirby Bauer) of Streptococcus suis

comparison of the sensitivity percentage of 1134 antibiograms performed in the following semesters:

	oct. 18 mar. 19	apr. 19 sep. 19	oct. 19 mar. 20	apr. 20 sep. 20
Gentamicin	84%	70%	72%	55%
Enrofloxacin	72%	59%	47%	52%
Tetracycline	6%	5%	4%	6%
Trimethoprim /sulfamethoxazole	51%	51%	53%	51%
Penicillin	91%	76%	81%	66%
Clindamycin	12%	10%	9%	10%
Ceftiofur	99%	93%	96%	91%
Ampicillin	95%	93%	97%	93%
Marbofloxacin	-	70%(1)	66%	67%
Tiamphenicol	95%	97%	97%	92%
Amoxicillin + clavulanic acid	98%	97%	99%	97%
Gamithromycin	22%	26%	20%	18%
Amoxicillin	91%(2)	76%	98%	92%
Florfenicol	93%	80%	59%	79%
Tilmicosin	18%	18%	14%	10%
Cefquinome	85%	91%	95%	87%
Tiamulin	94%	98%	98%	97%

We evaluated the evolution of the sensitivity for each antibiotic over time using the Chi-square test. We considered that time and sensitivity variables are dependent, i.e., there are significant differences between the % of sensitive ones for the different time periods, if p-value < 0.01 (*).

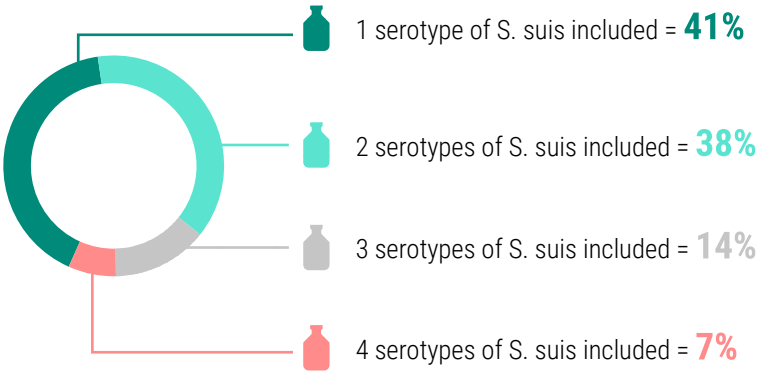
(1) Marbofloxacin susceptibility results have been studied as of July 2019.
(2) Amoxicillin results have been studied as of January 2019.

Studying a six-monthly evolution since October 2018, we observed a **significant decrease in the sensitivity of the strains against gentamicin, enrofloxacin, penicillin, florfenicol, marbofloxacin and ceftiofur.**

• statistical results: autovaccines

autovaccines produced for Streptococcus suis in the last 5 years

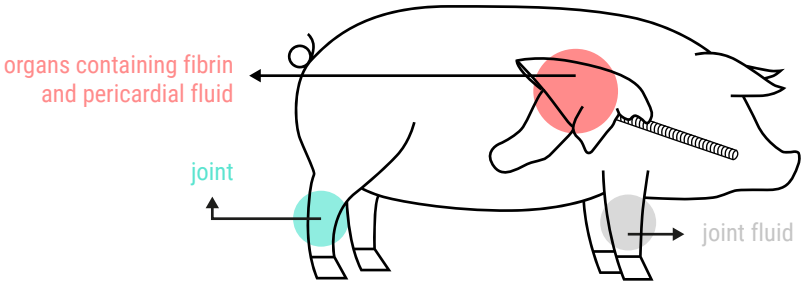
percentage of autovaccines produced with one or more serotypes



The application of Streptococcus suis autovaccines in sows helped **reduce the incidence of meningitis, polyserositis and arthritis in weaning animals.**

polyserositis

sampling



paneles diagnósticos

Poliserositis:

qPCR: Mycoplasma hyorhinis, Actinobacillus suis, Streptococcus suis - Serotyping, Glaesserella parasuis - Serotyping

Glaesserella parasuis - Serotyping:

qPCR: 1/2/11, 2, 3, 4, 5/12, 6, 7, 8, 9, 10, 11, 13, 14, virulent strain, non virulent strain

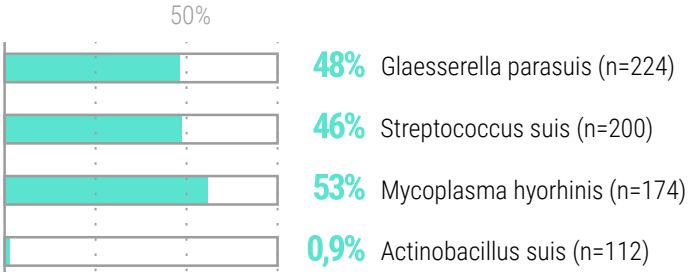
Streptococcus suis - Serotyping and virulence factors:

qPCR: S. suis, 1-14, 2-1/2, 1-1/2, 2-14, 3, 4, 5, 7, 8, 9, 14, suilysin (SLY), muramidase-released protein (MRP), extracellular protein factor (EPF)

statistical results: diagnosis

pathogens analyzed in polyserositis panel

% of positives in the last 5 years



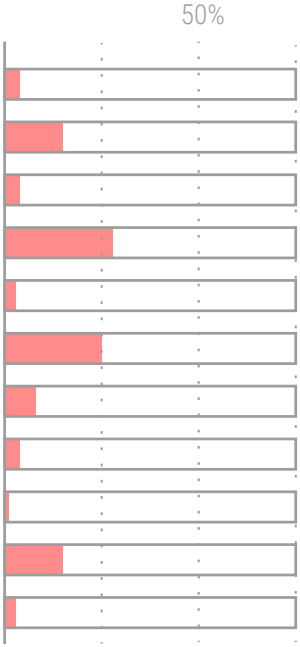
In cases of polyserositis, **Mycoplasma hyorhinis, Glaesserella parasuis and Streptococcus suis** show a similar incidence. Each of them was detected in **45-50% of the cases analyzed**.

Co infection with more than one of these agents is common. It was detected in **58% of the analyzed cases**.

