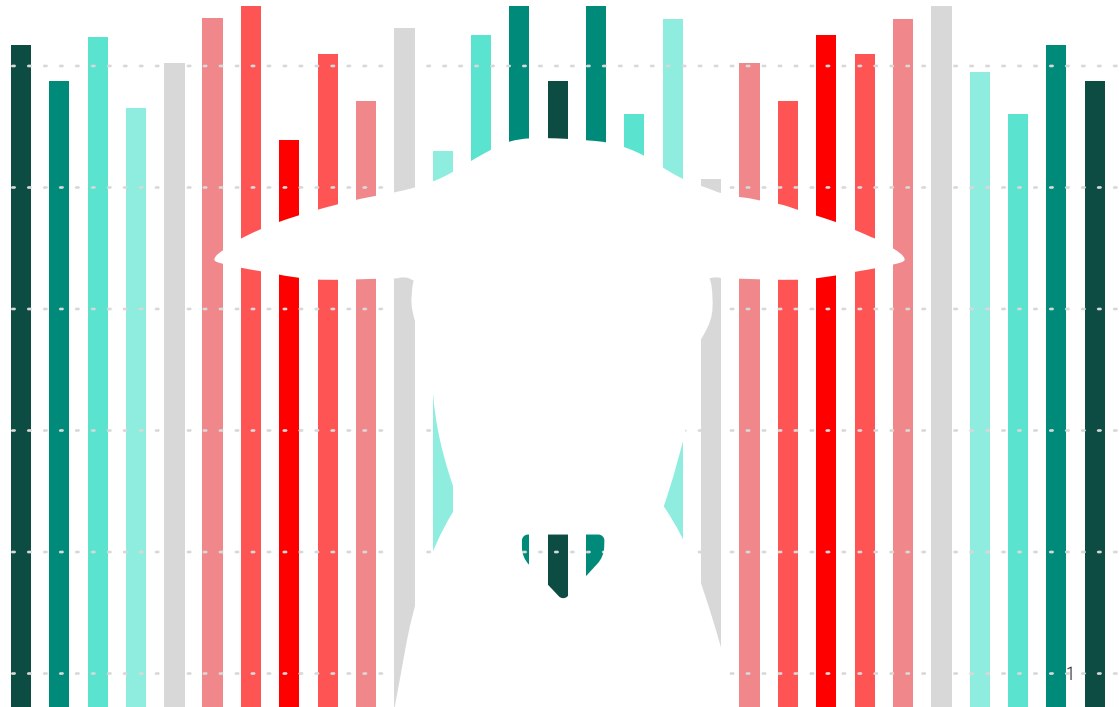
small ruminants: etiology in statistics

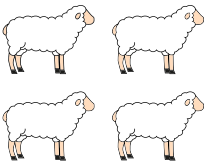
exopol



small ruminants: etiology in statistics

Published in 2021

which samples should be selected?



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

our diagnostic panels include the analysis of up to 5 samples, except for milk samples, which can include the analysis of up to 9 samples

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

if **organs or animals** are being sent, it is preferable for them to be **slaughtered** or, failing that, to 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

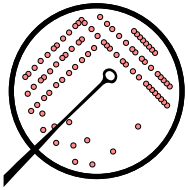
samples	considerations
swabs with medium	*can be shipped at room temperature*
organs	*if frozen, first take a swab to perform microbiological analyses*
bronchoalveolar lavages	
feces	*to be taken directly from the rectum*
foot rot (lesion swabs)	*specific sampling under anaerobic conditions*
whole blood	*use EDTA tubes* 
serum	*use tubes without anticoagulant* 
milk	*in specific cases of mastitis: freeze until several samples are collected*
air - CORIOLIS equipment	
dirty surfaces - boot swabs	
clean surfaces - wipes	

it is recommended to keep the samples refrigerated until they are received in 24h for shipments of more than 24h, consult the conditions of conservation of each sample.

microbial culture

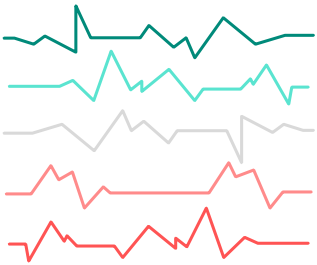
isolating and identify bacteria using MALDI-TOF

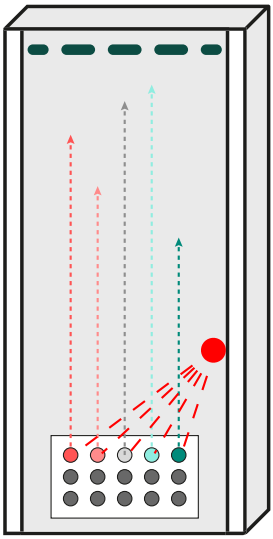
what is it?



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?

Dichelobacter nodosus, Mycoplasma sp.

antibiograms

antibiotic susceptibility testing (Kirby Bauer method)

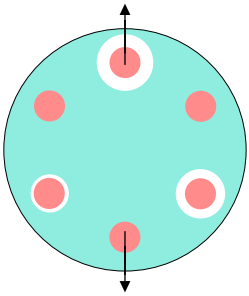
bacterial strains isolated in microbiological culture can be seeded in the right growth media where **discs containing a standardized antibiotic concentration are placed**

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)

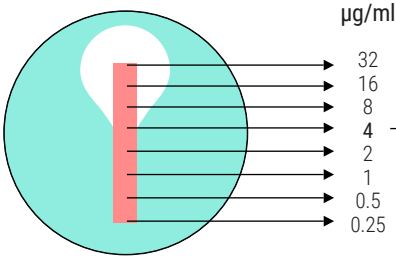
minimum inhibitory concentration (MIC)
antibiotic susceptibility testing

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

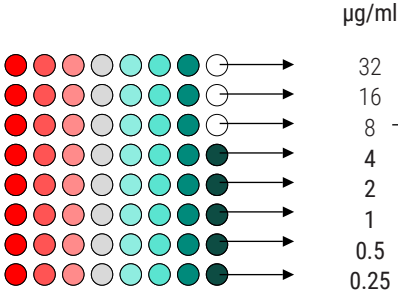


clinical cut-off points for antibiotic A
- sensitive: ≤0,25
- resistant: ≥1

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

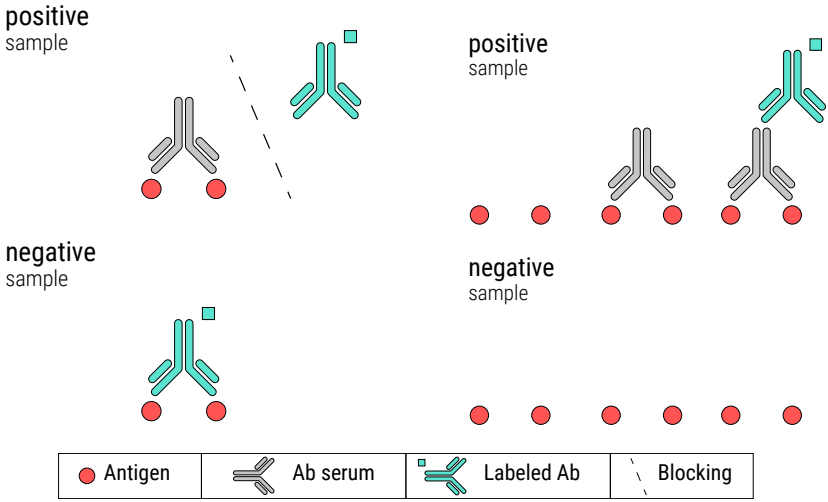
what is a serology?:

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

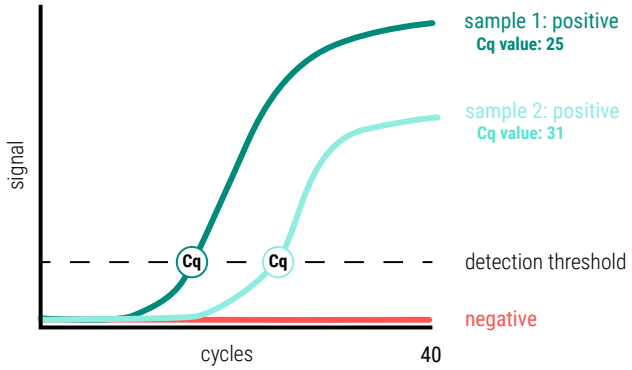
positivo — pathogen is present in sample

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

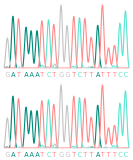
what is sequencing?:

determination of the nucleotide sequence of one or more genes

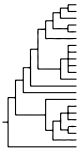
what are its applications?

<u>sequencing</u>

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

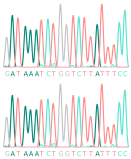


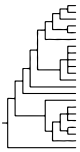
→ we generate phylogenetic trees



<u>sequencing + genotyping</u>

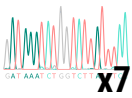
we compare the sequences obtained with those of vaccine strains or previous sequenced samples → we generate phylogenetic trees → we obtain the strain genotype





<u>MLST technique</u>

we sequence seven genes

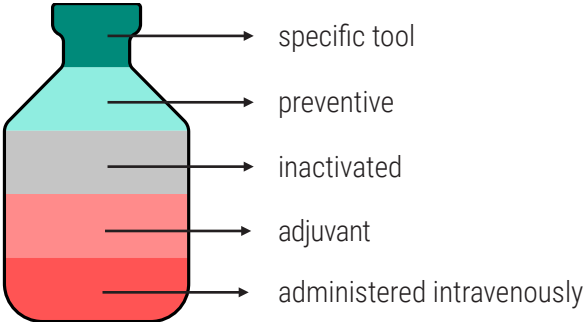


→ through changes in sequences we determine the alleles of each gene: the combination of the alleles of the 7 genes determines the ST (sequence type)

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?

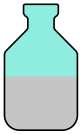
- presence of an infectious disease
- confirmatory laboratory diagnosis
- selecting the involved strains or serotypes
- under veterinary prescription
- produced by an authorized laboratory
  (Nª REG Exopol: 235/50/015-A)

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

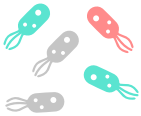
an example:



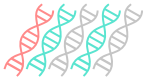
initial autogenous vaccine: two serotypes

→ shipping of new sample to monitor the farm

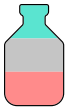
shipping of new **samples**



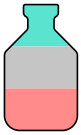
isolation of **bacteria**



tracking of the **new serotypes** via qPCR



incorporating the new serotypes into the **autovaccine**



final autogenous vaccine: three serotypes

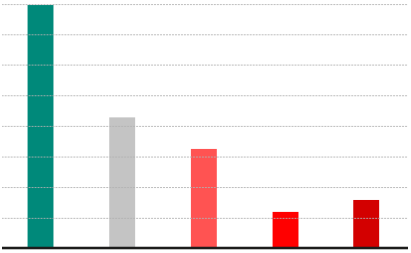
available autogenous vaccines for swine

Diarrhoea
Respiratory processes
Mastitis-agalactia
Keratoconjunctivitis
Septicaemia
Arthritis
Pedero
Pseudotuberculosis
Abscess disease

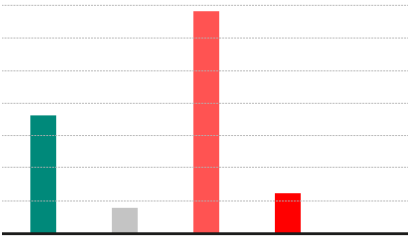
We prepare autovaccines specific to farms or epidemiological units based on laboratory diagnosis and in which it is possible to combine different pathogens

primary autogenous vaccines produced in 2021:

ovine



caprine



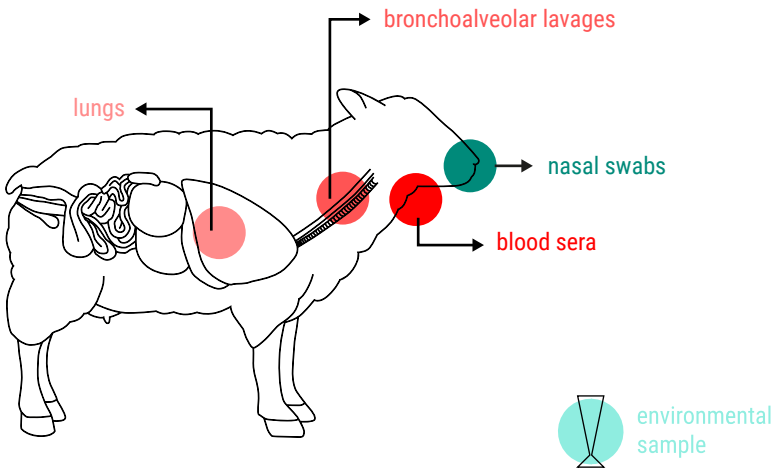
statistical results:

we compiled our laboratory data obtained since 2016 to provide you with statistics about the presence and incidence of pathogens in different processes, the evolution of antibiotic sensitivity in Spanish farms, what serotypes are present, autovaccines produced, etc.

