cattle etiology
in the Iberian Peninsula:
statistics

exopol

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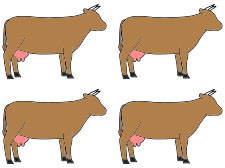
statistics

Published in 2022

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

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 temp. chilled frozen

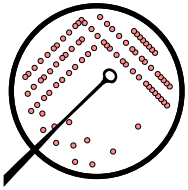
samples	remarks
swabs with medium	*can be shipped at room temperature*
organs	*if frozen, take a swab beforehand for microbiological analysis*
bronchoalveolar lavages	
feces	*directly from the rectum*
preputial scraping	
skin scraping	
blood	*use EDTA tubes* 
serum	*use tubes without anticoagulant* 
milk	*in specific cases of mastitis: freeze until several samples are collected*
samples in RNAlater	*for intestinal integrity studies and organs for molecular studies in aquaculture*
CORIOLIS (air)	
boot swabs (dirty surfaces)	
wipes (clean surfaces)	

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

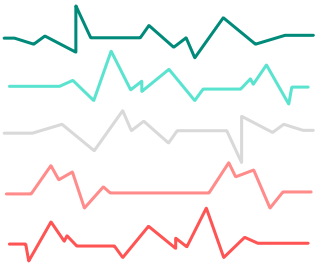
isolating and identify bacteria using MALDI-TOF

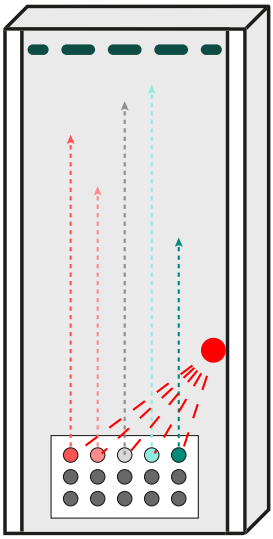
what is it?



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?

Actinobacillus lignieresii, Actinomyces bovis, Mycoplasma bovis, Prototheca spp.

antibiograms

antibiotic susceptibility testing (Kirby Bauer method)

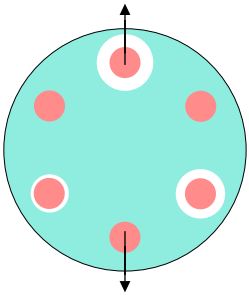
bacterial strains isolated in microbiological culture can be seeded in the right growth media where **discs containing a standardized antibiotic concentration are placed**

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)

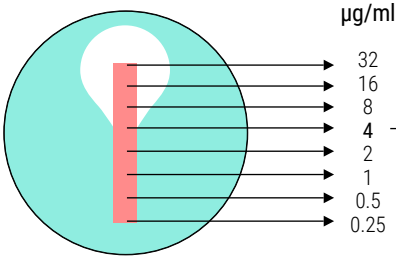
antibiotic susceptibility testing

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

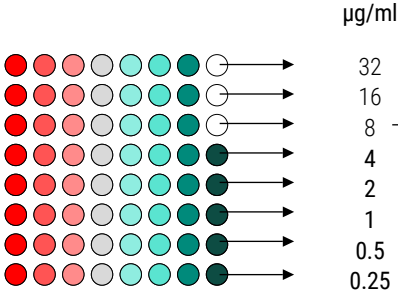
sensitive: ≤0,25

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

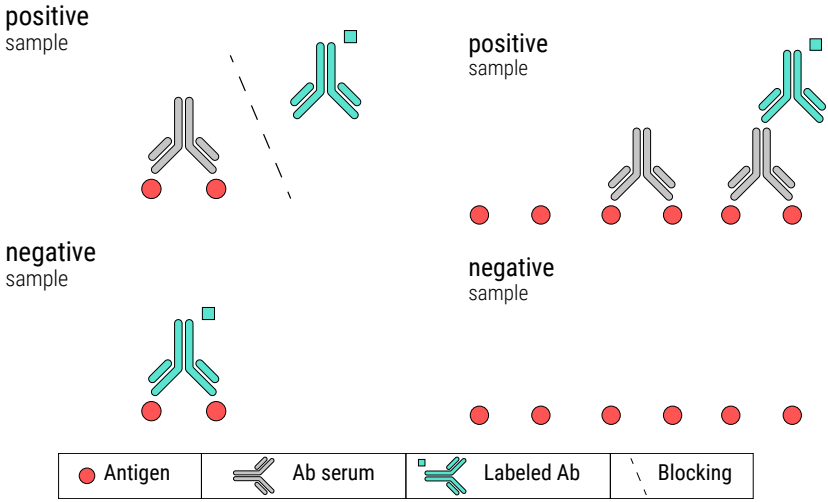
what is a serology?:

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

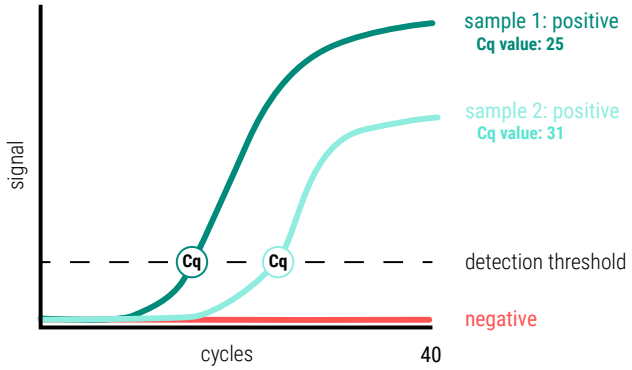
positivo — pathogen is present in sample