Meanwhile, **Actinobacillus suis** –that was also described as a potential etiologic agent of polyserositis– was detected in **less than 1% of the cases analyzed.**

Glaesserella parasuis serotypes identified

52 clinic cases analyzed in 35 farms in the last 5 years



4%	Glaesserella parasuis serotype 1
15%	Glaesserella parasuis serotype 2
4%	Glaesserella parasuis serotype 4
28%	Glaesserella parasuis serotype 5/12
3%	Glaesserella parasuis serotype 6
25%	Glaesserella parasuis serotype 7
8%	Glaesserella parasuis serotype 9
4%	Glaesserella parasuis serotype 10
1%	Glaesserella parasuis serotype 11
15%	Glaesserella parasuis serotype 13
3%	Glaesserella parasuis serotype 14

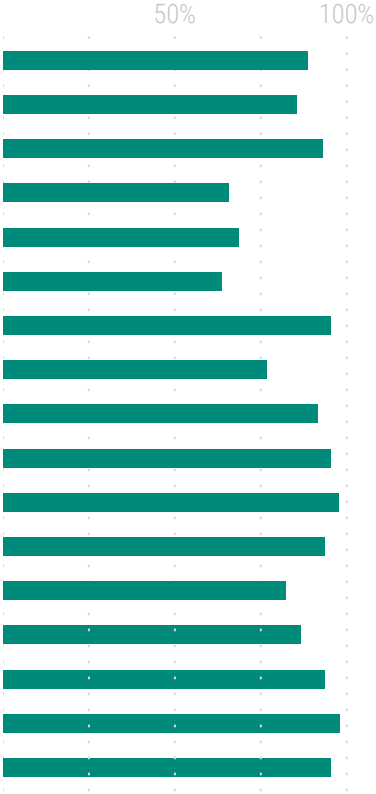
Serotyping of G. parasuis in clinical samples and isolates.
Most prevalent serotypes (in descending order): 5/12 > 7> 2 > 13

In 11% of cases, a co infection with different G. parasuis serotypes was detected and in 7% of cases it was not possible to determine the type of G. parasuis.

Serotyping is of great epidemiological interest so as to make preventive decisions, i.e. choosing a vaccine or autovaccine.

antibiotic susceptibility testing (Kirby Bauer) of Glaesserella parasuis

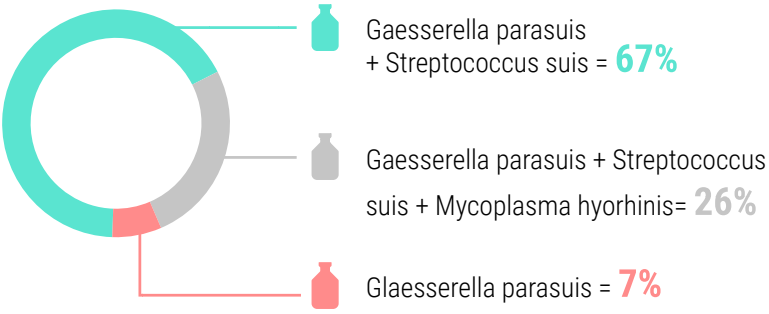
comparison of the sensitivity percentage of 82 antibiograms performed since October 2018



Gentamicin	88%
Enrofloxacin	84%
Tetraciclin	91%
Trimethoprim/sulfamethoxazole	65%
Neomycin	67%
Clindamycin	62%
Ceftiofur	94%
Ampicillin	76%
Tiamphenicol	90%
Amoxicillin + clavulanic acid	94%
Gamithromycin	96%
Cephalotin	91%
Amoxicillin	81%
Florfenicol	85%
Spectinomycin	93%
Tilmicosin	96%
Tiamulin	94%

autovaccines produced for polyserositis in the last 5 years

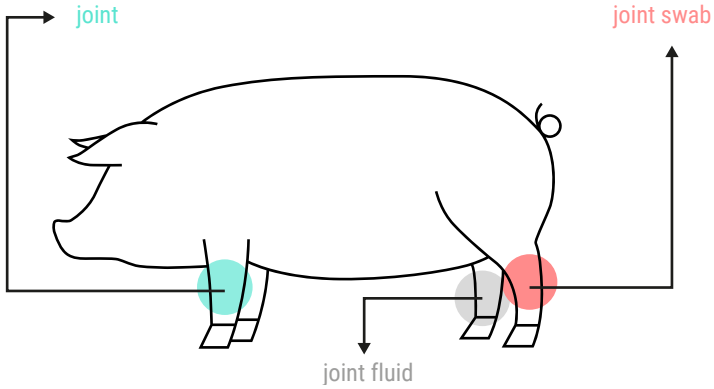
percentage of autovaccines produced with one or more pathogens



Autovaccines are specifically designed for both sows and piglets from each production unit with the strains obtained, **combining different agents and different serotypes** (Streptococcus suis and Glasesserella parasuis) from each of them.

joint processes

sampling



diagnostic panels

Joint disease:

qPCR: Mycoplasma hyorhinis, Mycoplasma hyosynoviae, Glaesserella parasuis, Erysipelothrix rhusiopathiae, Actinobacillus pleuropneumoniae, Actinobacillus suis, Streptococcus suis - Serotyping

Glaesserella parasuis - Serotyping:

qPCR: 1/2/11, 2, 3, 4, 5/12, 6, 7, 8, 9, 10, 11, 13, 14, virulent strain, non virulent strain

Streptococcus suis - Serotyping and virulence factors:

qPCR: S. suis, 1-14, 2-1/2, 1-1/2, 2-14, 3, 4, 5, 7, 8, 9, 14, suilysin (SLY), muramidase-released protein (MRP), extracellular protein factor (EPF)