- **respiratory processes**
- **joint processes and hoof profiles**
- **digestive processes**
- **reproductive processes**
- **mastitis and contagious agalactia**
- **abscess-causing processes**
- **other: hemoparasites, ocular and nervous processes**

• respiratory processes

• sampling



• diagnostic panels

Respiratory (suckling and fattening animals):

qPCR: Mesomycoplasma ovipneumoniae, Bibersteinia trehalosi, Pasteurella multocida - Capsular typing, Mannheimia haemolytica - 1, 2 y 6 serotype identification, Parainfluenza 3

Respiratory (adult and replacement animals):

qPCR: Mesomycoplasma ovipneumoniae, Bibersteinia trehalosi, Pasteurella multocida - Capsular typing, Mannheimia haemolytica - 1, 2 y 6 serotype identification, Parainfluenza 3, Ovine pulmonary Adenocarcinoma, Maedi Visna/CAE

Pasteurella multocida - Capsular typing:

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

Mannheimia haemolytica - 1, 2 and 6 serotype identification:

qPCR: serotypes 1, 2, 6

When respiratory problems are found on the holding, it is possible to take diverse types of samples. It is imperative to always choose untreated animals, with recent clinical signs as well as to assess an adequate number of animals.

nasal swabs

- ✓ easy sampling in livestock
- ✓ assessment of respiratory problems in the upper airways
- ✗ no lung sample is taken
- ⚠ potential false positives when detecting bacteria that are part of the nasopharyngeal microbiome and do not reach the lung

lungs

- ✓ allows for a complete diagnosis
- ✓ assessment of respiratory problems in lower airways
- ✗ requires animals that have recently died or been slaughtered
- ⚠ it might not be a representative sample of the group

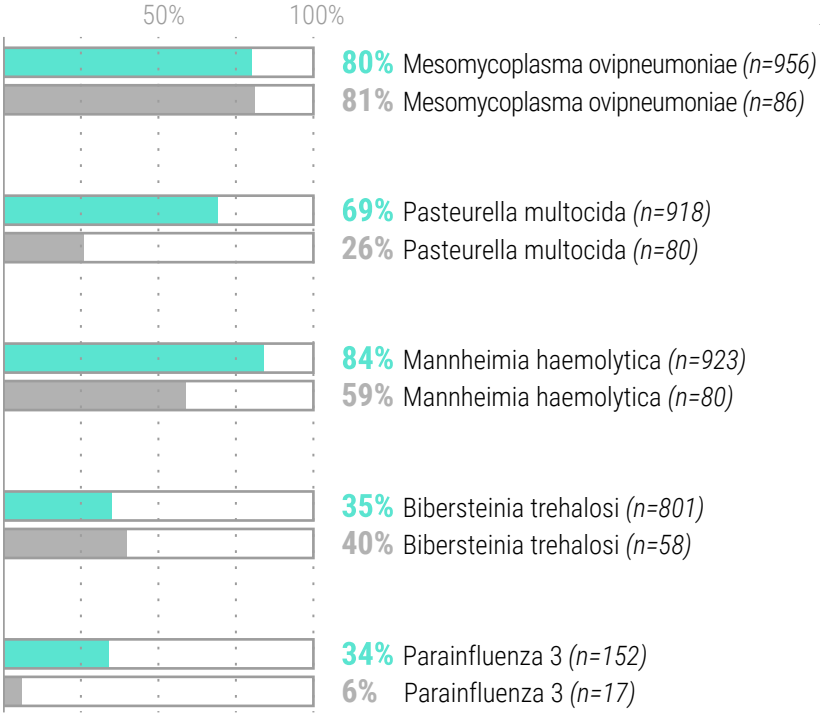
bronchoalveolar lavages

- ✓ sampling in livestock
- ✓ they allow for a greater number of animals to be sampled
- ✓ they provide information about the agents present in the lungs
- ⚠ specific materials and qualified personnel are necessary

• statistical results: diagnosis

pathogens analyzed in the respiratory panel: suckling and fattening animals (ovine & caprine)

% of positives analyzed via qPCR

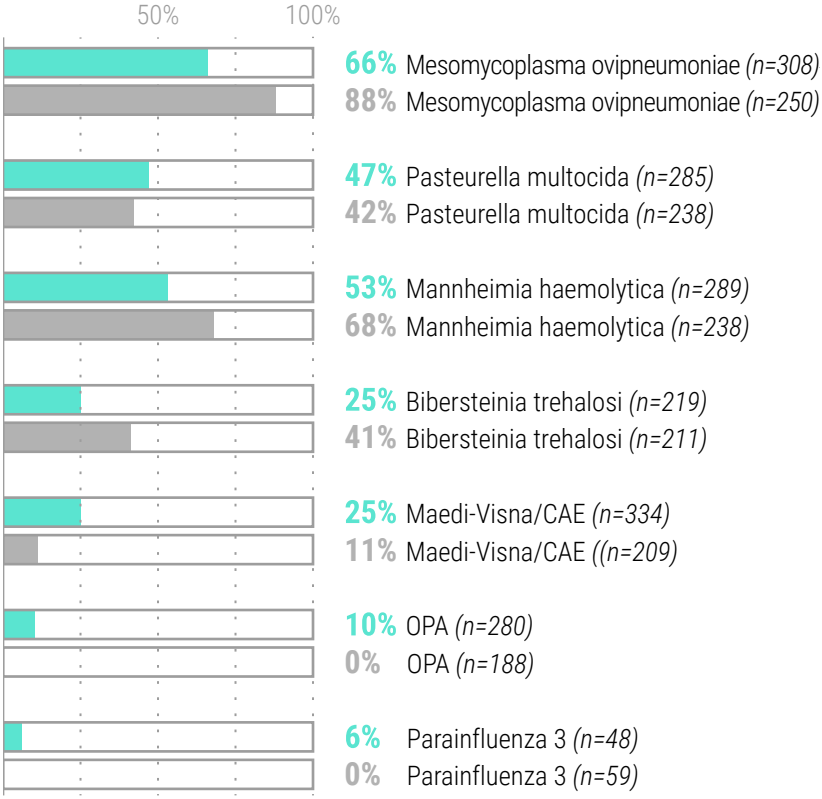


Mesomycoplasma ovipneumoniae and Mannheimia haemolytica are the most frequently detected agents in young animals.

Most analyzed clinical cases are respiratory complexes with presence of more than one agent involved.

pathogens analyzed in the respiratory panel: replacement and adult animals (ovine & caprine)

% of positives analyzed via qPCR

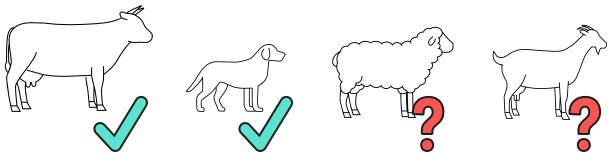


As in the case of young animals, Mesomycoplasma ovipneumoniae and Mannheimia haemolytica are the main agents detected. They are usually multifactorial diseases (presence of more than one agent).

Maedi-Visna virus/CAE (Caprine Arthritis and Encephalitis) and OPA (Ovine pulmonary adenocarcinoma) diseases are included in the respiratory differential diagnosis in this age group since they are slow-progressing diseases that occur in adults. Its analysis in replacement animals allows for the selection of future breeding females.

study of Parainfluenza 3 in respiratory processes in small ruminants

virus causing respiratory prolems widely described in cattle and dogs but of **unknown effect in small ruminants**



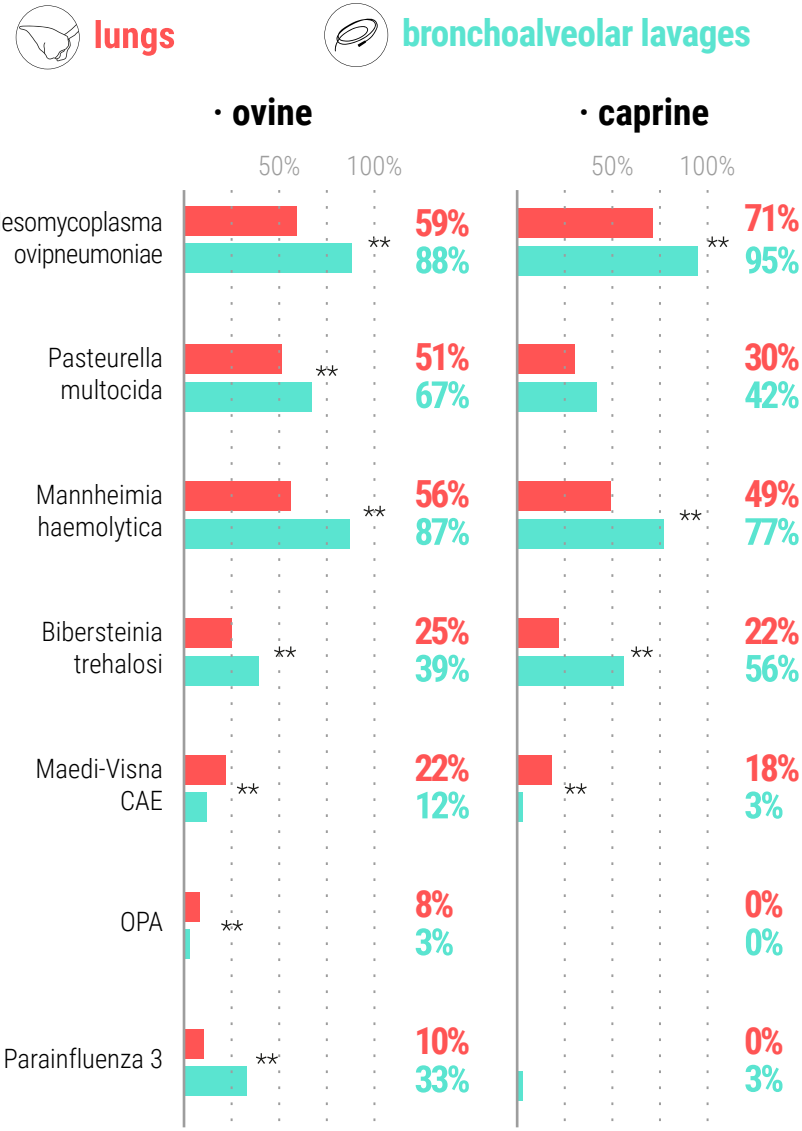
Bovine Parainfluenza-3 virus genotype
≠
Parainfluenza-3 genotype in small ruminant

until now its implication in respiratory problems in sheep and goats was unknown due to the lack of diagnostic techniques, although associated lesions have been identified in young animals as well as the presence of seropositive animals

it is now possible to **assess their presence** in small ruminants thanks to the **specific qPCR assay for sheep and goats**

detection of respiratory pathogens according to the type of sample analyzed

% of positives analyzed via qPCR



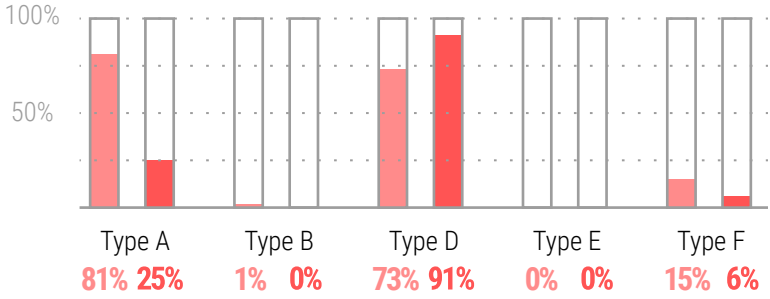
We evaluated the difference in the number of positives for each of the pathogens analyzed in the respiratory panel depending on the type of sample—lung or bronchoalveolar lavage—using the Chi-square statistical test. Significant differences were found to exist between the percentage (%) of positives of lungs and lavages, if p-value <0.05 (*) and <0.01 (**). These results are based on unpaired samples.

The viruses responsible for OPA and Maedi-Visna/CAE diseases, analyzed only in replacement and adult animals, are most often detected in the lungs, since their elimination to alveolar lumen occurs when an important part of the lung is injured. Therefore, the detection of said viruses using bronchoalveolar lavages is limited.

A higher percentage of the remaining pathogens is detected in bronchoalveolar lavages. This difference may be because the lavage analysis makes it possible to increase the number of animals sampled and to select those with early respiratory symptoms that have not yet received antibiotic treatment. This eliminates the need to sacrifice them in order to obtain the sample. Moreover, in summer, organs often undergo autolysis and contamination, making it difficult to isolate and detect pathogens of animal health significance.

detection of capsular serotypes of Pasteurella multocida on clinical samples (ovine & caprine)

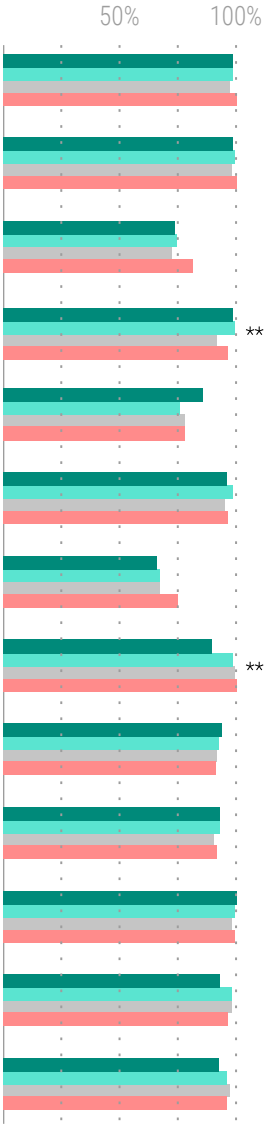
360 and 75 cases analyzed since February 2018 via qPCR



Co-infection of different capsular types of Pasteurella multocida was detected in 61% of cases in sheep, and 22% in goats. Co-infection of types A and D was found to be the most frequent case—46% in sheep and 19% in goats.

antibiotic susceptibility testing (Kirby Bauer method) of Pasteurella multocida (ovine & caprine)

comparison of the sensitivity percentage of 458 antibiograms performed in the following semesters:



	jul. 19 dec. 19	jan. 20 jun. 20	jul. 20 dec. 20	jan. 21 jun. 21
Ampicillin	98%	98%	97%	100%
Spectinomycin	98%	99%	98%	100%
Penicillin	73%	74%	72%	81%
Trimethoprim/ sulfamethoxazole	98%	99%	91%	97%
Tetracycline	85%	75%	78%	78%
Gentamicin	96%	98%	95%	96%
Streptomycin	65%	67%	67%	75%
Florfenicol	89%	98%	99%	100%
Thiamphenicol	94%	92%	91%	91%
Tilmicosin	93%	93%	90%	91%
Ceftiofur	100%	99%	98%	99%
Enrofloxacin	93%	98%	98%	97%
Marbofloxacin	92%	96%	97%	96%

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 <0.05 (*) and <0.01 (**).

In general, these strains show low antibiotic resistance, presenting more resistance to streptomycin, tetracycline, and penicillin.

Although this chart does not show the results sorted by species, we found higher percentages of sensitivity to almost all antibiotics in goat antibiograms. Streptomycin showed a difference of up to 20%.