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

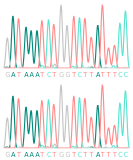
what is sequencing?:

determination of the nucleotide sequence of one or more genes

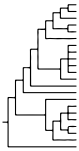
what are its applications?

sequencing *(e.g. BRSV)*

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

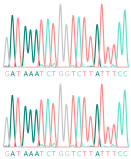


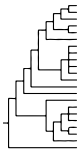
→ we generate phylogenetic trees



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

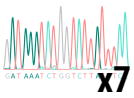
we compare the sequences obtained with those of vaccine strains or previous sequenced samples → we generate phylogenetic trees → we obtain the strain genotype





MLST technique *(e.g. Mycoplasma bovis)*

we sequence seven genes

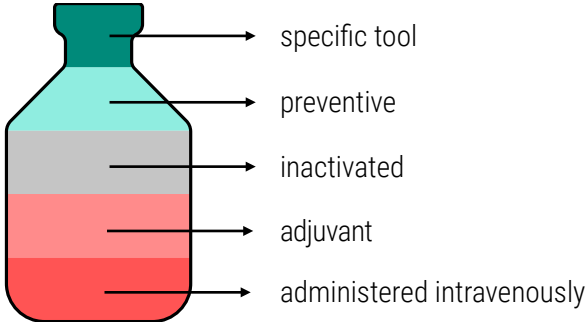


→ through changes in sequences we determine the alleles of each gene: the combination of the alleles of the 7 genes determines the ST (sequence type)

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?

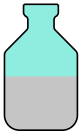
- presence of an infectious disease
- confirmatory laboratory diagnosis
- selecting the involved strains or serotypes
- under veterinary prescription
- produced by an authorized laboratory
  (Nª REG Exopol: 235/50/015-A)

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

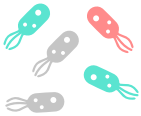
an example:



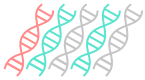
initial autogenous vaccine: two serotypes

→ shipping of new sample to monitor the farm

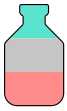
shipping of new **samples**



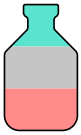
isolation of **bacteria**



tracking of the **new serotypes** via qPCR



incorporating the new serotypes into the **autovaccine**



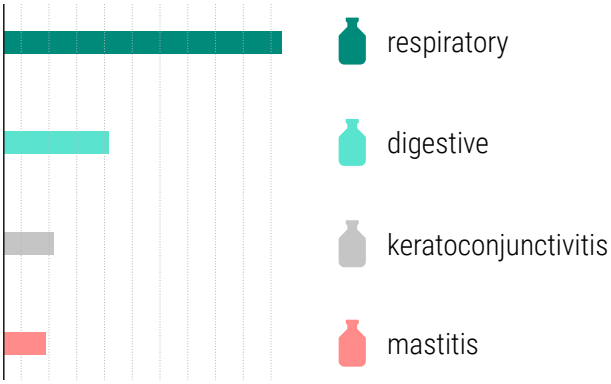
final autogenous vaccine: three serotypes

available autogenous vaccines for swine

- Actinobacillus lignieresii
- Escherichia coli
- Clostridium perfringens tipo A
- Histophilus somni
- Mannheimia haemolytica
- Moraxella bovis
- Moraxella bovoculi
- Mycoplasma bovis
- Pasteurella multocida
- Salmonella spp.
- Staphylococcus spp.
- Streptococcus spp.
- 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:



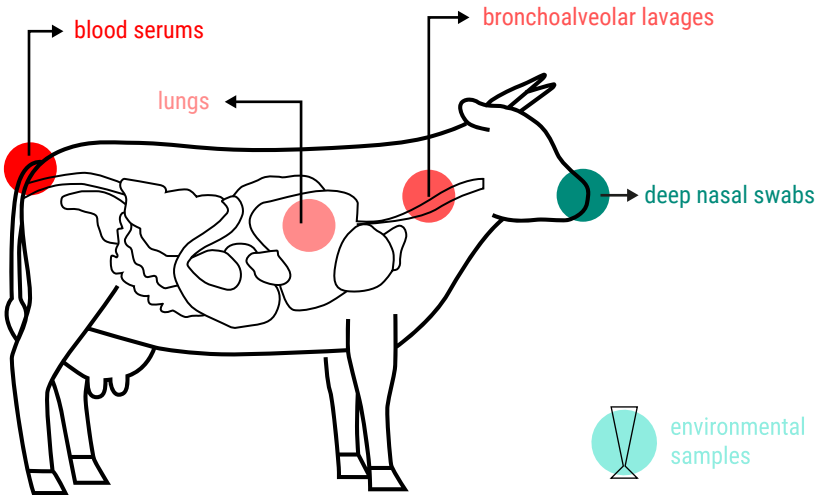
statistical results:

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 Iberian farms, what serotypes are present, autovaccines produced, etc.