• statistical results: diagnosis

pathogens analyzed in joint disease panel

% of positives in the last 5 years

3% Actinobacillus suis *(n=279)*

1% Erysipelothrix rhusiopathiae *(n=329)*

20% Glaesserella parasuis *(n=532)*

20% Mycoplasma hyorhinis *(n=445)*

6% Mycoplasma hyosynoviae *(n=368)*

45% Streptococcus suis *(n=564)*

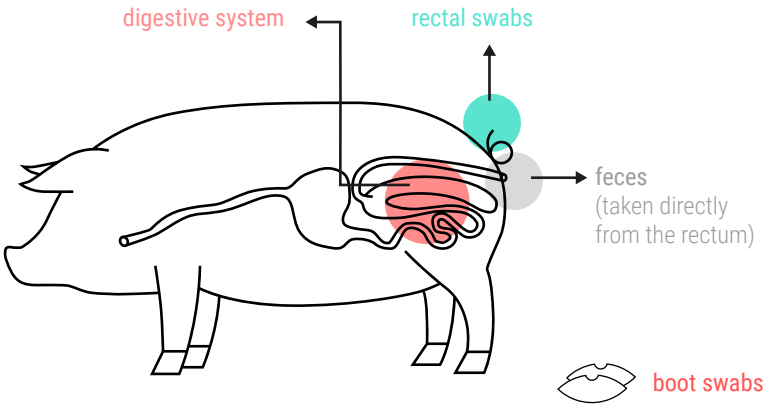
Differential diagnosis of infectious causes of joint processes that affect the porcine species.

Streptococcus suis is the most common infectious agent with 45% of positive cases, followed by Mycoplasma hyorhinis and Glaesserella parasuis.

Mycoplasma hyosynoviae was detected in 6% of cases. It is an agent that **causes arthritis mainly in growing/fattening animals, replacement female pigs and adult animals**. 12% of clinical cases of joint processes were positive for Mycoplasma hyosynoviae in these age groups.

• digestive processes

• sampling



• diagnostic panels

Digestive (piglets):

qPCR: Escherichia coli - Virulence factors, Clostridium perfringens - Toxins, Enterococcus hirae, Rotavirus A, Rotavirus C, PEDV, Transmissible gastro enteritis (TGE), Isospora suis, Clostridioides difficile

Digestive (postweaning):

qPCR: R: Escherichia coli - Virulence factors, Salmonella sp., Rotavirus A, PEDV

Digestive (fattening and adult animal):

qPCR: Lawsonia intracelullaris, Brachyspira hyodysenteriae, Brachyspira pilosicoli, Brachyspira intermedia, Salmonella sp., PEDV

Swine dysentery:

Microbiology: Brachyspira insolation, Brachyspira sp. MIC

qPCR: Brachyspira hyodysenteriae

Brachyspira - Isolation (up to 5 sample)

Coprological:

qPCR: Eimeria sp., Isospora suis, Entamoeba suis, Nematodes, Trichuris suis, Cestodes, Trematod

Escherichia coli - Virulence factors:

qPCR: E. coli gen eae, F4, F5, F6, F41, F18, STa, STb, LT, STX2e, AIDA, EAST, Escherichia coli

Clostridium perfringens - Toxins:

qPCR: Alpha, Beta, Epsilon, Iota, Enterotoxina, Beta-2

Brachyspira hyodysenteriae - Description (MLST)

Rotavirus A - Sequencing (VP7, VP4)

PEDV - Sequencing (Spike-S1)

Salmonellae swine:

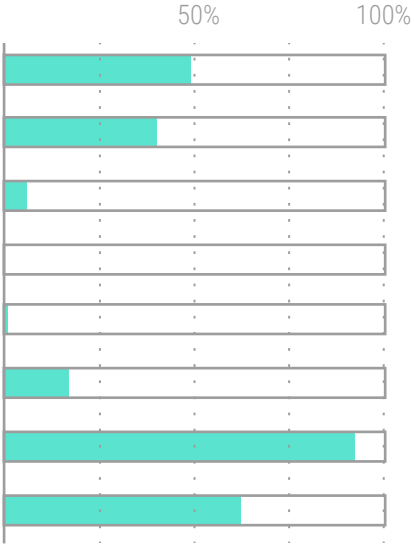
qPCR: Salmonella enteritidis, Salmonella typhimurium, Salmonella choleraesuis

· main Salmonella serovar detection via qPCR

	S. choleraesuis	S.typhimurium
affects	breeding sows	young animals (+8 weeks)
symptoms	fever, septicemia, depression, pneumonia, meningitis, arthritis, diarrhea	diarrhea
causes	clinical illnesses at times of elevated stress excretion	channel contamination (affects food hygiene)

pathogens analyzed in digestive panel during lactation

% of positives in the last 5 years



49% Rotavirus type A (n=1893)

40% Rotavirus type C (n=1606)

6% PEDV (n=1818)

0% TGE (n=295)

1% Eimeria sp. (n=1100)

17% Isospora suis (n=1110)

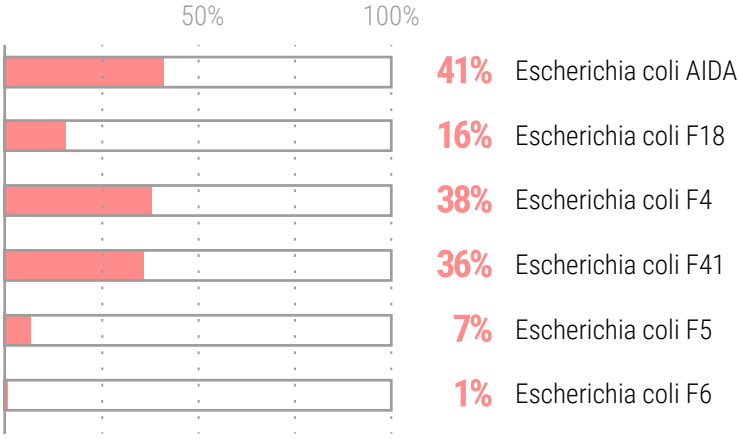
92% Clostridium perfringens (n=1703)

62% Clostridium difficile (n=1544)

Diarrhea in sucking piglets is a multifactorial process in which infectious agents and predisposing factors exist side-by-side. Detecting more than one infectious agent involved is the most common case scenario.

colibacillosis in sucking piglets:

% presence of fimbriae in clinical digestive samples from suspected cases of enterotoxigenic Escherichia coli (ETEC)* in 94 clinical samples analyzed



In lactating piglets, colibacillosis occurs mainly due to toxinogenic strains (ETEC) that adhere to the epithelium through fimbriae and adhesins, and produce the toxins that cause diarrhea (Sta, Stb, LT or EAST).