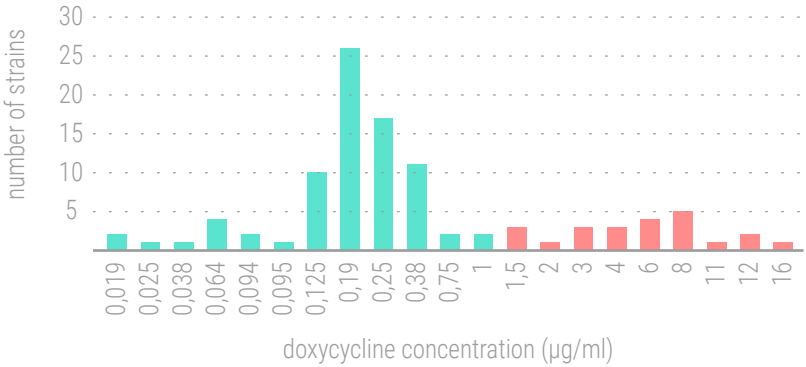
Minimum inhibitory concentration - P. multocida

MICs carried out since 2019

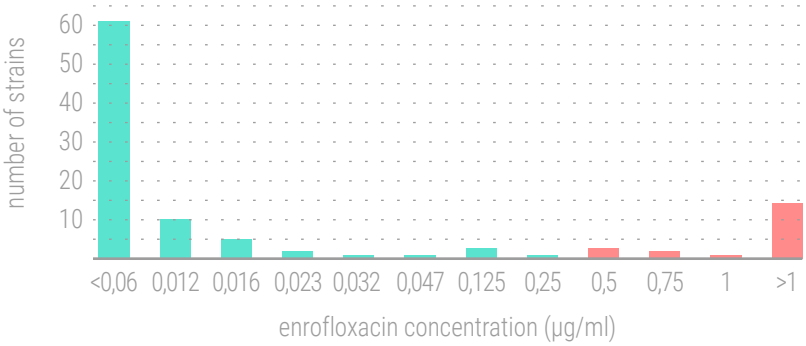
Antibiotic	MIC50 (µg/mL)	MIC90 (µg/mL)	sensitive if: (µg/mL)	analyzed samples
Amoxicillin	0,094	0,19	4	42
Clindamycin	>8	>8	0,5	43
Colistin	1	1,5	4	43
Doxycycline	0,25	6	1	102
Enrofloxacin	<0,06	>1	0,25	104
Florfenicol	0,5	1	2	43
Gamithromycin	2	>4	4	43
Penicillin	0,125	0,5	0,25	43
Spectinomycin	16	32	32	43
Tetracycline	1	>4	2	108
Tilmicosin	>8	>8	8	43
Tulathromycin	8	16	16	43
Tylosin	16	>16	8	43

MIC distribution of:

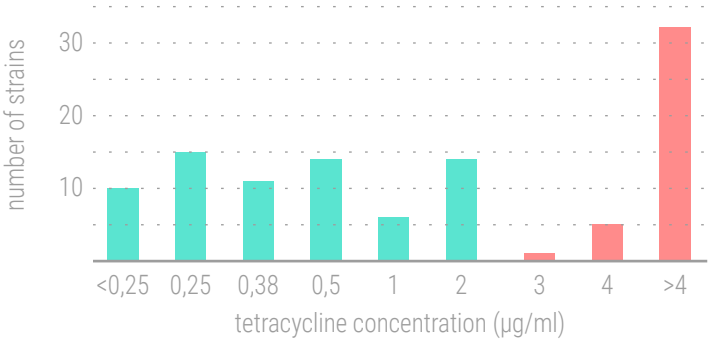
· doxycycline



· enrofloxacin



· tetracycline



This table shows the MIC50 and MIC90 values of the different antibiotics for Pasteurella multocida. MIC50 and MIC90 values are the minimum concentration of antibiotic 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).

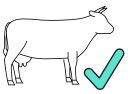
The charts show a significant difference between the MIC50 and MIC50 of gamithromycin, penicillin, doxycycline, enrofloxacin and tetracycline. That is the reason frequency charts of the distribution of MIC values are displayed for these last three antibiotics.

The doxycycline chart follows a normal distribution profile. In the case of enrofloxacin, we observed the existence of a population of very sensitive strains, although 19% of the strains analyzed were resistant. However, for tetracycline, we found a very large resistant subpopulation, which highlights the need to perform antibiotic sensitivity tests in order to choose the most appropriate treatment.

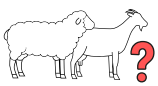
These results may be biased, since the MIC study is usually performed in the harshest environments, for example, when the antibiotic treatment is not working.

serotyping of Mannheimia haemolytica

12 different serotypes were detected:
A1, A2, A5, A6, A7, A8, A9, A12, A13, A14, A16, A17



most prevalent serotypes: A1, A2 and A6

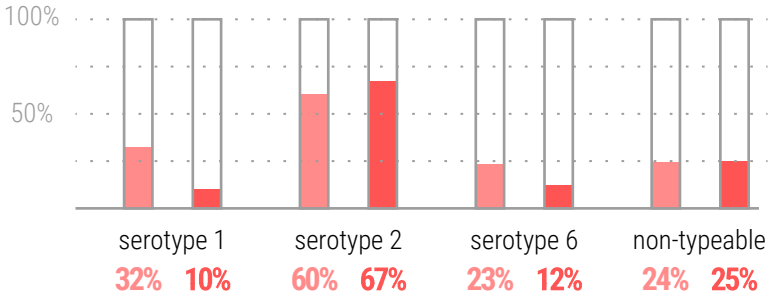


very few published prevalence studies

carrying out now the study of these serotypes in order to see their implication

detection of Mannheimia haemolytica serotypes on clinical samples (ovine & caprine)

435 and 129 cases analyzed since February 2018 via qPCR



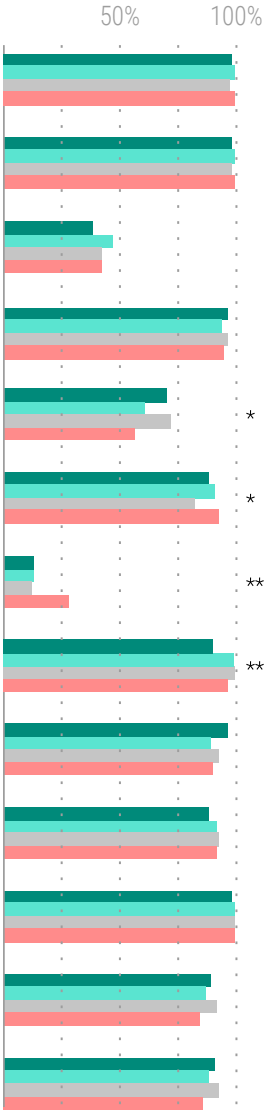
The most commonly detected Mannheimia haemolytica serotype is 2. In 25% of clinical samples, detection of Mannheimia haemolytica is different from the analyzed serotypes (serotypes 1, 2 and 6).

A co-infection of two or three detectable serotypes of Mannheimia haemolytica was found in 37% of cases for sheep and 12% of cases for goats.

The sensitivity profile of Mannheimia haemolytica is similar to that obtained with Pasteurella multocida. In the case of streptomycin, penicillin and tetracycline, lower percentages were obtained. A decrease in sensitivity as a function of time was observed for tetracycline. The results in goats also showed higher sensitivity.

antibiotic susceptibility testing (Kirby Bauer method) of Mannheimia haemolytica (ovine & caprine)

comparison of the sensitivity percentage of 651 antibiograms performed in the following semesters:



	jul. 19 dec. 19	jan. 20 jun. 20	jul. 20 dec. 20	jan. 21 jun. 21
Ampicillin	98%	99%	97%	99%
Spectinomycin	98%	99%	98%	99%
Penicillin	38%	47%	42%	42%
Trimethoprim/ sulfamethoxazole	96%	93%	96%	95%
* Tetracycline	70%	60%	72%	56%
* Gentamicin	88%	90%	82%	92%
** Streptomycin	13%	13%	12%	27%
** Florfenicol	89%	98%	99%	95%
Thiamphenicol	96%	89%	92%	90%
Tilmicosin	88%	91%	92%	91%
Ceftiofur	98%	99%	99%	99%
Enrofloxacin	89%	87%	91%	84%
Marbofloxacin	91%	88%	92%	86%

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 <0.05 (*) and <0.01 (**).

Concentración Mínima Inhibitoria - M. haemolytica

CMIs realizadas desde 2019

Antibiótico	CMI50 (µg/mL)	CMI90 (µg/mL)	sensible si: (µg/mL)	muestras analizadas
Amoxicilina	0,125	0,25	4	46
Clindamicina	>8	>8	0,5	47
Colistina	1	1,5	4	47
Doxiciclina	0,38	4	1	120
Enrofloxacina	0,064	>1	0,25	119
Florfenicol	1	1	2	47
Gamitromicina	4	>4	4	47
Penicilina	0,5	1	0,25	47
Espectinomicina	32	>32	32	47
Tetraciclina	2	8	2	123
Tilmicosina	>8	>8	8	47
Tulatromicina	32	>32	16	47
Tilosina	>16	>16	8	47

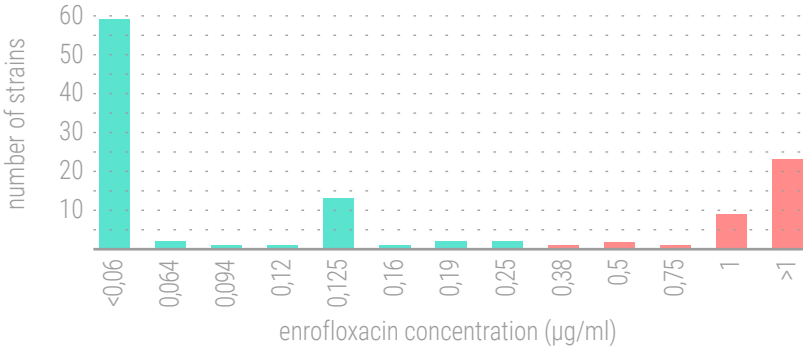
The results for clindamycin, penicillin, tilmicosin, tulathromycin and tylosin show that at least 50% of the strains analyzed are resistant to these antibiotics. The tulathromycin MIC value distribution frequency chart shows that most of the strains analyzed are resistant strains. In addition, the MIC of sensitive strains is close to the cut-off point. In the case of enrofloxacin, we find a large group of strains that are very sensitive to the antibiotic, although 30% showed resistance.

Regarding tetracycline, we observed that the distribution of 50% of the strains has MIC values found below the cut-off point. However, most of the resistant strains show very high MIC values. This highlights the existence of a high-resistance subpopulation.

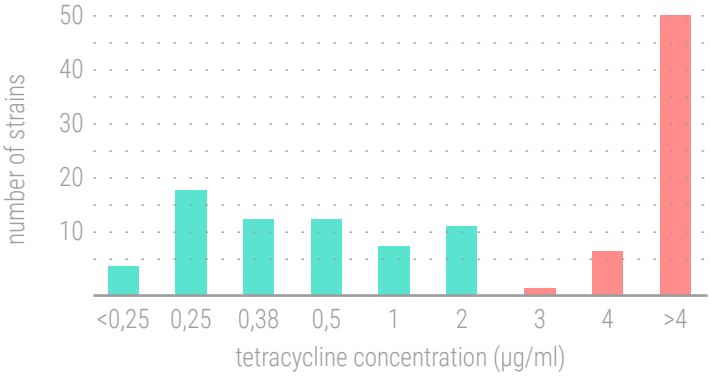
These results may be biased, since the MIC study is usually performed in the harshest environments, for example, if the antibiotic treatment is not working.

MIC distribution of:

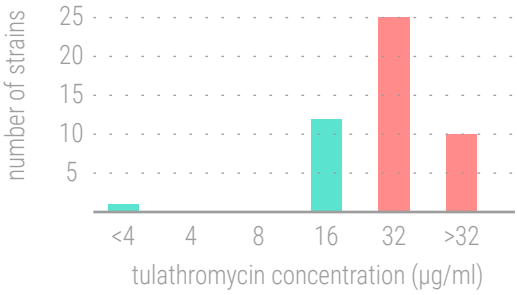
· enrofloxacin



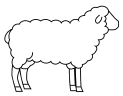
· tetracycline



· tulathromycin



Maedi-Visna / CAE



respiratorios
mamitis

nerviosos
articulares

al **no existir un tratamiento efectivo**, se realizan **programas de control y erradicación:** la sensibilidad serológica depende del kit comercial utilizado y la variante del virus, por lo que interesa **aumentar la sensibilidad** mediante diagnóstico por **qPCR sobre sangre con EDTA**

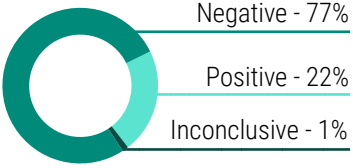
1 **diagnóstico serológico:** valoración de prevalencia de la enfermedad en la explotación

2 **diagnóstico por qPCR:** analizar a los animales con resultado negativo por serología

Maedi-Visna/CAE serological study

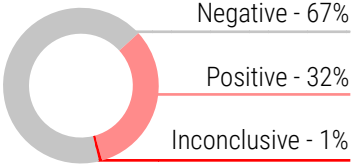
% of positive sera against the total number of sera analyzed

- **ovine:** 3099 sera



38% of positive farms

- **caprine:** 1921 sera



64% of positive farms

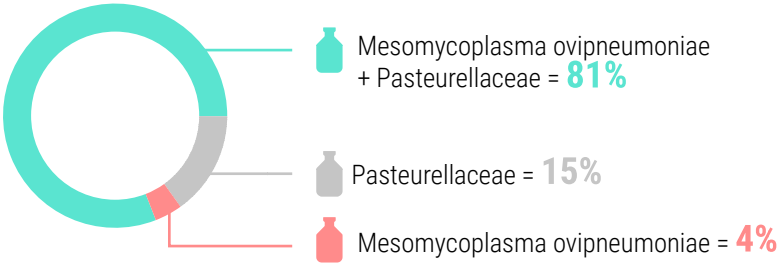
The majority of results are from farmed animals showing compatible symptomatology with Maedi-Visna/CAE (biased samples) and should not be taken as prevalence data.