- respiratory processes
- digestive processes
- reproductive processes
- mastitis
- other processes: hemoparasites and ocular processes

• respiratory processes

• sampling



• diagnostic panels

Respiratory:
qPCR: Pestivirus, IBR, BRSV , Parainfluenza 3, Bovine Coronavirus , Mycoplasma bovis, Histophilus somni, Pasteurella multocida - Capsular typing, Mannheimia haemolytica - 1, 2 y 6 serotype identification

Respiratory - Serology:
Serology: BVD p80/Border, IBR gE, Parainfluenza 3, BRSV, Mycoplasma bovis

BRSV - sequencing (gen G)

BVD (Bovela) - Vaccine strain differentiation:
qPCR: BVDV1 Bovela, BVDV2 Bovela

Mannheimia haemolytica - 1, 2 and 6 serotype identification

Pestivirus - Differentiation:
qPCR: BVDV1, BVDV2, BVDV3 (Hobi-like), Border Disease

Parainfluenza 3 - sequencing (gen P)

Pasteurella multocida - Capsular typing:
qPCR: type A, type B, type D, type E, type F

Always sample an adequate number of untreated animals with recent clinical symptoms

lungs

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

bronchoalveolar/transtracheal lavages

- ✓ on live animals
- ✓ 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
- ⚠ more invasive technique for the animals (transtracheal lavages)

deep nasal swabs

- ✓ easy sampling on live animals
- ✓ 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

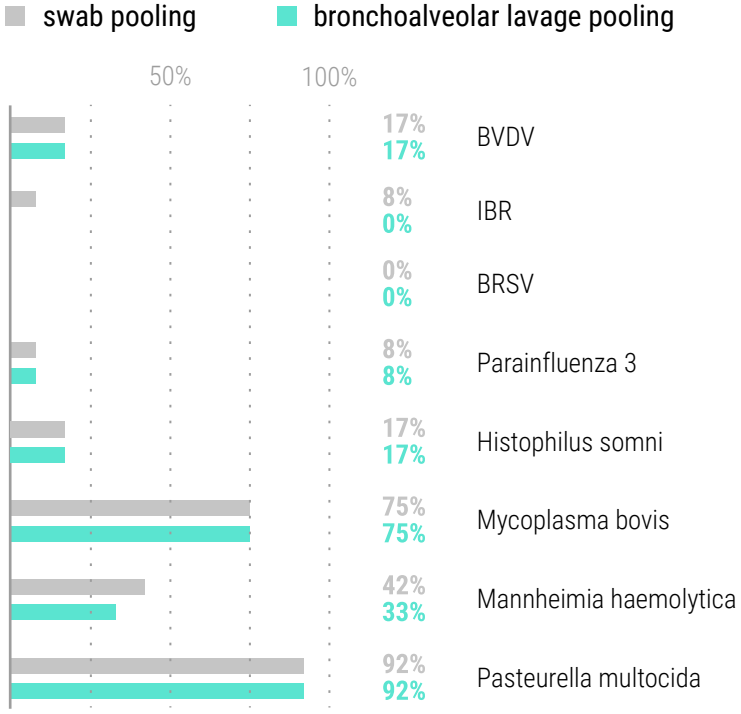
but... which sample is the best one?

Use of deep nasal swabs and bronchoalveolar lavages for the detection of respiratory pathogens

Poster presented in Anembe 2018

- paired sampling of 5 calves of less than 1 year of age from 12 holdings with a history of respiratory processes

% of positives analyzed via qPCR



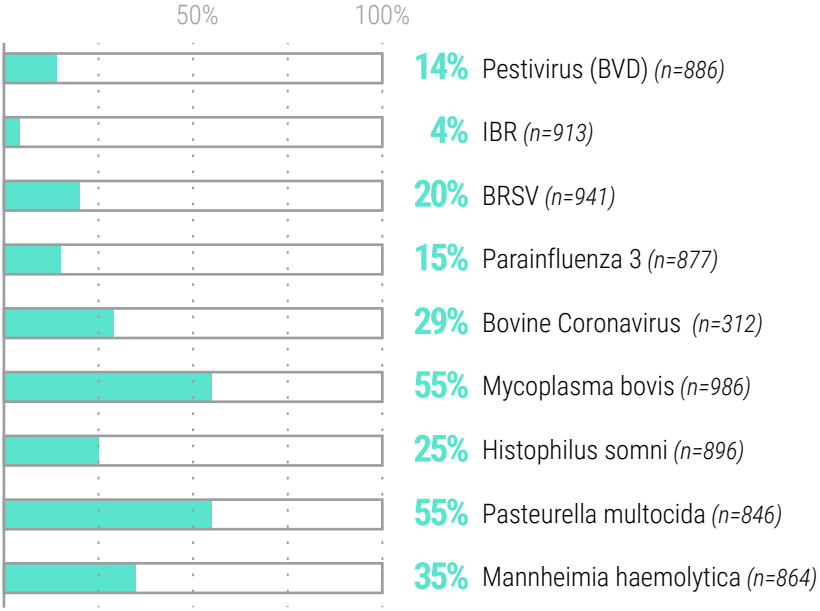
Pathogen detection is similar in both sampling methods and both are appropriate on live animals.

In the case of IBR, only one positive case was detected in the pool of swabs, which turned out negative in the pool of washes. The observed difference is not significant.

• statistical results: diagnosis

pathogens analyzed in respiratory panel

% of positives analyzed via qPCR



The most frequently detected agents are Mycoplasma bovis and Pasteurella multocida. Among viral agents, Bovine coronavirus and Syncytial virus (BRSV) are most detected.

PCR techniques detect live attenuated vaccines in recently vaccinated animals. The use of DIVA PCR or sequencing techniques allows to differentiate vaccine strains from field strains.

Bovine coronavirus is a primary agent responsible for digestive processes. However, recently it has been confirmed that it can affect the respiratory complex, which had so far been underestimated.