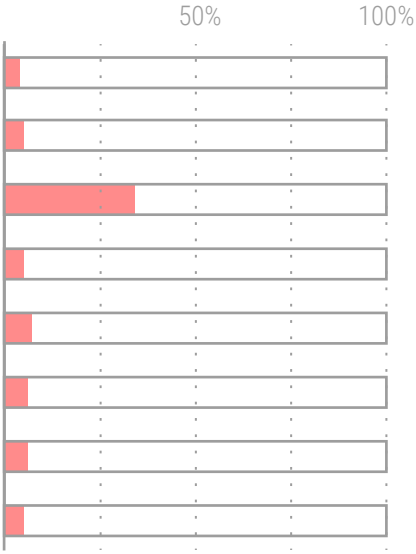
Patterns compatible with major ETEC strains were detected in 13% of the cases analyzed*. Enteropathogenic (EPEC) strains carriers of the EAE gene that lead to malabsorption due to the adhesion to the epithelium are also involved.

The presence of the EAE gene (EPEC strain) in clinical samples is extremely high (63% of cases), however high concentrations of its clinical implication are only found in 5% of the cases*.

**Results are obtained by comparing the concentration (inferred from Cq value) of the genes encoding fimbriae, adhesins and toxins with each other and with the total concentration of E. coli in the sample.*

colibacillosis in sucking piglets:

most common enterotoxigenic Escherichia coli (ETEC)* pathotypes in sucking piglets in 94 clinical samples tested



In ETEC strains, the most prevalent adhesion factors are the AIDA adhesin and the F4 and F41 fimbriae. However, the involvement of AIDA in cases of diarrhea is still under scientific discussion.

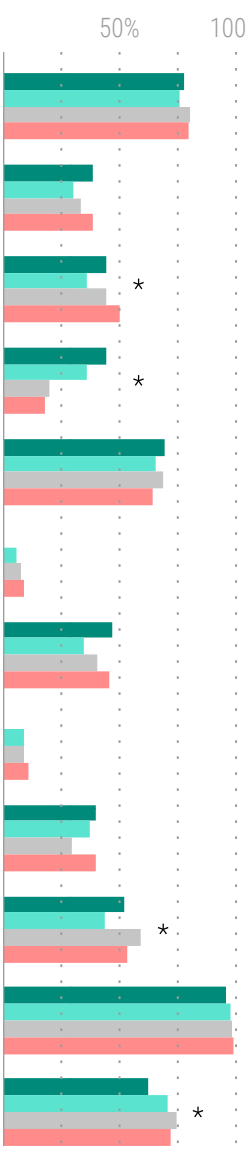
It should be stressed that we have found the presence of more than one of these adhesion factors in the same strain in 12% of the cases.

A wide variety of ETEC strains appear depending on the combination of fimbriae/adhesins and toxins. The predominant pathotype is the combination of AIDA adhesin and EAST and Stb toxins*.

**Results are obtained by comparing the concentration (inferred from Cq value) of the genes encoding fimbriae, adhesins and toxins with each other and with the total concentration of E. coli in the sample.*

antibiotic susceptibility testing (Kirby Bauer) of Escherichia coli in sucking piglets

comparison of the sensitivity percentage of 1203 antibiograms performed in the following semesters:



	oct. 18 mar. 19	apr. 19 sep. 19	oct. 19 mar. 20	apr. 20 sep. 20
Gentamicin	78%	76%	80%	80%
Enrofloxacin	39%	30%	33%	38%
Trimethoprim /sulfamethoxazolel	44%	36%	44%	50%
Neomicin	44%	36%	20%	18%
Ceftiofur	69%	66%	69%	64%
Ampicillin	-	6%	7%	9%
Amoxicillin + clavulanic acid	47%	35%	40%	45%
Amoxicillin	-	9%	9%	11%
Florfenicol	40%	38%	30%	40%
Spectinomycin	52%	44%	59%	53%
Colistin sulfate	96%	98%	98%	99%
Cefquinome	62%	71%	74%	72%

We evaluated the evolution of the sensitivity for each antibiotic over time using the Chi-square test. We considered that time and sensitivity variables are dependent, i.e., there are significant differences between the % of sensitive ones for the different time periods, if p-value < 0.01 (*).

When studying the evolution, there was a **significant increase in neomycin resistant strains**. However, there was a slight increase in the sensitivity of the strains against trimethoprim-sulfamethoxazole and cefquinome.

Toxin typing of Clostridium perfringens helps us know **what type of toxins produced by the strain have been detected**. Since C. perfringens is a pathogen thar is part of the gut microbiome, we can complete the diagnosis. **The isolation of C. perfringens does not necessarily indicate that it is the cause of the process.**

In addition, it will help us t**o make decisions about preventive measures** such as selecting vaccines that include the toxinotypes present on the holding.

· toxin typing of Clostridium perfringens

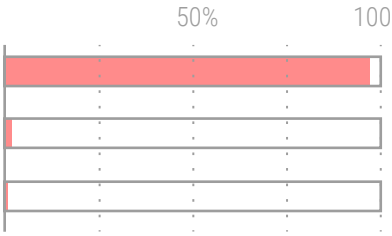
Clostridium perfringens strains are classified into five toxinotypes based on the production of four toxins

	Alpha	Beta	Epsilon	Iota
tipo A	+	-	-	-
tipo B	+	+	+	-
tipo C	+	+	-	-
tipo D	+	-	+	-
tipo E	+	-	-	+

the strains of these toxinotypes can also produce ENT and β2 toxins.

typification of Clostridium perfringens in the last 5 years

653 clinic cases analyzed in 333 farms



97% C. perfringens type A

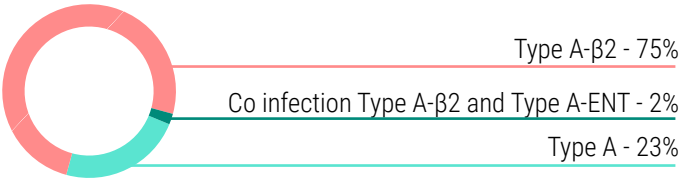
2% C. perfringens type C

1% C. perfringens type A + type C

97% of cases of diarrhea in lactating piglets positive for Clostridium perfringens are type A, 2% are type C, and in 1% of cases both toxinotypes are detected. Type C was detected in such a small number of cases. This is probably due to widespread use of vaccination plans against this agent.