• statistical results: autovaccines

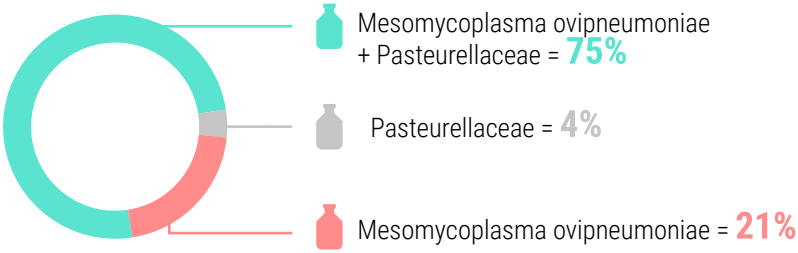
autovaccines produced for respiratory processes

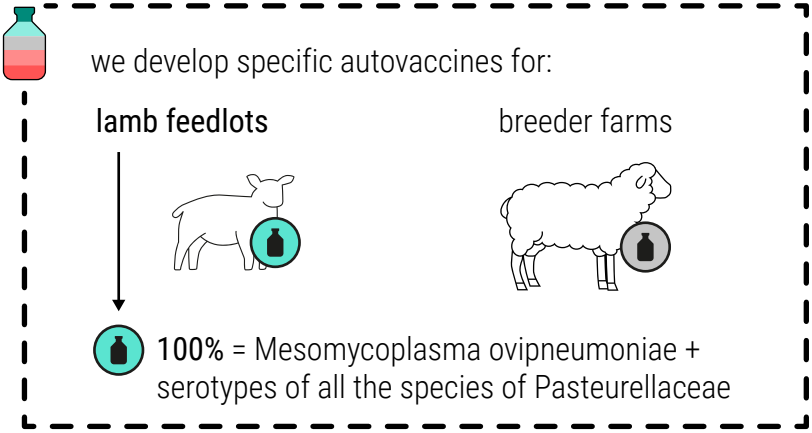
% of autovaccines containing the different agents

ovine



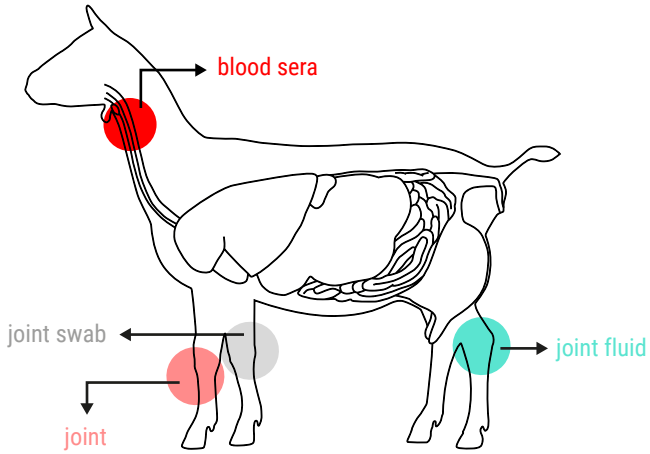
caprine





• joint processes

• sampling



• diagnostic panels

Ovine: Joint disease (lambs):

qPCR: Mycoplasmopsis agalactiae, Streptococcus dysgalactiae, Erysipelothrix rhusiopathiae

Goats: Joint disease (lambs):

qPCR: Mycoplasmopsis agalactiae, Mycoplasma mycoides cluster, Mycoplasma putrefaciens, Streptococcus dysgalactiae, Erysipelothrix rhusiopathiae

Ovine: Joint disease (adults):

qPCR: Mycoplasmopsis agalactiae, Maedi-Visna/CAE

Goats: Joint disease (adults):

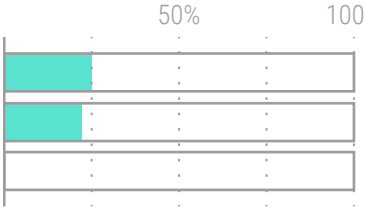
qPCR: Mycoplasmopsis agalactiae, Mycoplasma mycoides cluster, Mycoplasma putrefaciens, Maedi-Visna/CAE

• statistical results: diagnosis

pathogens analyzed in the joint panel during lactation

% of positives analyzed via qPCR

· ovine

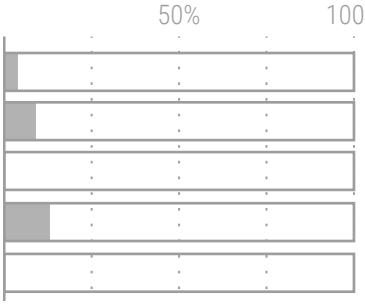


25% Erysipelothrix rhusiopathiae *(n=60)*

22% Streptococcus dysgalactiae *(n=60)*

0% Mycoplasmopsis agalactiae *(n=70)*

· caprine



4% Erysipelothrix rhusiopathiae *(n=23)*

9% Streptococcus dysgalactiae *(n=23)*

0% Mycoplasmopsis agalactiae *(n=25)*

13% Mycoplasma mycoides cluster *(n=24)*

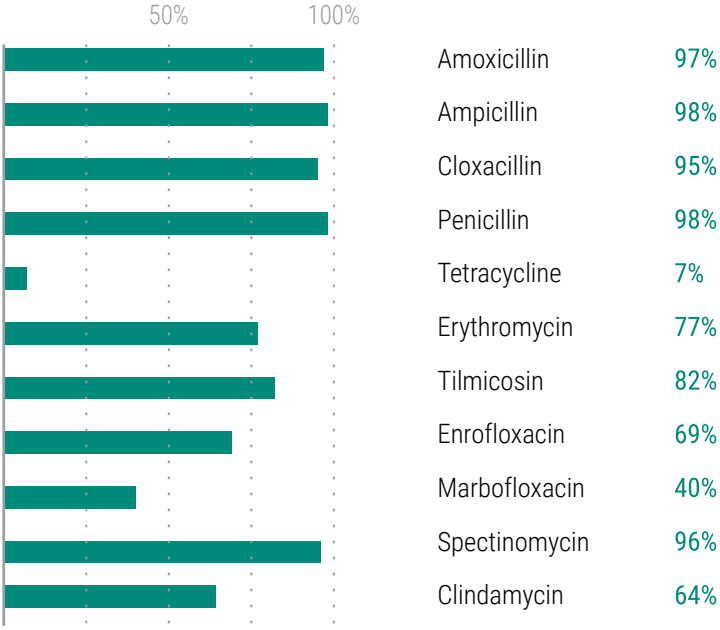
0% Mycoplasma putrefaciens *(n=10)*

Young animals frequently suffer from arthritis with a bacterial infection as its etiology. Erysipelothrix rhusiopathiae and Streptococcus dysgalactiae are detected more frequently in lambs.

For a correct sampling, it is crucial to select animals with a case of initial arthritis, since the presence of a chronic inflammatory process or joints with abundant purulent, hemorrhagic, or fibrous content make the diagnosis more challenging.

antibiotic susceptibility testing (Kirby Bauer method) of Streptococcus dysgalactiae (ovine & caprine)

comparison of the sensitivity percentage of 45 antibiograms performed since 2016*:



** strains isolated in joint processes and mastitis*

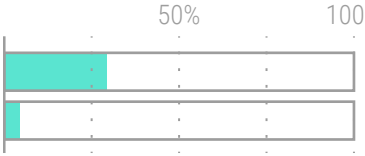
The sensitivity results of the analyzed Streptococcus dysgalactiae strains show that most of them are sensitive to ampicillin, penicillin, tilmicosin, cloxacillin and amoxicillin. Tetracycline and marbofloxacin were found to be the most resistant antibiotics.

This is consistent with the literature, which describes resistance genes for tetracycline that are prevalent in this bacterium.

pathogens analyzed in joint panel (adults)

% of positives analyzed via qPCR

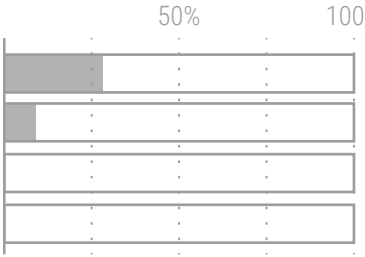
· ovine



30% Maedi-Visna *(n=33)*

5% Mycoplasmopsis agalactiae *(n=38)*

· caprine



27% CAE *(n=15)*

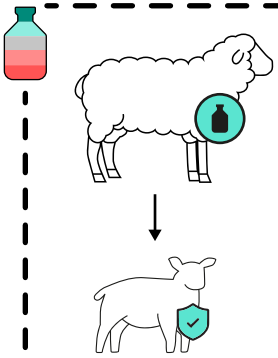
9% Mycoplasmopsis agalactiae *(n=22)*

0% Mycoplasma mycoides cluster *(n=21)*

0% Mycoplasma putrefaciens *(n=10)*

As in young animals, the selection of individuals with early signs of arthritis is important.

At these ages, the most common cause of infectious arthritis is Maedi-Visna/CAE disease. Its diagnosis is usually made on samples that are more easily collected, such as milk or serum samples.



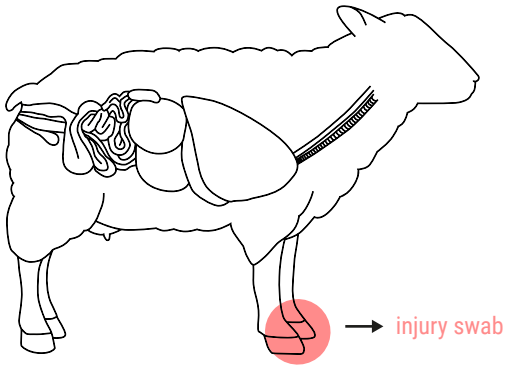
Streptococcus dysgalactiae and/or Erysipelothrix rhusiopathiae autovaccines given to prepartum sheep

↓

prevention of joint processes in sucking lambs or kids

• hoof profiles

• sampling



• diagnostic panels

Hoof profile:

qPCR: R: Dichelobacter nodosus, Dichelobacter nodosus (virulent strains), Fusobacterium necrophorum, Treponema sp., Treponema - Pathogenic phylogrou

Dichelobacter nodosus - Typing:

qPCR: serogroups A, B, C, D, E, F, G, H, I, M

Treponema - Pathogenic phylogroups:

qPCR: Treponema pedis, Treponema phagedenis, Treponema medium

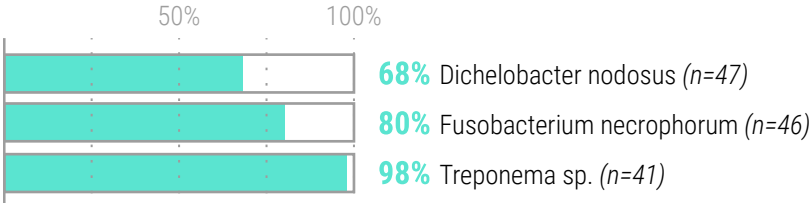
In cases of hoof disease, it is important to take a substantial number of samples to reach a laboratory diagnosis of the disease. In order to do that, it is necessary to select the animals accordingly:

- ✓ animals with early hoof disease signs that have not been treated with systemic antibiotics or hoof baths
- ✓ animals with moderate initial injuries, never serious
- ⚠ in case of interest in the isolation of the strain, the shipment must be made under anaerobic conditions

• statistical results: diagnosis

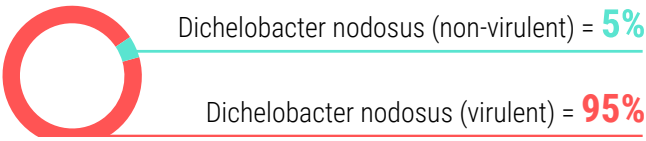
pathogens analyzed in ovine hoof panel

% of positives analyzed via qPCR ever since November 2018



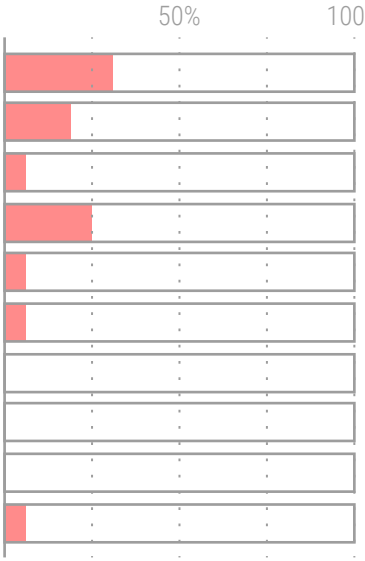
identification of virulent and avirulent strains of Dichelobacter nodosus

% of the 40 analyzed cases since November 2018 via qPCR



Dichelobacter nodosus strain Serotyping

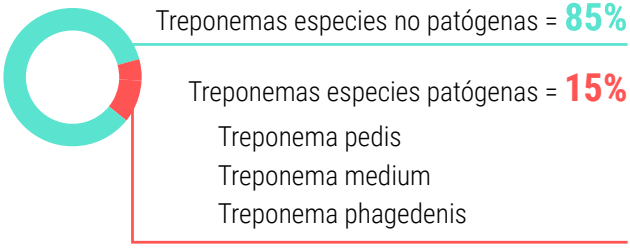
of 16 analyzed cases via qPCR



Dichelobacter nodosus serogroup A	31%
Dichelobacter nodosus serogroup B	19%
Dichelobacter nodosus serogroup C	6%
Dichelobacter nodosus serogroup D	25%
Dichelobacter nodosus serogroup E	6%
Dichelobacter nodosus serogroup F	6%
Dichelobacter nodosus serogroup G	0%
Dichelobacter nodosus serogroup H	0%
Dichelobacter nodosus serogroup I	0%
Dichelobacter nodosus serogroup M	6%

identificación a nivel de especie de Treponema sp.

de 40 casos en los que se ha detectado desde noviembre de 2018 mediante qPCR



Dichelobacter nodosus, the primary etiologic agent of foot rot, was detected in 68% of the analyzed cases.

The study of the isolated strains determined that 95% are virulent strains, positive to the aprV gene. In addition, the typing results determined that 76% of the strains belonged to serogroups A, B and D.

The result accurately reflects what was described in previous studies conducted in the Iberian Peninsula. However, it is necessary to continue to expand the study in order to assess the presence of the remaining serogroups.

Other agents involved in hoof processes are Fusobacterium necrophorum (causing interdigital pododermatitis) and pathogenic phylogroups of the genus Treponema (causing Contagious Digital Ovine Dermatitis, CODD). Detection of F. necrophorum and Treponema sp. in most samples is because they are regularly found in the environment, so it is important to assess the existing lesions.

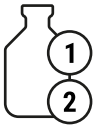
In 15% of the cases, pathogenic phylogroups of Treponema sp. are detected. Although studies on this disease in the peninsula are scarce, this confirms the presence of CODD in Spain. In other countries such as the UK its prevalence can reach up to 50%.

Dichelobacter nodosus

- ✓ high antigenic diversity
- ✓ different serogroups:

A B C D E F G H I M

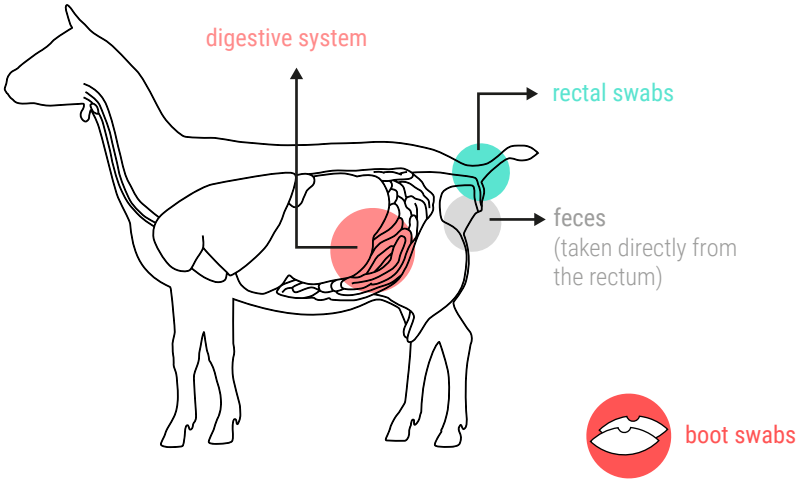
autovaccines against Dichelobacter nodosus



- ✓ two serogroups maximum
- ✓ highly effective in the control and reduction of its prevalence and transmission

• digestive processes

• sampling



• diagnostic panels

Digestive (suckling lamb/kid):

qPCR: Clostridium perfringens - Toxins, Escherichia coli, F17, eae gene , Rotavirus A, Cryptosporidium parvum, Eimeria sp.