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

Bovine coronavirus

Neonatal calf diarrhea

symptoms: profuse and hemorrhagic watery diarrhea, anorexia, dehydration, and often, death

Winter dysentery in adult cattle

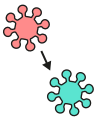
symptoms: diarrhea, decreased milk production, depression, anorexia, nasolacrimal discharge

Respiratory infections (calves > adults)

symptoms: fever, shortness of breath, mild to severe cough, conjunctivitis

what do we know about them?

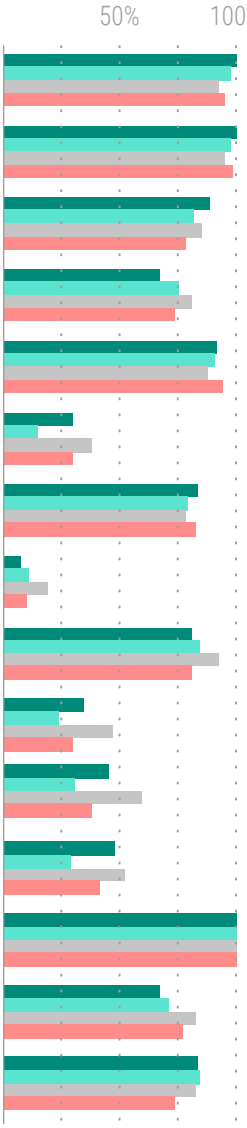
enteric and respiratory viruses:
there is cross-protection even though there are genetic differences



a digestive infection can evolve into a respiratory infection and viremia

antibiotic susceptibility testing (Kirby Bauer method) of Pasteurella multocida

comparison of the sensitivity percentage of 194 antibiograms performed in the following semesters:



	jul. 19 dec. 19	jan. 20 jun. 20	jul. 20 dec. 20	jan. 21 jun. 21
Amoxicillin	100%	98%	93%	96%
Ampicillin	100%	98%	95%	99%
Spectinomycin	89%	82%	86%	79%
Penicillin	68%	76%	81%	74%
Trimethoprim/ Sulfamethoxazole	92%	91%	88%	94%
Tetracycline	30%	16%	38%	30%
Gentamicin	84%	80%	79%	83%
Streptomycin	8%	11%	19%	10%
Florfenicol	81%	84%	93%	81%
Thiamphenicol	35%	24%	48%	30%
Gamithromycin	46%	31%	60%	39%
Tilmicosin	49%	29%	52%	41%
Ceftiofur	100%	100%	100%	100%
Enrofloxacin	68%	71%	83%	77%
Marbofloxacin	84%	84%	83%	74%

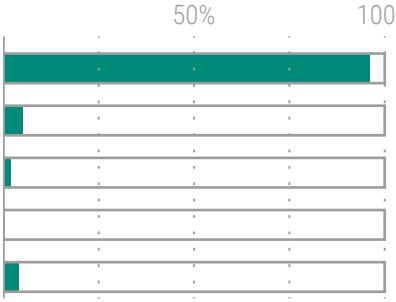
We evaluated the evolution of sensitivity to each antibiotic over time using the Chi-square test. No significant differences were observed for any antibiotic.

Sensitivity results of the analyzed Pasteurella multocida strains show that most of them are sensitive to most antibiotics, except for tetracycline, streptomycin, thiamphenicol and tilmicosin, for which greater resistance is shown.

These strains have a high resistance to streptomycin. According to the scientific literature, there is an increase in strains that contain resistance genes to this antibiotic.

detection of capsular serotypes of Pasteurella multocida on clinical samples

189 cases analyzed since July 2017 via qPCR



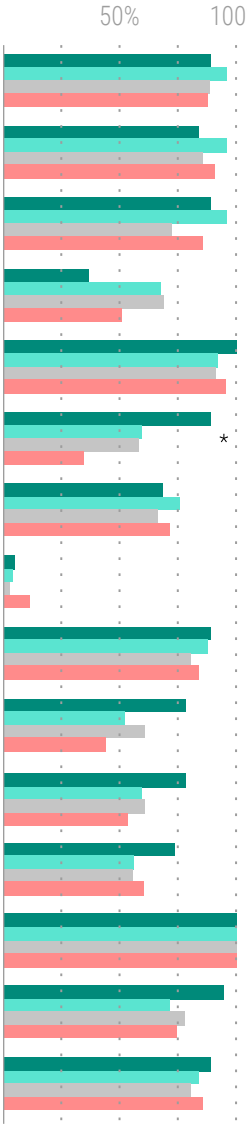
Pasteurella multocida type A	96%
Pasteurella multocida type B	5%
Pasteurella multocida type D	2%
Pasteurella multocida type E	0%
Pasteurella multocida type F	4%

Pasteurella multocida type A was detected in 96% of cases.