A potential co-infection of different strains has been found in the sample based on the concentration of each gene inferred from Cq.

C. perfringens type A positive for toxin β2 and ENT



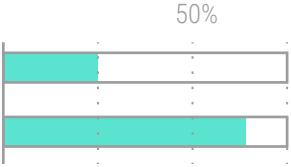
The presence of enterotoxins (ENT), and especially β2 toxin, relates to strains of Clostridium perfringens type A of greater pathogenic capacity.

75% of positive cases compatible with Clostridium perfringens type A were also positive for β2 toxin. 2% of cases showed genes encoding both β2 toxin and enterotoxin, signs of co-infection.

β2 toxin was found in all positive cases for C. perfringens type C.

viral pathogens tested in digestive panel in weaning pigs

% of positives in the last 5 years



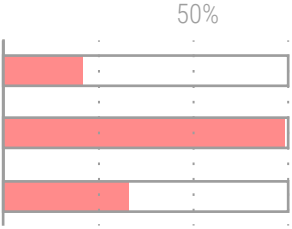
14% PEDV (n=1182)

64% Rotavirus type A (n=1057)

Rotavirus type A was found in 64% of the cases of diarrhea in weaning animals in the last 5 years, while PEDV was only identified in 14%.

colibacillosis in weaning animals:

% of presence of fimbriae in digestive samples of suspected cases of enterotoxigenic Escherichia coli (ETEC)* in 503 clinical samples analyzed



21% E. coli AIDA

74% E. coli F18

33% E. coli F4

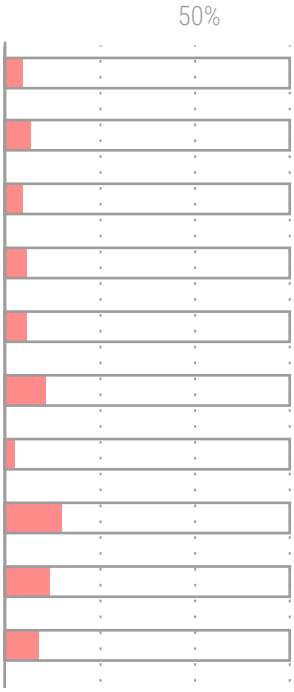
In weaning animals, colibacillosis is characterized by two processes: **post-weaning diarrhea caused by enterotoxigenic (ETEC) strains and, to a lesser extent, diarrhea caused by enteropathogenic strains (EPEC) producing intimin encoded by the eae gene.**

In 37% of cases, virulence factors compatible with the majority presence of an ETEC strain were detected, while 6% of cases are related to an EPEC strain*.

**Results are obtained by comparing the concentration (inferred from Cq value) of the genes encoding fimbriae, adhesins and toxins with each other and with the total concentration of E. coli in the sample.*

colibacillosis in weaning animals:

most common enterotoxigenic Escherichia coli (ETEC)* pathotypes in weaning piglets in 503 clinical samples tested



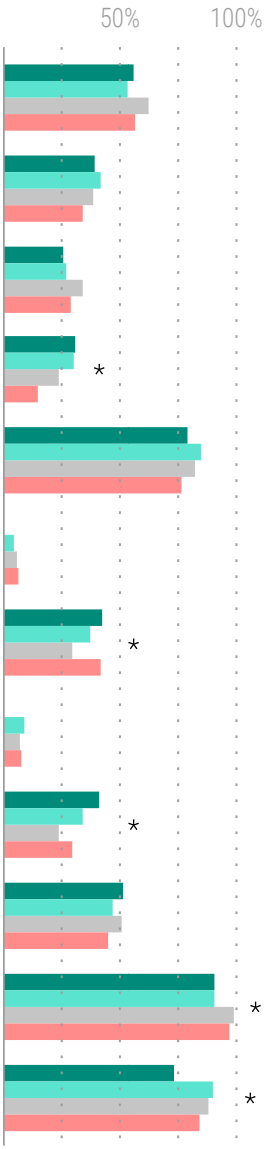
- **5%** E. coli F18 + EAST + LT + STb
- **7%** E. coli F18 + EAST + LT + STa + STb
- **5%** E. coli AIDA + EAST + STb
- **6%** E. coli F18 + EAST + LT + STa
- **6%** E. coli F4 + EAST + LT + STa + STb
- **11%** E. coli F4 + EAST + LT + STb
- **3%** E. coli F4 + STa + STb
- **15%** E. coli F18 + STa + STb
- **12%** E. coli F18 + STa
- **9%** E. coli F18 + LT + STa

In case of post-weaning diarrhea due to ETEC strains, **the most prevalent fimbria is F18, present in 74% of the strains considered as majority.** In 13% of the strains there was a combination of more than one adhesion factor. **The combination of F18 and Sta and Stb toxins is the most common one.**

**Results are obtained by comparing the concentration (inferred from Cq value) of the genes encoding fimbriae, adhesins and toxins with each other and with the total concentration of E. coli in the sample.*

antibiotic susceptibility testing (Kirby Bauer) of Escherichia coli in weaning piglets

comparison of the sensitivity percentage of 1323 antibiograms performed in the following semesters:



	oct. 18 mar. 19	apr. 19 sep. 19	oct. 19 mar. 20	apr. 20 sep. 20
Gentamicin	54%	51%	60%	54%
Enrofloxacin	38%	40%	38%	33%
Trimethoprim /sulfamethoxazole	25%	26%	33%	28%
Neomicin	30%	29%	23%	14%
Ceftiofur	76%	82%	80%	74%
Ampicillin	-	4%	6%	6%
Amoxicillin + clavulanic acid	41%	36%	29%	41%
Amoxicillin	-	9%	7%	7%
Florfenicol	40%	33%	23%	28%
Spectinomycin	50%	46%	49%	44%
Colistin sulfate	88%	88%	96%	94%
Cefquinome	71%	87%	85%	81%

We evaluated the evolution of the sensitivity for each antibiotic over time using the Chi-square test. We considered that time and sensitivity variables are dependent, i.e., there are significant differences between the % of sensitive ones for the different time periods, if p-value < 0.01 (*).