Clostridium perfringens - Toxins:

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

Ovine Coccidia:

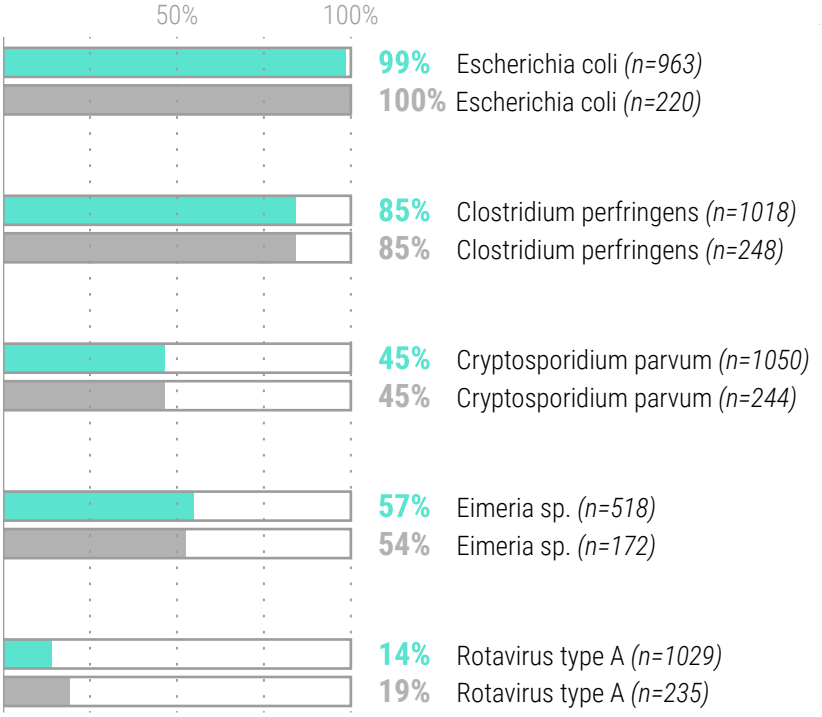
qPCR: Eimeria sp., Eimeria ovinoidalis, Eimeria crandallis/ahsata

Goat Coccidia:

qPCR: Eimeria sp., Eimeria arloingi, Eimeria ninakohlyakimovae, Eimeria christenseni

pathogens analyzed in digestive panel (lambs & kids)

% of positives analyzed via qPCR



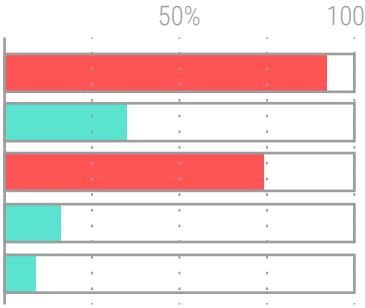
In most cases, we detected more than one infectious agent. This confirms that diarrhea in young animals is multi-etiological in nature.

There is a higher percentage of detection of bacterial agents: Escherichia coli and Clostridium perfringens. In order to confirm its involvement in the digestive process, the concentration detected, and the virulence factors must be evaluated.

In small ruminants only two virulence factors of Escherichia coli (F17 and eae gene) were detected.

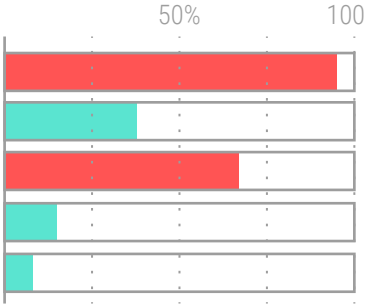
identification of E. coli virulence factors

- **ovine:** 932 cases analyzed via qPCR



92% samples with presence of F17
35% major strain* with F17
74% samples with presence of eae gene
16% major strain* with eae gene
9% major strain* with F17 and eae gene

- **caprine:** 210 cases analyzed via qPCR



95% samples with presence of F17
38% major strain* with F17
67% samples with presence of eae gene
15% major strain* with eae gene
8% major strain* with F17 and eae gene

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

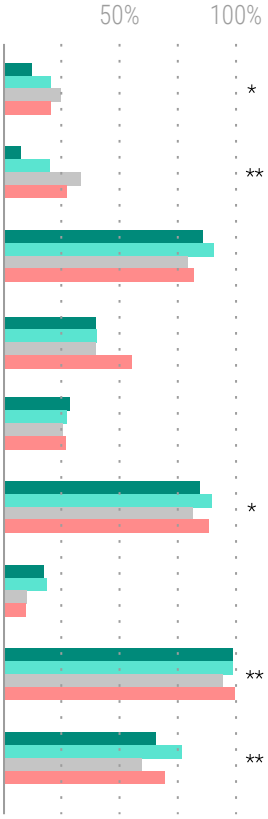
F17 fimbria was detected in the majority of cases. The intimin eae gene was detected in approximately 70%.

However, we can confirm the clinical implication of Escherichia coli strains carrying virulence factors if found in high concentration. In this case, the percentage of enteropathogenic strains is reduced. Less than 40% have F17 as the majority strain and less than 20% have the eae gene. Finally, approximately 10% of cases have both virulence factors in high concentrations.

It should be noted that in cases of septicemic colibacillosis, the strains involved may be negative to both virulence factors.

antibiotic susceptibility testing (Kirby Bauer method) of Escherichia coli (ovine & caprine)

comparison of the sensitivity percentage of 926 antibiograms performed in the following semesters:



	jul. 19 dec. 19	jan. 20 jun. 20	jul. 20 dec. 20	jan. 21 jun. 21
Amoxicillin	12%	20%	25%	20%
Ampicillin	8%	20%	33%	27%
Spectinomycin	85%	89%	79%	81%
Trimethoprim/ sulfamethoxazole	39%	40%	39%	54%
Tetracycline	28%	27%	25%	26%
Gentamicin	84%	89%	80%	88%
Neomycin	17%	18%	10%	10%
Colistin sulfate	98%	98%	93%	98%
Enrofloxacin	65%	76%	59%	69%

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 <0.05 (*) and <0.01 (**).

Overall, these strains have medium-high antibiotic resistance. They are more resistant to amoxicillin, ampicillin, sulfatrimethoprim, tetracycline and neomycin. Similar percentages are found in both goats and sheep.

These results may be related to the existence of resistance genes described in the literature, although an increase in sensitivity to amoxicillin and ampicillin has been observed as a function of time.

Minimum inhibitory concentration - Escherichia coli

MICs carried out since 2019

Antibiotic	MIC50 (µg/mL)	MIC90 (µg/mL)	sensitive if: (µg/mL)	analyzed samples
Amoxicillin	4	>256	4	30
Ampicillin	4	>16	8	31
Clindamycin	>16	>16	0,5	31
Colistin	1,5	3	4	26
Enrofloxacin	≤0,12	≤0,12	0,25	48
Florfenicol	8	>8	2	31
Spectinomycin	32	>64	32	31
Tetracycline	8	>256	2	47
Trimethoprim/Sulfamethoxazole	>2	>2	2	48

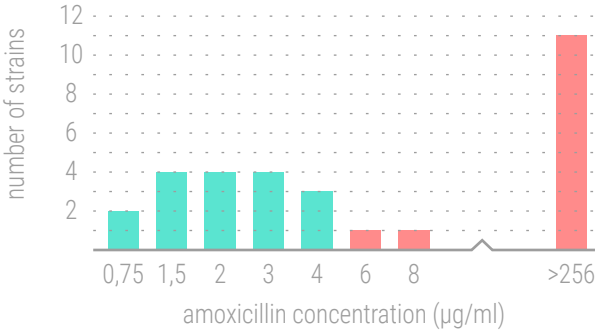
There is a significant difference between the MIC50 and the MIC90 of amoxicillin, ampicillin, and tetracycline. Therefore, frequency charts of the distribution of MIC values of some of these antibiotics are shown.

Amoxicillin and ampicillin charts display the existence of a more resistant subpopulation. This highlights the need to perform antibiotic sensitivity tests in order to choose the most appropriate treatment.

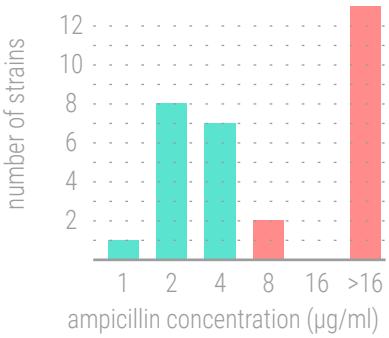
However, the analyzed strains showed high sensitivity to enrofloxacin and colistin. All strains are sensitive to the latter.

number of MIC distribution of:

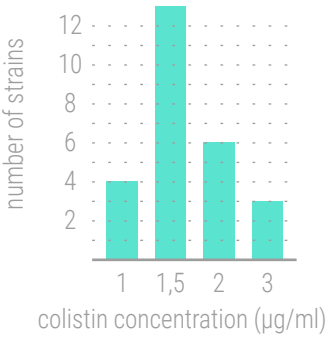
· amoxicillin



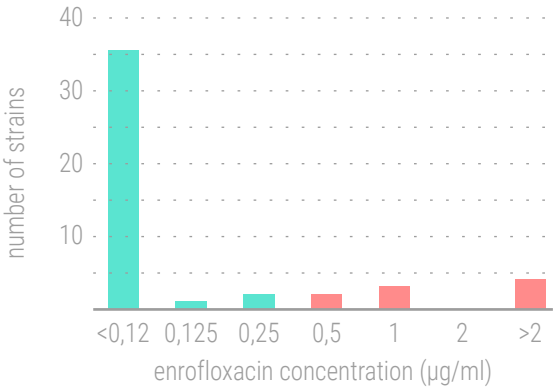
· ampicillin



· colistin



· enrofloxacina



Toxinotyping of Clostridium perfringens helps us know what type of toxins produced by the strain have been detected. Since C. perfringens is a pathogen that 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 to make decisions about preventive measures such as selecting vaccines that include the toxinotypes present on the holding.

· toxinotyping of Clostridium perfringens

A
causes: yellow lamb disease
age: lambs and kids mostly

B
causes: dysentery
age: lambs and kids

C
causes: hemorrhagic enterotoxemia (suckling animals)
causes: Struck (adult animals)

D
causes: enterotoxemia or purple gut
age: fattening animals and adults

E
causes: necrotic hemorrhagic enteritis
age: young animals

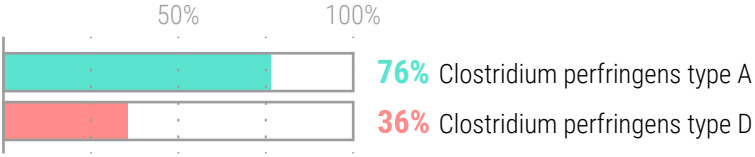
Clostridium perfringens is grouped into 5 toxinotypes (A, B, C, D, E) according to the production of 4 toxins (alpha, beta, epsilon, iota).

Strains of the above toxinotypes can also produce enterotoxin (ENT) and β2 toxin.

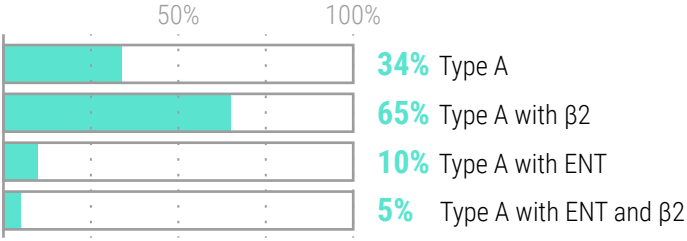
	A	B	C	D	E
alpha	+	+	+	+	+
beta	-	+	+	-	-
epsilon	-	+	-	+	-
iota	-	-	-	-	+

typification of Clostridium perfringens since 2016 in sucking and fattening lambs

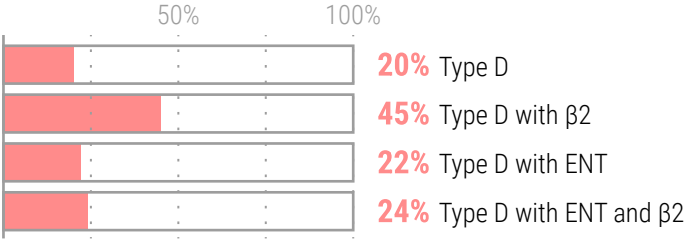
387 clinic cases analyzed in 316 farms via qPCR



Clostridium perfringens type A - 76%



Clostridium perfringens type D - 36%

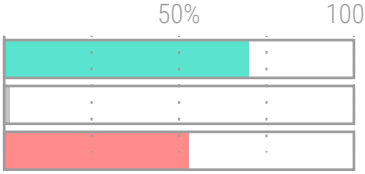


In the case of lambs, the majority of Clostridium perfringens toxinotypes are A and D. Toxinotypes E and B were found to a lesser extent (0.3%). A co-infection of toxinotype A with D is the most frequent case.

In the case of Clostridium perfringens type A, a high presence of β2 toxin-producing strains was observed. In other animal species, this toxin was related to strains of Clostridium perfringens type A with a greater pathogenic capacity.

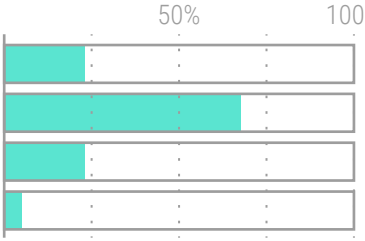
typification of Clostridium perfringens since 2016 in sucking and fattening goats

89 clinic cases analyzed in 74 farms via qPCR



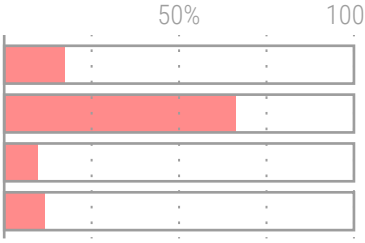
70% Clostridium perfringens type A
1% Clostridium perfringens type C
53% Clostridium perfringens type D

Clostridium perfringens type A - 70%



23% Type A
69% Type A with β2
23% Type A with ENT
5% Type A with ENT and β2

Clostridium perfringens type D - 53%



15% Type D
66% Type D with β2
9% Type D with ENT
11% Type D with ENT and β2

Among the cases of Clostridium perfringens-positive kid diarrhea, 70% were type A, while 53% were type D.