In 7% of analyzed cases, a type A coinfection was detected together with another capsular type.

antibiotic susceptibility testing (Kirby Bauer method) of Mannheimia haemolytica

comparison of the sensitivity percentage of 123 antibiograms performed in the following semesters:



	jul. 19 dec. 19	jan. 20 jun. 20	jul. 20 dec. 20	jan. 21 jun. 21
Amoxicillin	89%	96%	89%	88%
Ampicillin	84%	96%	86%	91%
Spectinomycin	89%	96%	72%	86%
Penicillin	37%	68%	69%	51%
Trimethoprim/ Sulfamethoxazole	100%	92%	92%	95%
Tetracycline	89%	60%	58%	35%
Gentamicin	68%	76%	67%	72%
Streptomycin	5%	4%	3%	12%
Florfenicol	89%	88%	81%	84%
Thiamphenicol	79%	52%	61%	44%
Gamithromycin	79%	60%	61%	53%
Tilmicosin	74%	56%	56%	60%
Ceftiofur	100%	100%	100%	100%
Enrofloxacin	95%	72%	78%	74%
Marbofloxacin	89%	84%	81%	86%

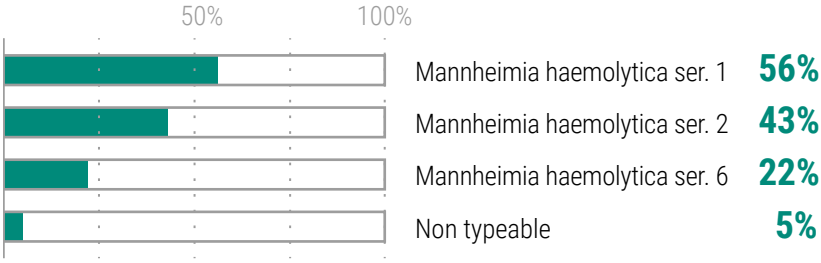
We evaluated the evolution of sensitivity to each antibiotic over time using the Chi-square test. We considered that time and sensitivity variables are dependent, i.e., there are significant differences between the % of sensitive ones for the different time periods, if p-value <0.01 (*).

Sensitivity results of the analyzed Mannheimia haemolytica strains show that most of them are sensitive to almost all the antibiotics tested. However, a general trend towards reduced antibiotic sensitivity is seen over time.

In the case of tetracycline, a statistically significant decrease in sensitivity is seen in the course of time. As with Pasteurella multocida, Mannheimia haemolytica strains are particularly resistant to streptomycin due to the increased presence of resistance genes to this antibiotic.

detection of capsular serotypes of Mannheimia haemolytica on clinical samples

130 cases analyzed since February 2018 via qPCR

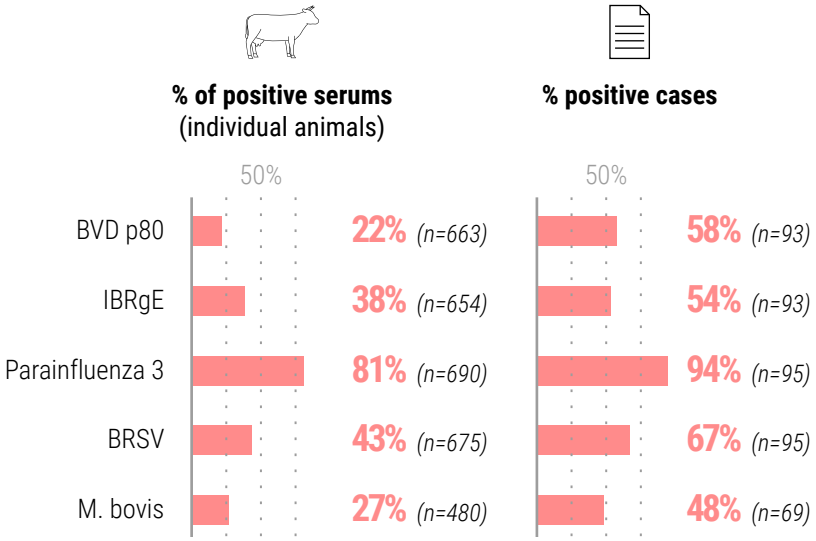


The most frequently detected serotypes are serotype 1 and 2. In 5% of the clinical samples, detection of Mannheimia haemolytica is different from the analyzed serotypes (serotypes 1, 2 and 6).

A coinfection of two or three detectable serotypes of Mannheimia haemolytica was found in 25% of cases.

pathogens analyzed in respiratory-serological panel

seropositivity study using ELISA techniques

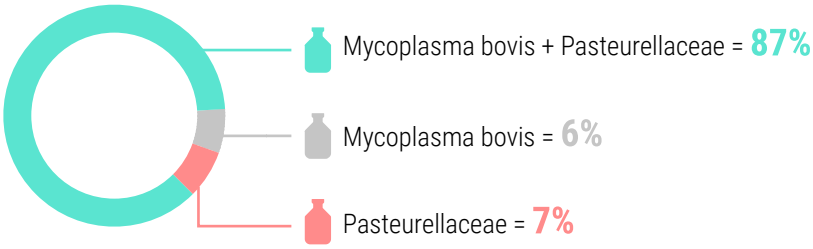


These results are from farmed animals with respiratory symptoms and should not be taken as prevalence data. A positive result may be indicative of recent infection, prior contact with the agent, or presence of vaccine antibodies (in the case of unlabeled vaccines).

• statistical results: autovaccines

autovaccines produced for respiratory processes

% of autovaccines including the different agents

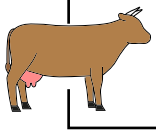


We develop specific autovaccines for calf feedlots and breeder farms. 100% of the autovaccines developed for fattening calves are combined vaccines which contain Mycoplasma bovis and the different isolated serotypes of Pasteurellaceae.

Bacteria of Pasteurella multocida, Mannheimia haemolytica and Histophilus somni are part of the Pasteurellaceae family. Autovaccines can contain several strains of the same or distinct species, depending on what was found in the diagnostics.