Colistin, cefquinome and ceftiofur are the antibiotics with greater effectiveness *in vitro* against E. coli strains isolated from weaning animals with diarrhea.

In the last two years there has been a **significant decrease in the proportion of strains sensitive to neomycin and florfenicol. The proportion of strains sensitive to colistin has significantly increased**, most likely due to the reduction in the use of this antibiotic.

antibiotic susceptibility testing (Minimum Inhibitory Concentration) of Escherichia coli in weaning animals

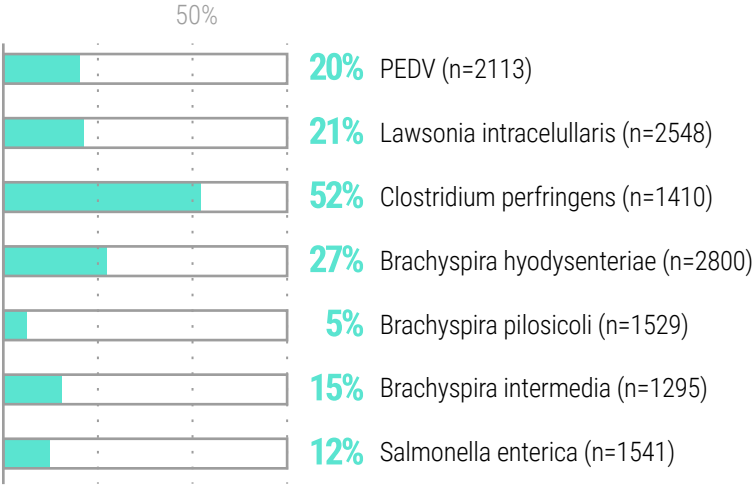
MICs carried out in the last three years

Antibiotic	MIC50 (µg/mL)	MIC90 (µg/mL)	sensitive if: (µg/mL)	analyzed samples
Gentamicin	>16	>16	≤2	59
Enrofloxacin	>2	>2	≤0,25	60
Trimethoprim/sulfamethoxazole	>2	>2	≤2	56
Neomycin	>32	>32	≤6	58
Ceftiofur	0,5	>8	≤2	56
Ampicillin	>16	>16	≤8	57
Amoxicillin + clavulanic acid	16	64	≤0,25	54
Florfenicol	>8	>8	≤2	56
Spectinomycin	>64	>64	≤32	56
Colistin	2	4	≤2	55

MIC study is usually carried out in the most complicated cases, e.g. when the established antibiotic treatment is not working properly, so results are expected to be biased towards particularly resistant strains.

pathogens analyzed in digestive panel in grow-finish phase animals

% of positives in the last 5 years



In cases of digestive processes in adult animals, **Brachyspira hyodysenteriae, Lawsonia intracellularis and PEDV are the main infectious agents** found to be the etiological cause.

Clostridium perfringens was detected in 52% of samples, but its presence does not necessarily presuppose a case of enterotoxaemia since it is part of the gut microbiome. The symptomatology, lesions and concentration detected must be jointly evaluated.

antibiotic susceptibility testing (Minimum Inhibitory Concentration) of Brachyspira hyodysenteriae

MICs carried out in the last three years

Antibiotic	MIC50 (µg/mL)	MIC90 (µg/mL)	sensitive if: (µg/mL)	samples analyzed	% sensitive
Lincomycin	16	>64	≤50	172	76,7%
Tiamulin	1	>8	≤0,5	172	42,4%
Tylosin Tartrate	>128	>128	≤32	172	12,8%
Tylvalosin	4	16	≤32	172	95,3%
Valnemulin	0,5	4	≤2	172	86,0%

The antibiotic susceptibility study* against Brachyspira hyodysenteriae is not routinely performed in all cases. This leads to a bias towards cases caused by especially resistant strains.

** Used cut-off points were established by the Clinical & Laboratory Standards Institute (CLSI), except for lincomycin. In this case, cut-off points have not been officially established, and therefore cut-off points described in literature have been used. These cut-off points vary depending on the author and the concentration of antibiotic used in animal feed. A vast number of strains show resistance to tylosin, which is consistent with field results obtained in recent years.*

Characterization of B. hyodysenteriae via MLST makes implementing epidemiological control measures, limiting the extent of the disease and designing autovaccines easier.

sequencing seven genes →

identifying corresponding alleles →

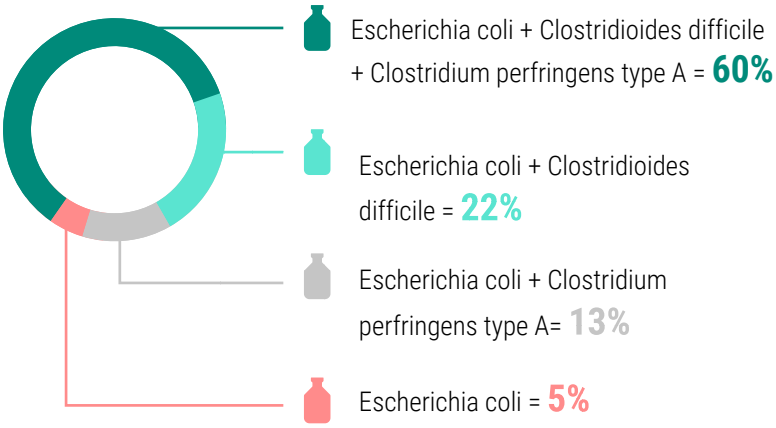
ST123

combining alleles = sequence type (ST)

• statistical results: autovaccines

autovaccines produced for neonatal diarrhea in the last 5 years

percentage of autovaccines produced with one or more pathogens



These autovaccines are administered to sows and are complementary to vaccines commercially available. It is appropriate to redesign the autovaccines periodically to adapt them to the new emerging strains.