In 37% of the cases, a co-infection of toxinotypes A and D was detected. Again, there is a minority presence of the other types.

digestive problems in feedlots...

are always due to coccidiosis ?

laboratory results → multifactorial problems

In the case of **multifactorial processes**
we recommend a **differential diagnosis**

✓ virus ✓ bacteria ✓ parasites

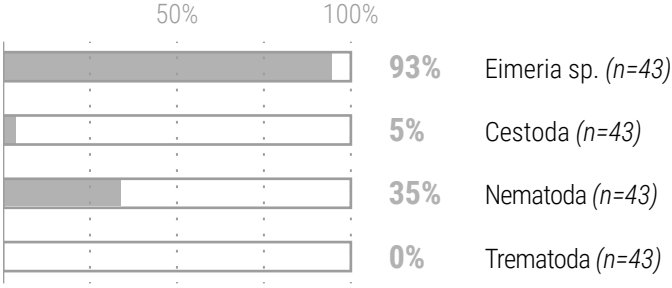
1 conducting a correct selection of samples

2 in order to reach a conclusive diagnosis

3 and establish preventive measures

pathogens analyzed in coprological panel

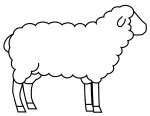
% of positives analyzed via qPCR ever since November 2020



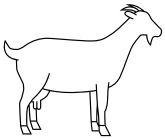
The presence of a high percentage of Eimeria sp. is observed. Nematoda are detected in second place, since we found 35% of positive samples.

Parasite detection via qPCR provides results with greater sensitivity, specificity, reproducibility, automation, and speed. In addition, it allows for the identification at genus and species levels, as in the case of Fasciola hepatica, Dricocoelium dendriticum, etc.

Eimeria sp.



Eimeria crandalis/ahsata
Eimeria ovinoidalis



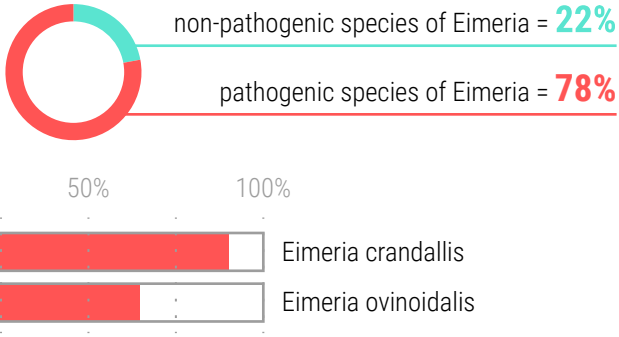
Eimeria ninakohlyakimovae
Eimeria arloingi
Eimeria christenseni

diagnosis via qPCR on stool pools

- ✓ assessment of infestation by batch, age, etc.
- ✓ assessment of the efficacy of a treatment by evaluating the concentration (Cq)
- ✓ detection of the pathogenic species of Eimeria sp. in each animal species

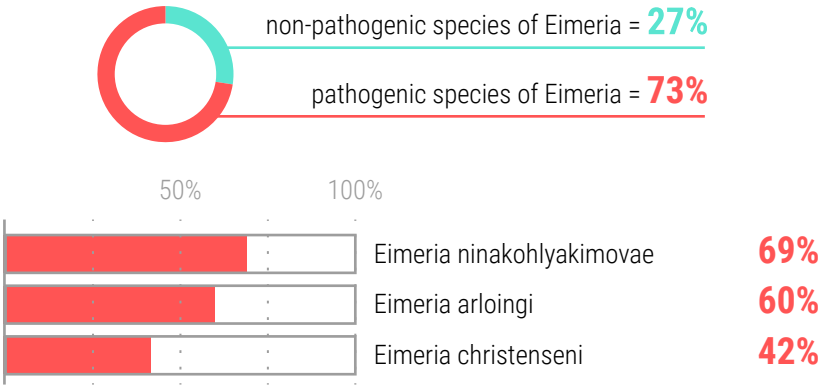
identification to species level of Eimeria in sheep

145 analyzed cases via qPCR



identification to species level of Eimeria in goats

62 analyzed cases via qPCR

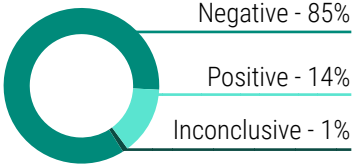


The presence of pathogenic species of Eimeria sp. has been detected in more than 70% of the cases. These data highlight its wide distribution on holdings. Therefore, it is necessary to evaluate the treatment applied as well as the use of a drug rotation protocol.

serological study of Paratuberculosis

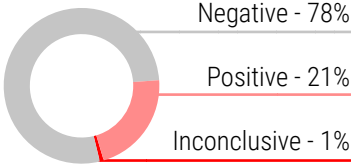
% of positive sera against the total number of sera analyzed

· ovine: 1077 sera



37% of positive farms

· caprine: 2399 sera



66% of positive farms

These results are from farmed adult animals with digestive symptoms or for health monitoring (biased samples) and should not be taken as prevalence data.

A positive result is indicative of an animal infected with Paratuberculosis, while in the case of a seronegative animal, the study should be extended by performing qPCR on feces to increase diagnostic sensitivity.

Paratuberculosis: chronic infectious disease that causes weight loss and diarrhea

· serological diagnosis

on serum
+ cost-effective

· qPCR diagnosis

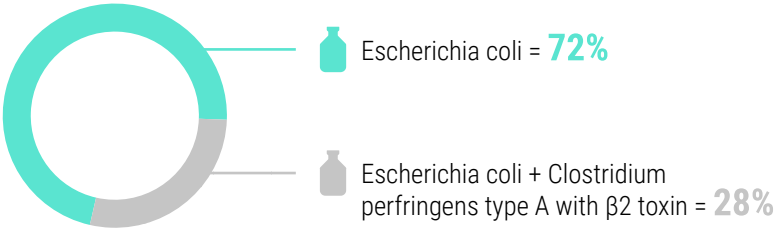
on feces, digestive samples, and swabs

	if serological result is	and qPCR result is	the animal would be
case 1	negative	negative	**negative**
case 2	negative	positive	**positive**
case 3	positive	positive or negative	**positive**

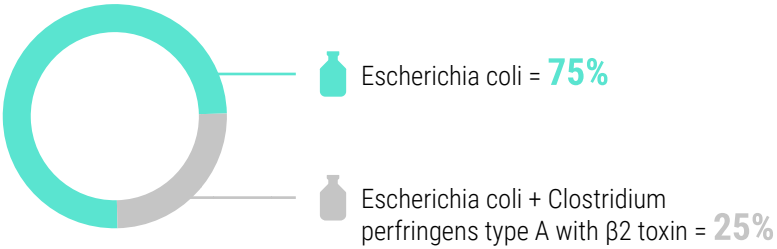
autovaccines produced for digestive processes

% of autovaccines containing the different agents

ovine



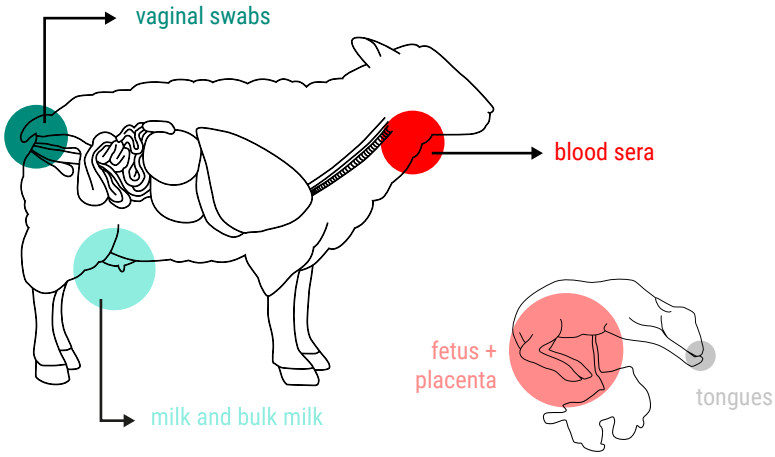
caprine



Escherichia coli strains (even in digestive autovaccines) are selected based on the virulence factors they present (F17 and eae gene).

reproductive processes

sampling



diagnostic panels

Abortion:

qPCR: Coxiella burnetii, Chlamydia abortus, Salmonella sp., Campylobacter sp., Pestivirus, Toxoplasma gondii, Neospora caninum

Chlamydia abortus - Differentiation of vaccine strains from field strains:

qPCR: CAB175 vaccine, CAB283 vaccine, CAB636 vaccine, CAB175 field strain, CAB283 field strain, CAB636 field strain

Reproductive (bulk milk):

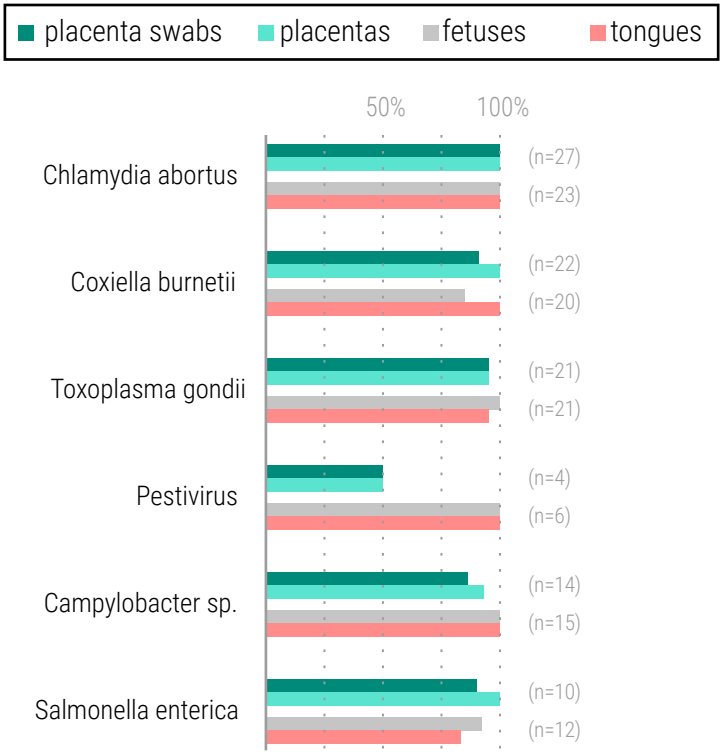
qPCR: Coxiella burnetii, Chlamydia abortus, Pestivirus, Toxoplasma gondii

Reproductive - Serology:

Serología: Coxiella burnetii, Chlamydia abortus, Brucella spp. (Rose Bengal test), BVD p80/Border, Toxoplasma gondii

new sampling types for the analysis of reproductive problems causing abortions: **tongues and placenta swabs**

- ✓ easy-to-take-samples
- ✓ possibility of increasing the number of animals sampled

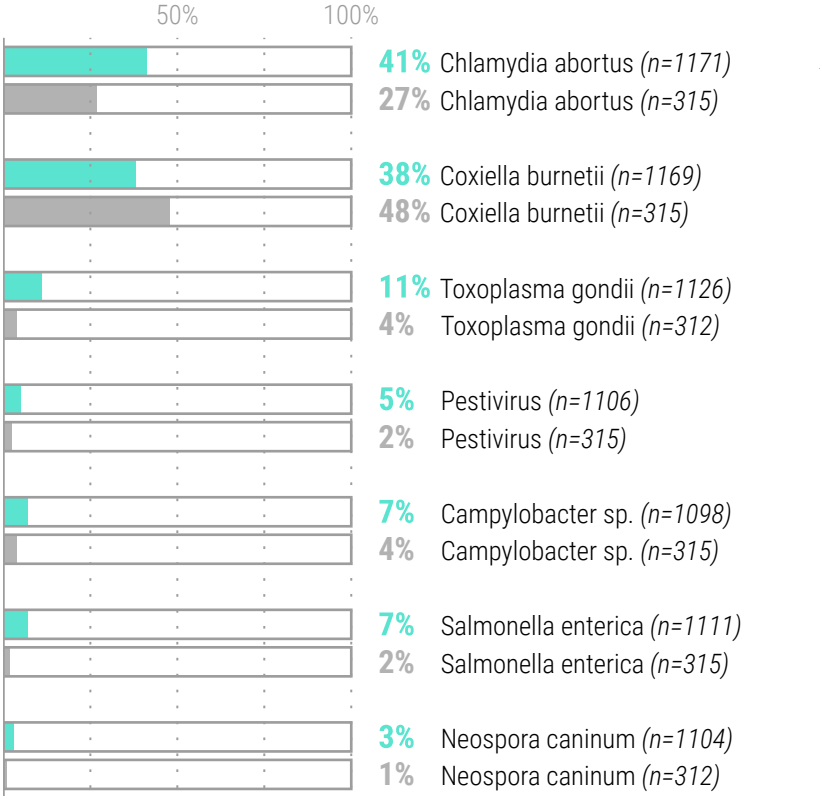


swabs + tongues ≈ fetus + placenta

- ✓ facilitates sample collection and shipment to the laboratory
- ✓ increases to 5 the number of abortions to be included (we now analyze a maximum of 2 fetuses per panel)

pathogens analyzed in abortion panel (ovine & caprine)

% of positives analyzed via qPCR

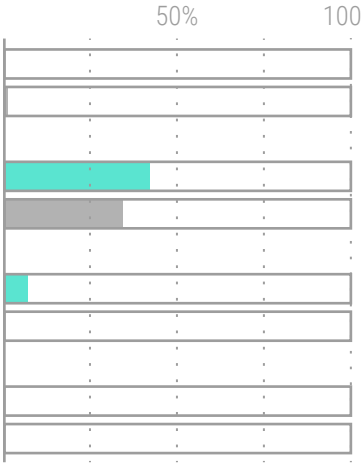


Chlamydia abortus and Coxiella burnetii are the most prevalent agents in abortions.

Out of the 203 positive cases found in goats and 816 found in sheep, we detected more than one etiological agent in 30% in goats and 39% in sheep. In these cases, the concentration of each agent should be assessed in order to evaluate its involvement in abortion. The presence of some agents, such as Border disease, is relevant regardless of their concentration.

pathogens analyzed in reproductive panel: bulk milk (ovine & caprine)

% of positives analyzed via qPCR



0% Chlamydia abortus *(n=42)*
1% Chlamydia abortus *(n=102)*

43% Coxiella burnetii *(n=42)*
35% Coxiella burnetii *(n=102)*

7% Pestivirus *(n=42)*
0% Pestivirus *(n=102)*

0% Toxoplasma gondii *(n=42)*
0% Toxoplasma gondii *(n=102)*

Monitoring of these pathogens on farms through tank analysis helps monitor diseases after a clinical process and anticipate infertility and abortion problems.

Elimination via milk is not continuous, so a negative tank result does not guarantee freedom from disease.