Mycoplasma bovis

responsible for:
pneumonia
arthritis
mastitis
otitis



✖ **problems:** increased resistance to antibiotics

✓ **alternatives: autovaccines** (no commercial vaccine)

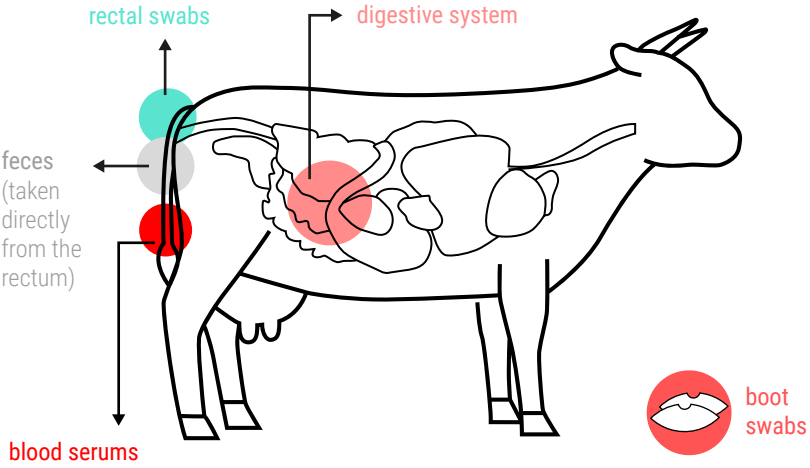
characterization of Mycoplasma bovis through (MLST)

- ✓ detection of strains present on the farm
- ✓ selection strains to develop autovaccines

preliminary study: ST8 and ST122 are the most frequently found strains in Iberian farms

digestive processes

sampling



diagnostic panels

Digestive (calf):

qPCR: Rotavirus A, Bovine Coronavirus , Pestivirus, Bovine Torovirus, Norovirus genotype 3, Nebovirus, Clostridium perfringens - Toxins, Salmonella sp., Escherichia coli - Virulence factors, Cryptosporidium parvum, Eimeria sp.

Coccidia:

qPCR: Eimeria bovis, Eimeria zuernii, Eimeria alabamensis, Eimeria sp.

Coprological:

qPCR: Eimeria sp., Nematodes, Cestodes, Trematodes

BVD (Bovela) -) - Vaccine strain differentiation:

qPCR: BVDV1 Bovela, BVDV2 Bovela

Clostridium perfringens - Toxins:

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

Escherichia coli - Virulence factors:

qPCR: F5, F17, F4, F41, gen eae, STa, STb, LT, STX1, STX2, E. coli

Pestivirus - Differentiation:

qPCR: BVDV1, BVDV2, BVDV3 (Hobi-like), Border Disease

Salmonellae bovine:

qPCR: S. typhimurium, S. dublin, S. enteritidis, S. infantis

Rotavirus A - sequencing (VP7, VP4)

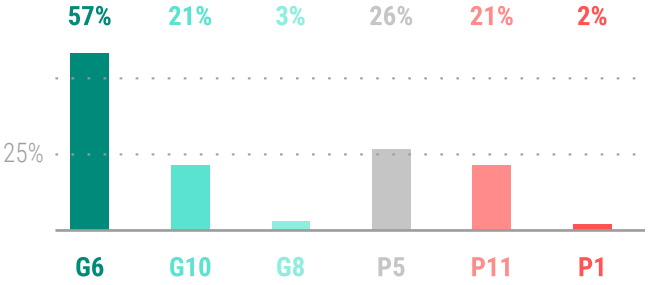
sequencing conducted on Rotavirus type A

most frequently detected virus in neonatal calf diarrhea

VP7 genotype (G type): at least 12

VP4 genotype (P type): at least 11

most frequent genotypes:



most frequent combinations:

G6P5 G6P11 G10P11

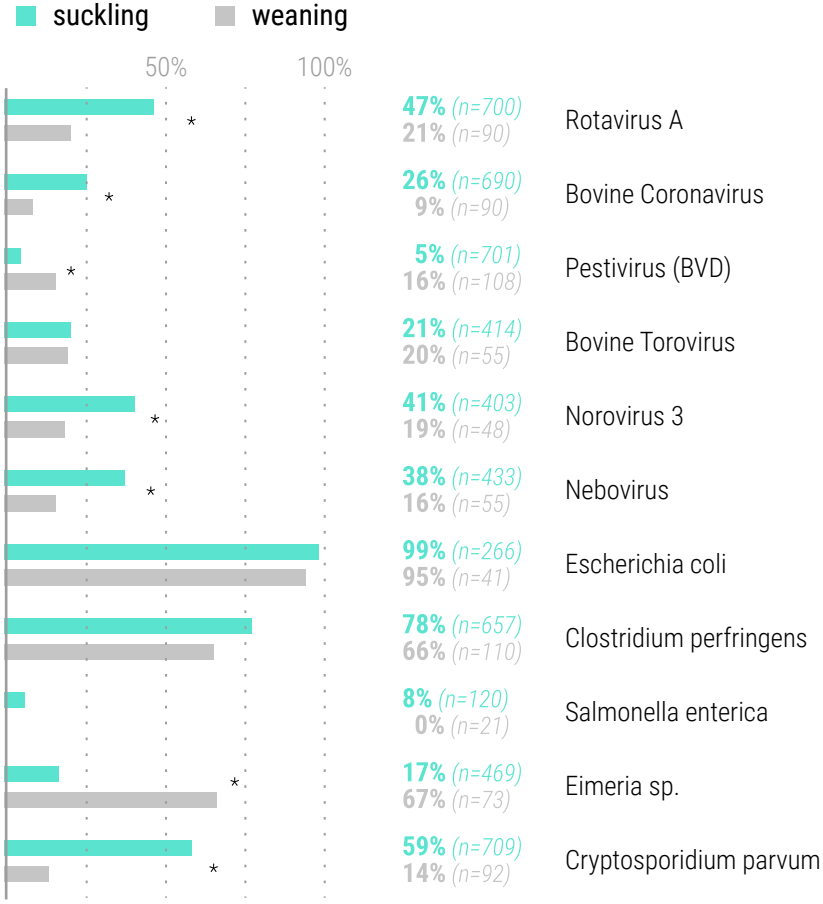
what is the role of sequencing?