autovaccines: Brachyspira hyodysenteriae

sow vaccination

- ↓ infection pressure
- ↓ transmission to piglets

highly effective in systems of **production in phases**

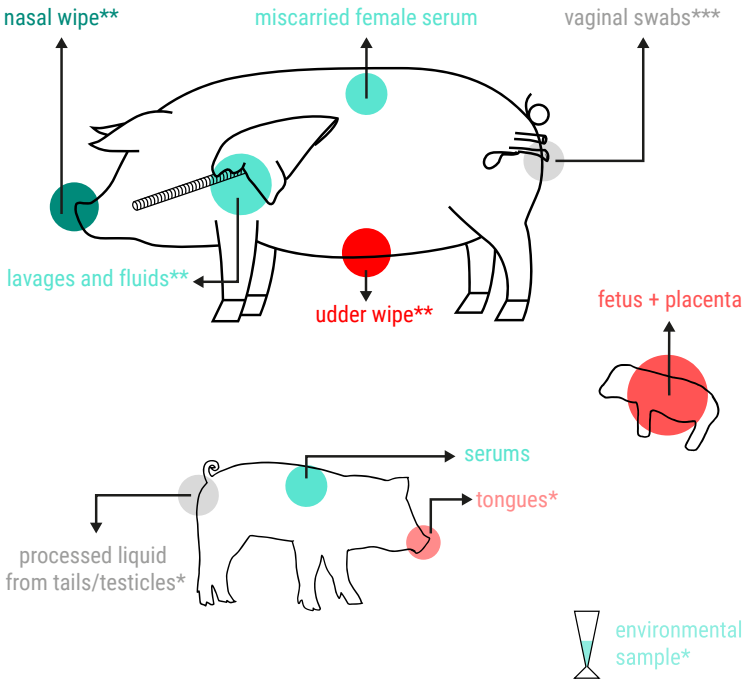
sow and piglet vaccination

necessary in:

- · **farrow-to-finish** holdings
- · other production systems e.g. **Iberian pig**

• reproductive processes

• sampling



*samples for specific diagnosis of: *PRRS **PRRS and Influenza ***metritis*

• diagnostic panels

Infertility / Metritis:

Microbioloy: : Bacteria isolation, Antimicrobial susceptibility test

qPCR: Pathogenic Leptospira, Chlamydiaceae (all species), Brucella sp.

Reproductive:

qPCR: PRRS (EU/NA), Circovirus type 2, Circovirus type 3, Porcine Parvovirus, Pathogenic Leptospira, Chlamydiaceae, Brucella sp.

Reproductive - Serology:

Serology: Brucella spp. (Rose Bengal test), PRRS (EU/NA), Porcine Parvovirus, Influenza A, Erysipelothrix rhusiopathiae, Circovirus type 2 (IgG and IgM)

Leptospira sp. - Typing

Leptospirosis can be a **serious reproductive disease** found throughout the world with a considerable economic impact in the pig industry.

Every host has serovars of Leptospira interrogans. **Host-adapted serovars usually cause a more insidious disease and not adapted serovars cause acute forms of leptospirosis.**

· typing of Leptospira sp. through sequencing

pathogen involved in reproductive diseases

knowing the serovar involved

is essential to treatingand controlling the disease

what does sequence typing add to the process?

- ✓ greater speed
- ✓ automated technique
- ✓ carried out in isolation and in tissues

pathogens analyzed in reproductive panel

% of positives in the last 5 years

3% Brucella sp. *(n=512)*

8% Chlamydiaceae *(n=523)*

7% Circovirus type 2 *(n=551)*

2% Circovirus type 3 *(n=126)*

11% Pathogenic Leptospira *(n=553)*

2% Porcine Parvovirus *(n=531)*

0% PRRS (NA) *(n=591)*

14% PRRS (EU) *(n=595)*

qPCR results on samples collected from **fetuses, placentas, endocervical swabs or serums from weak-born piglets.**

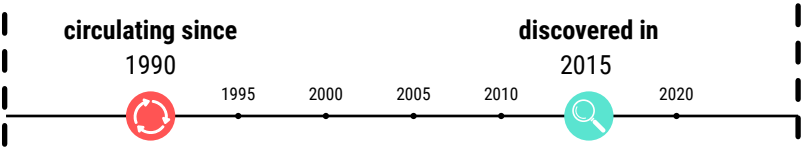
PRRS is the most common infectious agent, present in **14% of cases**, followed by pathogenic Leptospiras (11%) and Chlamydiaceae (8%).

Influenza A and Erysipelothrix rhusiopathiae do not cross the placental barrier, causing **indirect abortions** due to the female's inflammatory and febrile process. This is the reason this graph does not show the incidence of Influenza A and Erysipelothrix rhusiopathiae.

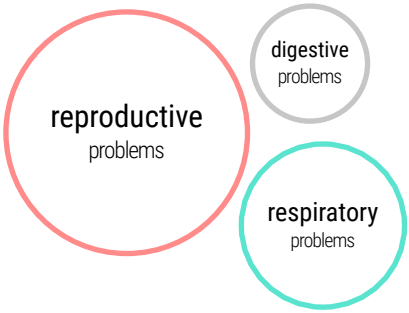
The **Circovirus type 3 (PCV3)** virus was discovered recently, although it has been confirmed that the virus has been circulating at least since the 1990s. It was detected in clinical samples of **reproductive, respiratory, and even digestive diseases.**

Although its role as a causal agent is still under study, there is increasing evidence that it **is a pathogenic agent inducing disorders of the reproductive system.**

· PCV3 diagnosis



where was PCV3 detected?