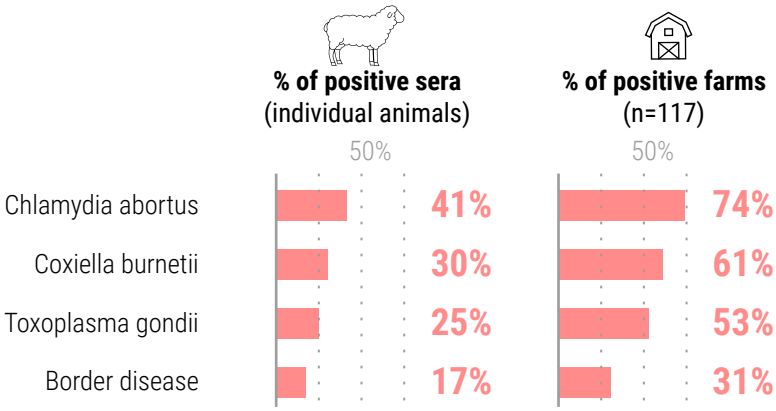
differentiation panel of C. abortus strains

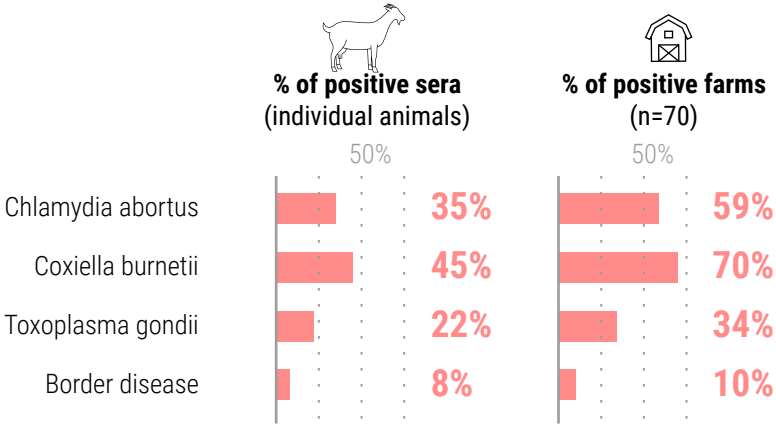
✓ positive to the vaccine strain
the involvement of C. abortus in abortion is ruled out

✗ negative to the vaccine strain
presence of field strain with possible implication

pathogens analyzed in serological-reproductive panel

seropositivity at individual and farm levels through ELISA techniques





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

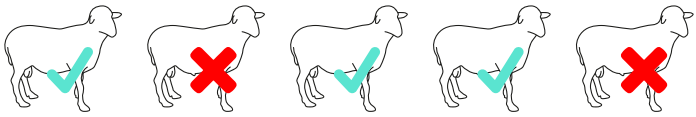
A positive result may indicate a recent infection, the presence of vaccine antibodies or previous contact with the agent.

Brucella ovis

etiological agent of ovine contagious epididymitis:

- → rams: infertility due to the inflammation of genital organs
- → breeding females: sporadic abortions

diagnosis by palpation?



- ✓ males with obvious lesions
- ✗ infected animals without clinical signs that
  - ⚠ maintain the disease on the holding

serological diagnosis

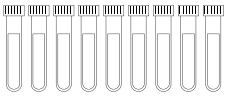
- ✓ detection of animals with subclinical infection
- ✓ assessment of disease prevalence on the holding

diagnosis via qPCR

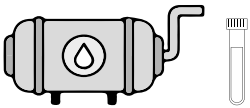
- ✓ epididymitis, infertility, carrier control on semen samples
- ✓ abortions on samples of fetuses, placentas, and vaginal swabs

mastitis & contagious agalactia

sampling



9 individual milk samples



1 bulk milk sample

diagnostic panels

Ovine - Mastitis 9 + bulk:

Microbiology: Bacteria isolation and id., Antimicrobial susceptibility test
qPCR: Mycoplasmopsis agalactiae, Maedi Visna/CAE, Mycoplasmopsis agalactiae, Maedi Visna/CAE, Staphylococcus sp., Staphylococcus aureus

Ovine - Mastitis (bulk milk):

qPCR: Mycoplasmopsis agalactiae, Maedi Visna/CAE, Mycoplasmopsis agalactiae, Maedi Visna/CAE, Staphylococcus sp., Staphylococcus aureus

Goat - Mastitis 9 + bulk:

Microbiology: Bacteria isolation and id., Antimicrobial susceptibility test
qPCR: Mycoplasmopsis agalactiae, Mycoplasma mycoides cluster, Mycoplasma putrefaciens, Maedi Visna/CAE, Mycoplasmopsis agalactiae, Mycoplasma mycoides cluster, Mycoplasma putrefaciens, Staphylococcus sp., Staphylococcus aureus, Maedi Visna/CAE

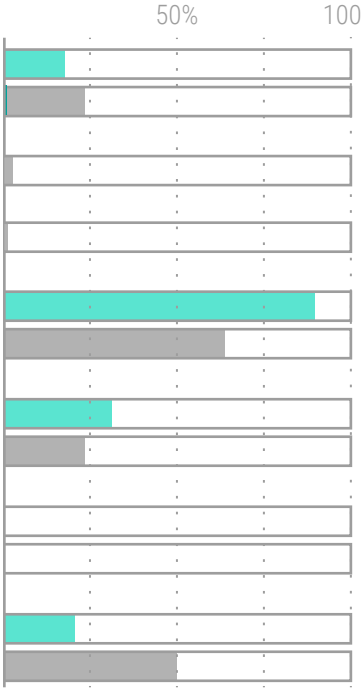
Goat - Mastitis (bulk milk):

qPCR: Mycoplasmopsis agalactiae, Mycoplasma mycoides cluster, Mycoplasma putrefaciens, Maedi Visna/CAE, Mycoplasmopsis agalactiae, Mycoplasma mycoides cluster, Mycoplasma putrefaciens, Staphylococcus sp., Staphylococcus aureus, Maedi Visna/CAE

• statistical results: diagnosis

pathogens analyzed in mastitis panel: bulk milk (ovine & caprine)

% of positives analyzed via qPCR



18% Mycoplasmopsis agalactiae *(n=274)*
24% Mycoplasmopsis agalactiae *(n=591)*

3% Mycoplasma mycoides cluster *(n=583)*

1% Mycoplasma putrefaciens *(n=403)*

92% Staphylococcus spp. *(n=73)*
65% Staphylococcus spp. *(n=168)*

32% Staphylococcus aureus *(n=268)*
24% Staphylococcus aureus *(n=590)*

0% Streptococcus equi *(n=118)*
0% Streptococcus equi *(n=257)*

21% Maedi Visna/CAE *(n=87)*
50% Maedi Visna/CAE *(n=215)*

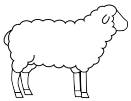
We found a high percentage of Staphylococcus sp. detection in tank samples. This genus of bacteria, mostly environmental, serves as a parameter of milk quality.

For goats, we found 50% of positives for CAE virus. This highlights its high prevalence in dairy goat farms.

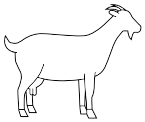
Tank monitoring is used to detect pathogens that are shed via milk even if a disease is not observed.

detection of asymptomatic carriers of Mycoplasmas:

- ✓ reducing the transmission of the disease on the holding
- ✓ avoiding the introduction of infected animals into free-range farms



nasal swabs - qPCR
lactation milk - qPCR
sera (non-vaccinated) - serology



ear swabs - qPCR
lactation milk - qPCR
sera (non-vaccinated) - serology

pathogens analyzed in mastitis panel: individual milk samples (ovine & caprine)

% of positives analyzed via qPCR

27% Maedi Visna/CAE *(n=158)*
39% Maedi Visna/CAE *(n=275)*

19% Mycoplasmopsis agalactiae *(n=657)*
23% Mycoplasmopsis agalactiae *(n=1133)*

5% Mycoplasma mycoides cluster *(n=1051)*

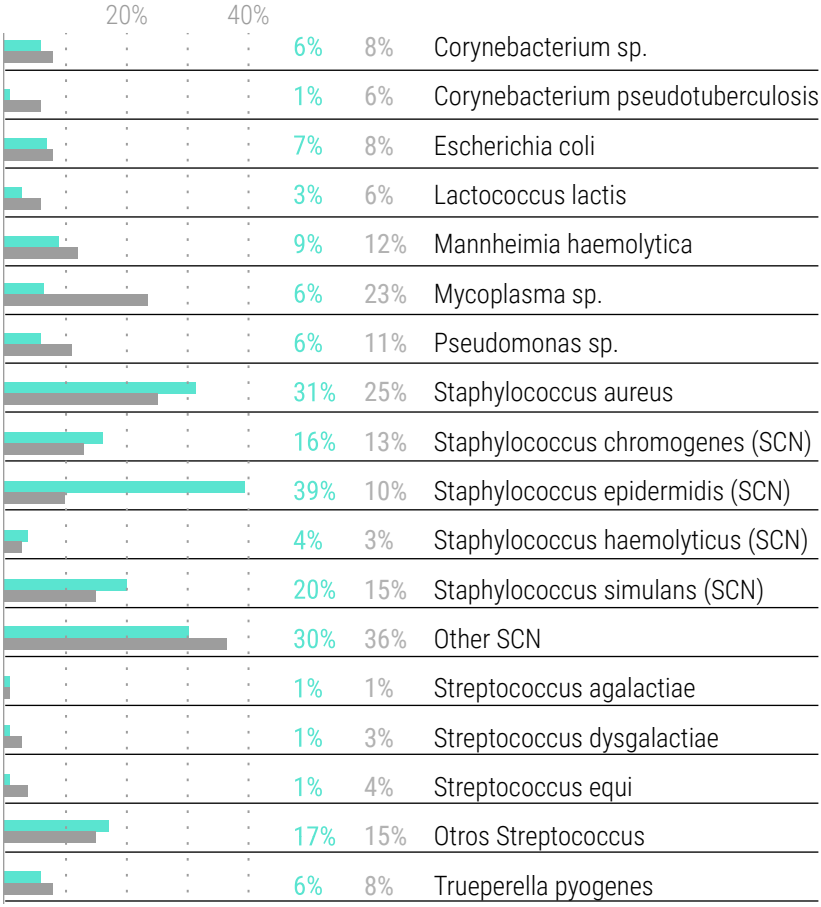
4% Mycoplasma putrefaciens *(n=607)*

In individual milk samples, there is also an important percentage of detection of Maedi-Visna/CAE virus as well as in tank analysis. This highlights the regular presence of the disease on the holding.

For goats, cases of contagious agalactia can be caused by distinct species of mycoplasmas. Mycoplasma agalactiae is the most prevalent one.

bacteria most frequently isolated in cases of mastitis (ovine & caprine)

438 and 674 cases analyzed since August 2018

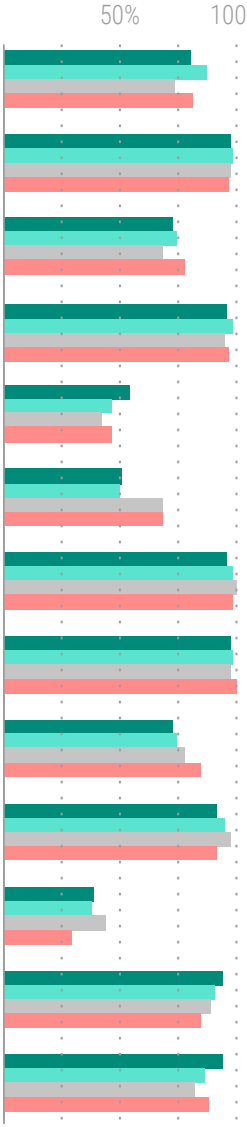


Thanks to the microbiological culture of the individual milk samples, we were able to isolate Staphylococcus aureus (gangrenous mastitis) and coagulase-negative Staphylococcus (CoNS).

The isolation of Mycoplasma requires specific conditions and a specific culture. Also, the culture of pure strains is more difficult. For this reason, combining both techniques (qPCR and microbiological cultures) is important in the diagnosis of agalactia to increase diagnostic sensitivity.

antibiotic susceptibility testing (Kirby Bauer method) of Staphylococcus aureus (ovine & caprine)

comparison of the sensitivity percentage of 222 antibiograms performed in the following semesters:

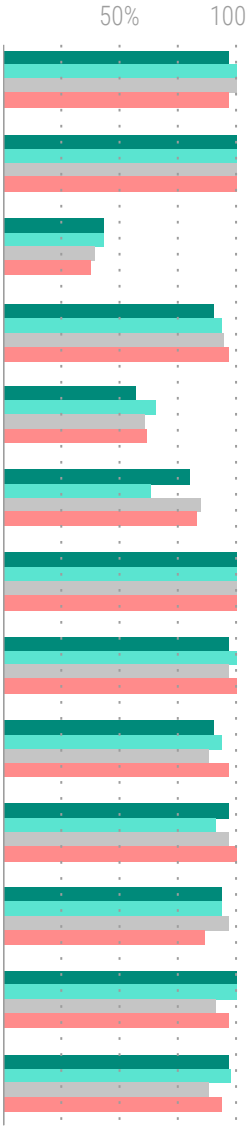


	jul. 19 dec. 19	jan. 20 jun. 20	jul. 20 dec. 20	jan. 21 jun. 21
Ampicillin	80%	88%	73%	82%
Cloxacillin	98%	98%	98%	97%
Penicillin	73%	74%	69%	78%
Trimethoprim/ sulfamethoxazole	96%	98%	96%	97%
Tetracycline	55%	47%	42%	47%
Streptomycin	51%	50%	69%	68%
Amoxicillin + clavulanic acid	96%	98%	100%	98%
Cephalothin	98%	98%	98%	100%
Clindamycin	73%	74%	78%	85%
Tilmicosin	92%	95%	98%	92%
Tylosin	39%	38%	44%	30%
Enrofloxacin	94%	91%	89%	85%
Marbofloxacin	94%	86%	82%	88%

We evaluated the evolution of the sensitivity for each antibiotic over time using the Chi-square test. No significant differences have been obtained for any antibiotic.

antibiotic susceptibility testing (Kirby Bauer method) of Staphylococcus epidermidis (ovine & caprine)

comparison of the sensitivity percentage of 143 antibiograms performed in the following semesters:



	jul. 19 dec. 19	jan. 20 jun. 20	jul. 20 dec. 20	jan. 21 jun. 21
Ampicillin	97%	100%	100%	97%
Cloxacillin	100%	100%	100%	100%
Penicillin	43%	43%	39%	38%
Trimethoprim/ sulfamethoxazole	90%	93%	94%	97%
Tetracycline	57%	65%	61%	62%
Streptomycin	80%	63%	85%	83%
Amoxicillin + clavulanic acid	100%	100%	100%	100%
Cephalothin	97%	100%	97%	100%
Clindamycin	90%	93%	88%	97%
Tilmicosin	97%	91%	97%	100%
Tylosin	93%	93%	97%	86%
Enrofloxacin	100%	100%	91%	97%
Marbofloxacin	97%	98%	88%	93%

We evaluated the evolution of the sensitivity for each antibiotic over time using the Chi-square test. No significant differences have been obtained for any antibiotic.