- ✓ evaluating if the strain present is the same or different from the one in the vaccine
- ✓ observing the presence of new genotypes in farms

• statistical results: diagnosis

pathogens analyzed in digestive panel by specific age

% of positives analyzed via qPCR



We evaluated the difference in the number of positives for each of the pathogens analyzed using the Chi-square statistical test. We considered that there are significant differences between the percentage of positives among the different age groups, if p-value <0.01 (*).

In suckling animals, we observed a higher percentage of positive cases for Rotavirus type A, Salmonella enterica and Cryptosporidium parvum. Torovirus, Norovirus and Nebovirus are considered emerging agents in digestive problems, mainly in lactating calves.

A greater presence of Eimeria sp. was observed in weaned and fattening calves.

toxin typing of Clostridium perfringens

- knowing what type of toxins are produced by the detected strain
- making decisions about preventive measures

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

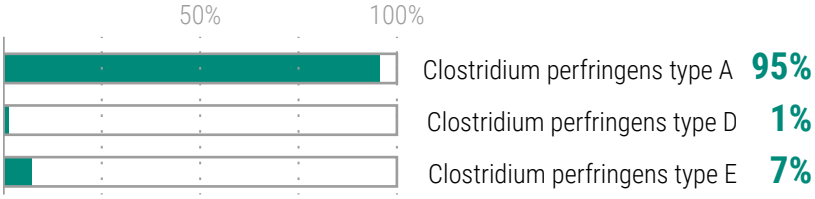
Strains of the above toxinotypes **can also produce enterotoxin (ENT) and β2 toxin**.

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

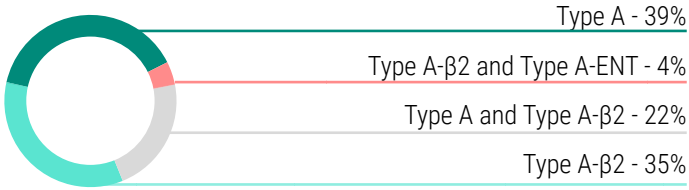
- **A** hemorrhagic enteritis and gas gangrene in calves under 3 weeks
- **B** dysentery (exceedingly rare)
- **C** necrotic enteritis in calves under 3 weeks
- **D** enterotoxaemia or purple gut in fattening animals and adults
- **E** necrotic hemorrhagic enteritis in calves

toxin typing of Clostridium perfringens

261 clinic cases analyzed in 209 farms via qPCR



presence of virulence factors associated with Clostridium perfringens type A



The most frequent toxinotype is type A. In 3% of cases a coinfection of toxinotype A with toxinotype E was detected.

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

Depending on their virulence factors, Escherichia coli strains can be classified into:

ETEC
enterotoxigenic strains

enterotoxigenic strains have **fimbriae** for attachment to the intestinal epithelium (F5, F17, F4 or F41) and **produce toxins** (Sta, Stb or LT) which can cause **clinical symptoms**

F5: most frequent one found in clinical cases

EPEC
enteropathogenic strains

enteropathogenic strains have the **eae gene**. An adhesin protein (**intimin**) is encoded by the eae gene and causes **diarrhea due to malabsorption**. The bacteria adheres to the intestine and its pathogenesis is not related to the production of toxins

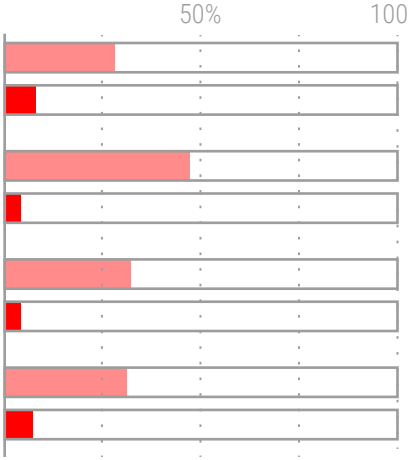
STEC
shiga toxin–producing strains

can cause diarrhea due to the **cytotoxic effect of shigatoxins (Stx1 or Stx2)**, although the importance of these strains lies in the fact that they can cause a **human food poisoning**

EHEC
enterohemorrhagic strains

can cause **hemorrhagic diarrhea** in calves due to Intimin Gene (eae) and Shigatoxins (Stx1 or Stx2)

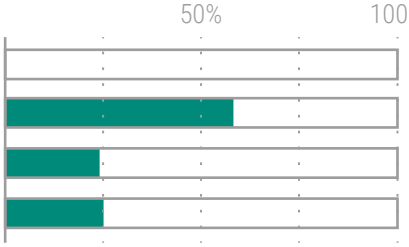
classification of Escherichia coli strains detected in cases of colibacillosis



samples with presence of:	
ETEC strains	28%
major ETEC strain*	8%
EPEC strains	47%
major EPEC strain*	4%
STEC strains	32%
major STEC strain*	4%
EHEC strains	31%
major EHEC strain*	7%