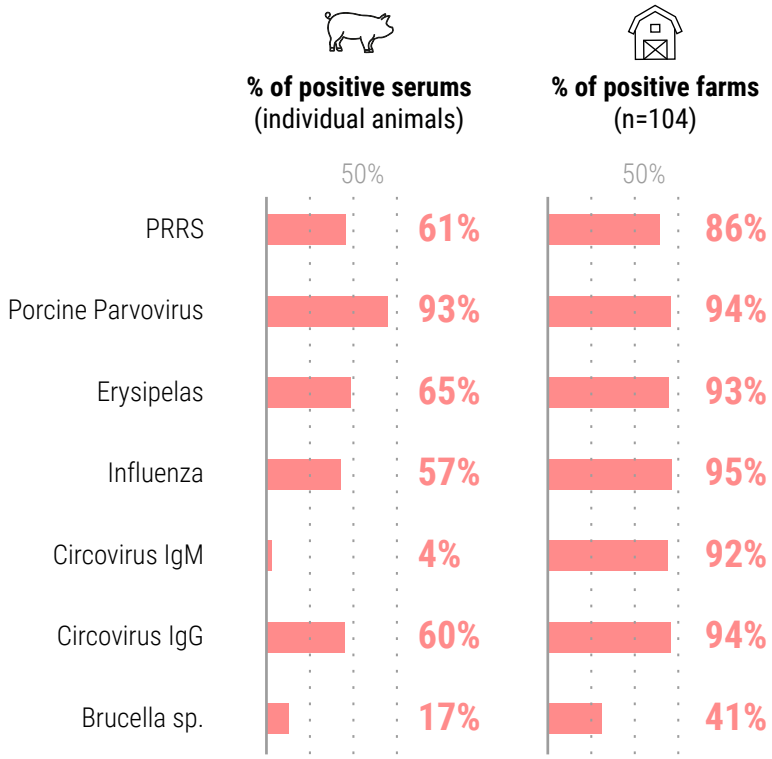


Including PCV3 in the **differential diagnosis** of reproductive and respiratory we can assess:

- ✓ **incidence** in clinical cases
- ✓ **concentration** in samples
- ✓ **coexistence** with other pathogenic agents

pathogens analyzed in serological-reproductive panel

seropositivity at individual and farm level through ELISA techniques



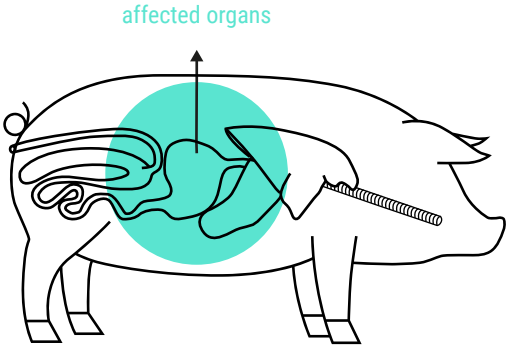
These results should not be taken as prevalence data since they are biased samples of animals with reproductive problems.

They have been analyzed using the ELISA technique for all diseases except in the case of Brucella sp. Rose Bengal Test was carried out in this case. This technique is extremely sensitive but not specific due to its cross-reaction with other bacteria such as Yersinia enterocolitica or certain serovars of E. coli.

Finally, **it should be noted that vaccinated animals will show positive serological results**.

septicemia

sampling



diagnostic panels

Sudden death

qPCR: Pasteurella multocida type B, Salmonella sp., Erysipelothrix rhusiopathiae, Paeniclostridium sordellii, Clostridium septicum, Clostridium chauvoei, Clostridium novyi, Clostridium haemolyticum

Systemic clostridiosis

qPCR: Paeniclostridium sordellii, Clostridium septicum, Clostridium chauvoei, Clostridium novyi, Clostridium haemolyticum

Pasteurella multocida - Capsular typing

qPCR: capsular types A, B, D, E, F

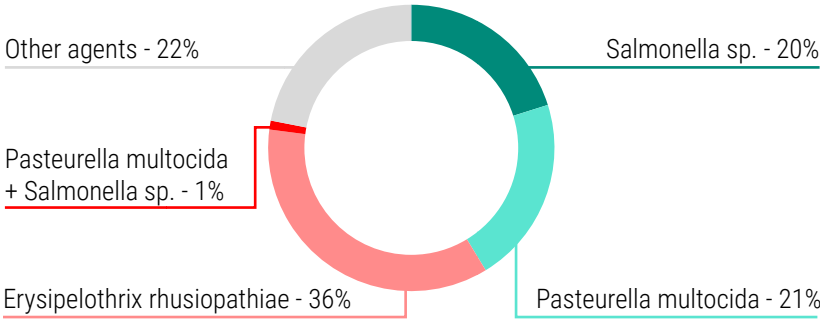
Erysipelothrix rhusiopathiae - Serotyping

qPCR: 1a, 1b, 2, 5

• statistical results: diagnosis

isolated bacteria in cases of septicemia

% of positives in the last 5 years

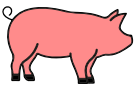


78% of cases of sudden septicemic casualties caused by aerobic bacteria are due to one of the following agents: E. rhusiopathiae, P. multocida or Salmonella sp. Cases of Erysipelas are the most common (mainly in Iberian pigs). These data do not include casualties due to Clostridium sp. since these bacteria are not isolated in cultures in aerobiosis.

Serotyping of Erysipelothrix rhusiopathiae via qPCR makes characterizing strains, detecting circulating serotypes and designing potential autovaccines possible.

· serotyping of Erysipelothrix rhusiopathiae

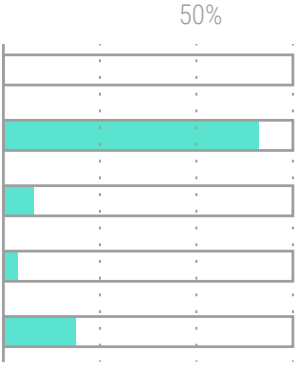
erysipela polyarthritis endocarditis septicemia miscarriage



most common serotypes in swine:

· 1A · 1B · 2

pathogens analyzed in systemic clostridiosis panel via qPCR



0% Clostridium chauvoei (n=53)

66% Clostridium haemolyticum (n=53)

8% Clostridium novyi (n=53)

4% Clostridium septicum (n=53)

19% Clostridium sordelli (n=53)

In case of sudden deaths, it will also be necessary to carry out a differential diagnosis with Systemic clostridiosis (C. Sordellii, C. septicum, C. chauvoei, C. novyi, C. haemolyticum) **and with C. perfringens enterotoxemia.**

In cases of septic processes due to Pasteurella multocida only type B was detected. No involvement of other capusllar types (A, D, E or F) in pigs was found.

Pasteurella multocida with capsular types A and D were found to be involved in respiratory processes.

• autovaccines: Pasteurella multocida type B

Vaccination of piglets and sows with autovaccines of P. multocida type B is recommended in the absence of vaccines commercially available that include this capsular type in swine.

septicemia caused by **P. multocida type B**, leading to sudden death and neck edema, mainly affects **Iberian piglets during the fattening period**

Polígono Río Gállego D/14
50840, San Mateo de Gállego
Zaragoza, SPAIN

www.exopol.com
+34 976 69 45 25
exopol@exopol.com