The sensitivity percentages of Staphylococcus aureus and Staphylococcus epidermidis are similar for most antibiotics.

In the case of Staphylococcus aureus, we observed a lower sensitivity to ampicillin, penicillin, tetracycline, streptomycin, clindamycin and especially tylosin.

On the other hand, the Staphylococcus epidermidis strains that were analyzed showed lower percentages of sensitivity to penicillin, as well as a tendency to decrease their sensitivity over time.

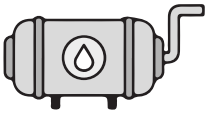
Listeria monocytogenes

✗ does not cause mastitis in small ruminants

✓ zoonoses

⚠ excretion through the milk, which leads to its withdrawal by dairy plants

how can we monitor the presence of Listeria monocytogenes on our holdings?



sample:
tank milk from several consecutive days:
⚠ excretion is intermittent

diagnostic techniques for its detection:

✓ microbial culture ✓ molecular techniques (qPCR)

In Exopol, 55% of the cases received for suspected Listeria monocytogenes turned out to be positive.

in order to control the presence of Listeria monocytogenes in tank milk:

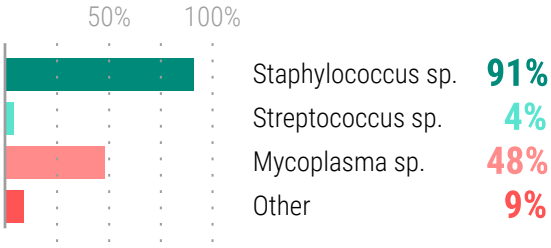
- ✓ **hygiene and periodic disinfection** of the milking machine and pipelines
- ✓ **carrier detection** by analyzing individual milk samples and eliminating positive animals
- ✓ **application of an autovaccine** against this bacterium to control excretion through milk

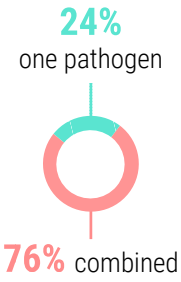
• statistical results: autovaccines

autovaccines produced for mastitis processes

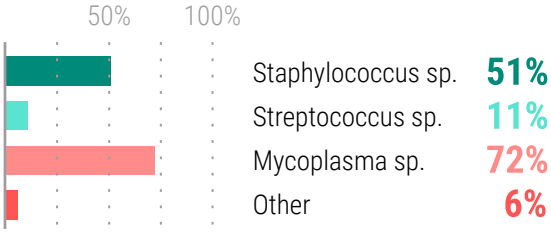
% of autovaccines containing the different agents

ovine



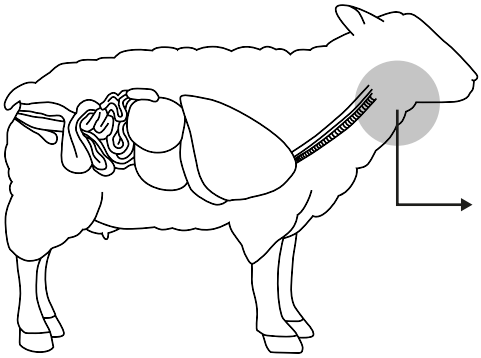


caprine



abscess-causing processes (skin level)

sampling

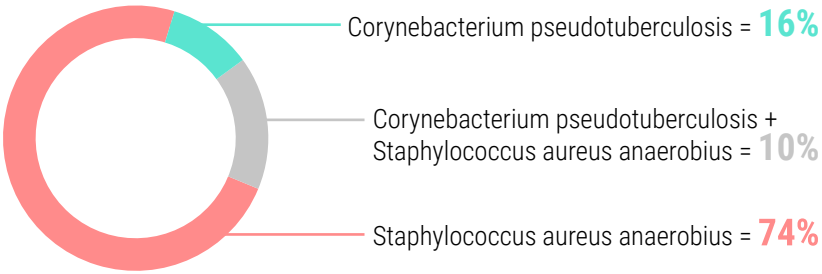


	abscess disease	caseous lymphadenitis
distribution	Europe - Asia West Africa	worldwide
etiology	Staphylococcus aureus anaerobius	Corynebacterium pseudotuberculosis
morbidity age	high	low
group affected	young animals	adult & replacement
form	superficial only (not always in lymph nodes)	superficial (always lymph nodes), visceral
abscess size	Ø to 7 cm	Ø 0,5-15 cm
abscess content	semi-liquid creamy pus	caseous pus concentric calcifications

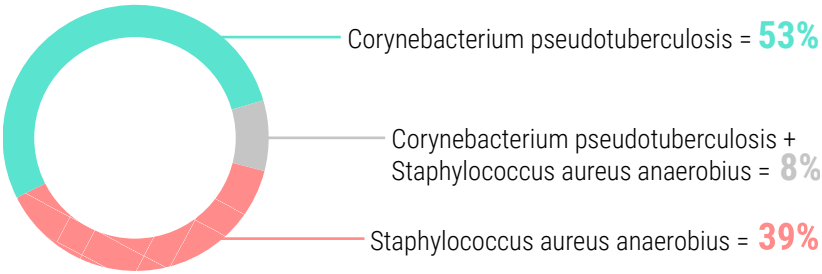
• statistical results: diagnosis

bacteria causing Morel's disease and lymphadenitis

· ovine



· caprine



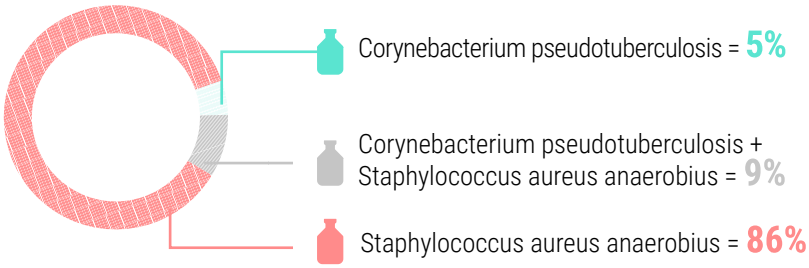
A higher isolation of Corynebacterium pseudotuberculosis (53%) is observed in goats compared to sheep. Morel's disease is more common (74%) amongst sheep.

• statistical results: autovaccines

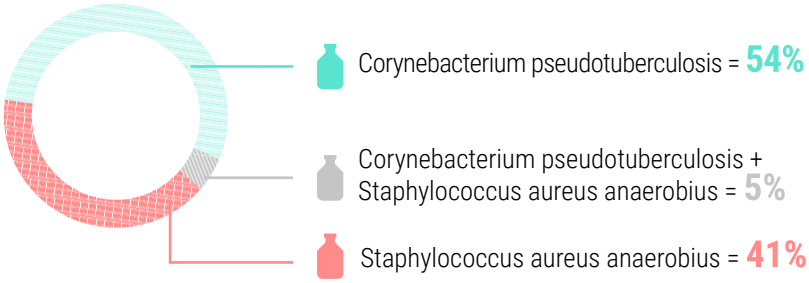
autovaccines produced for abscesses processes

% of autovaccines containing the different agents

ovine

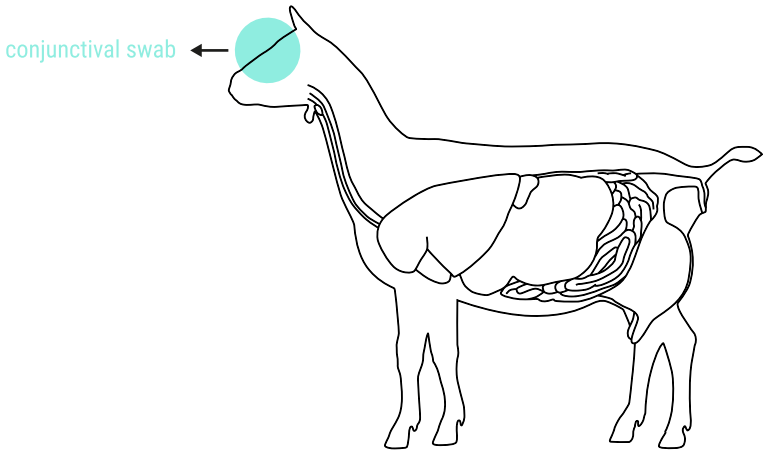


caprine



ocular processes

sampling



diagnostic panels

Ovine - Ocular disease:

qPCR: Mycoplasmopsis agalactiae, Mesomycoplasma conjunctivae, Moraxella ovis

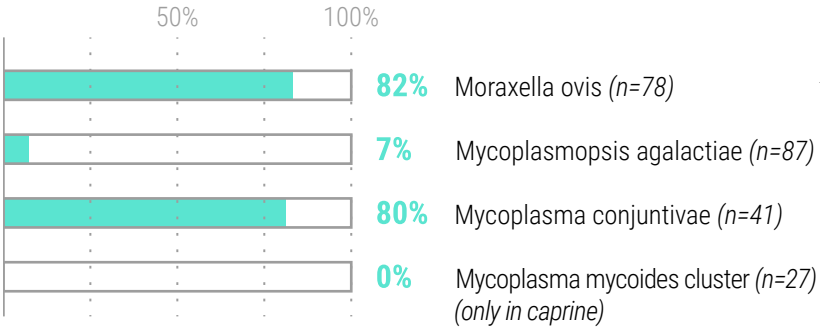
Goat - Ocular disease:

qPCR: Mycoplasmopsis agalactiae, Mycoplasma mycoides cluster, Mesomycoplasma conjunctivae, Moraxella ovis

• statistical results: diagnosis

pathogens analyzed in ovine and caprine hoof panel

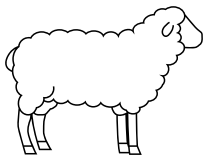
% of positives analyzed via qPCR



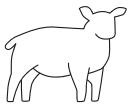
Moraxella ovis, the causative agent of ovine keratoconjunctivitis, was detected in most of the cases analyzed (82%). In addition, Mycoplasma conjunctivae was detected in 80% of the cases, but its implication as a primary agent in ocular processes has not been proved.

autovaccines against Moraxella ovis

effective for the control of ovine keratoconjunctivitis



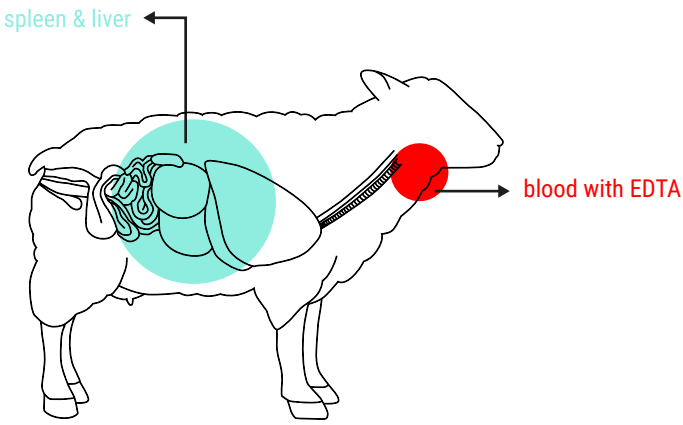
given to breeder farms with problems



given to sheep feedlots

hemoparasites

sampling



diseases caused by hemoparasites

Anaplasmosis

symptoms: fever, jaundice, anemia, and cachexia

⚠ chronic cases associated with Anaplasma sp.

seemingly healthy lambs with jaundiced carcasses

predisposing factor for other infectious diseases in adult lambs and ewes

Piroplasms: Babesia and Theileria

Babesia ovis and Babesia mutasi

✓ pathogenic species in small ruminants recently added to the diagnostic panel

• diagnostic panels

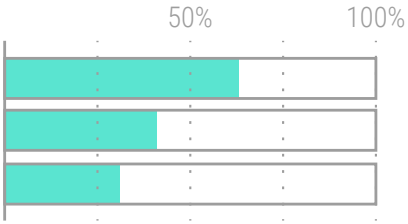
Hemoparasite:

qPCR: Piroplasmas, Anaplasma sp., Mycoplasma ovis, Babesia ovis, Babesia mutasi

• statistical results: diagnosis

pathogens analyzed in hemoparasite panel (ovine)

% of positives analyzed via qPCR



63% Anaplasma sp. *(n=93)*

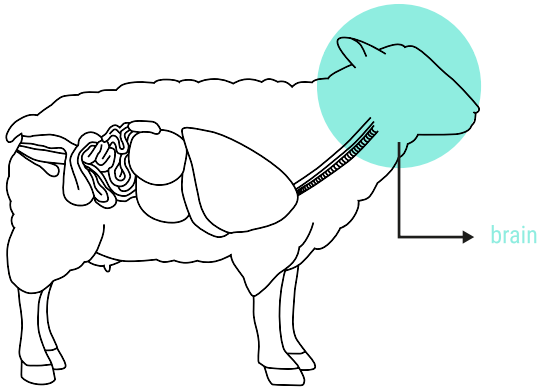
41% Mycoplasma ovis *(n=93)*

31% Piroplasm *(n=90)*

A high percentage of cases positive for Anaplasma sp. was found in sheep. Problems caused by hemoparasites are more prevalent in flocks of grazing sheep. Sporadic cases were detected in goats (results not shown).

nervous processes

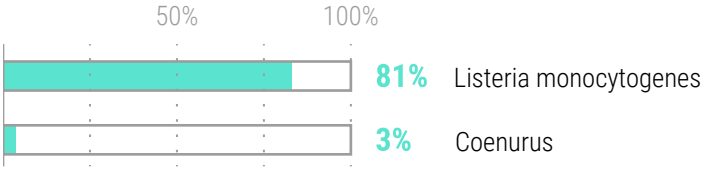
sampling



statistical results: diagnosis

pathogens analyzed in nervous processes

189 analyzed cases via qPCR and microbiology



From all the cases that were analyzed (mainly replacement and adult animal cases), 81% were diagnosed as listeriosis, while 3% were cases of cenurosis, which was confirmed by anatomopathological studies.

Cerebrocortical necrosis or polioencephalomalacia is a nervous pathology caused by vitamin B1 deficiency (thiamine). It is not displayed in the chart as samples are rarely received in the laboratory. It is usually diagnosed clinically, as thiamine treatment corrects the problem when thiamine disease is suspected.

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