**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.*

% of fimbriae present in major ETEC strains



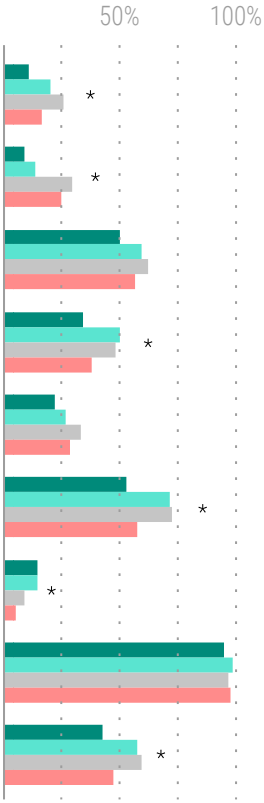
E. coli F4	0%
E. coli F5	58%
E. coli F17	17%
E. coli F41	25%

EPEC strains are the ones detected in the highest percentage in the analyzed cases (47%). However, they are the major strains of E. coli only in 4% of the samples. This suggests their involvement in the symptoms.

F17 fimbria was detected in 82% of cases. Nevertheless, only in 27% of cases F17 fimbria was the major strain without being associated with toxins. These strains cannot be classified as ETEC, but they are associated to digestive symptoms.

antibiotic susceptibility testing (Kirby Bauer method) of Escherichia coli

comparison of the sensitivity percentage of 652 antibiograms performed in the following semesters:



	jul. 19 dec. 19	jan. 20 jun. 20	jul. 20 dec. 20	jan. 21 jun. 21
Amoxicillin	11%	20%	26%	17%
Ampicillin	9%	14%	29%	25%
Spectinomycin	51%	60%	63%	57%
Trimethoprim/ sulfamethoxazole	34%	51%	49%	39%
Tetracycline	23%	27%	33%	29%
Gentamicin	54%	72%	73%	58%
Neomycin	15%	14%	9%	5%
Colistin sulfate	95%	99%	97%	98%
Enrofloxacin	43%	58%	60%	48%

We evaluated the evolution of sensitivity to 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 (*).

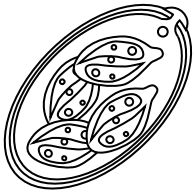
In general, these strains have high antibiotic resistance and present lower percentages of sensitivity to amoxicillin, ampicillin, tetracycline and neomycin. The latter shows a significant decrease over time.

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

detection of pathogenic Eimeria species

most important pathogenic species in the bovine species:

- Eimeria bovis
- Eimeria zuernii
- Eimeria alabamensis



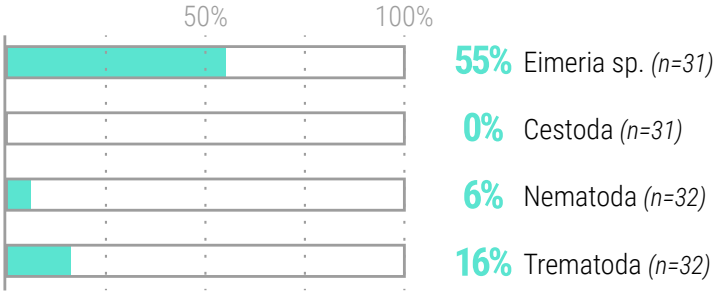
⚠ *it is important to assess the presence of the most pathogenic species, since the clinical signs are more related to the presence of pathogenic species than to the total count of Eimeria sp. (species with different degree of pathogenicity)*

diagnosis via qPCR on stool pools (> 5 g)

- ✓ assess **infestation** by lots, ages, etc.
- ✓ assess the **efficiency of a treatment** (Cq)
- ✓ assess the **pathogenicity of the current process**

pathogens analyzed in coprological panel

% of positives analyzed via qPCR ever since November 2020

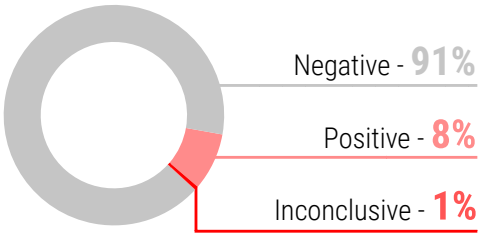


The presence of a high percentage of Eimeria sp. is observed. Trematodes are detected in second place, since a 16% of positive samples was found.

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 level, as in the case of Fasciola hepatica and Dicrocoelium dendriticum.

serological study of Paratuberculosis

% of positive serums against 19153 analyzed serums



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 performing qPCR on feces to increase diagnostic sensitivity.

Paratuberculosis: chronic infectious disease that causes weight loss and diarrhea

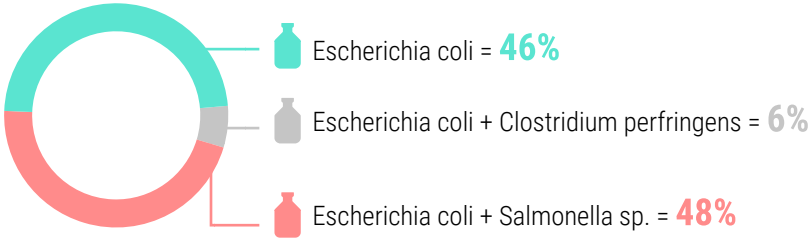
- **serological diagnosis**
  on serum
  more cost-effective
- **qPCR diagnosis**
  on feces, digestive. samples and swabs

	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**

• statistical results: autovaccines

autovaccines produced for digestive processes

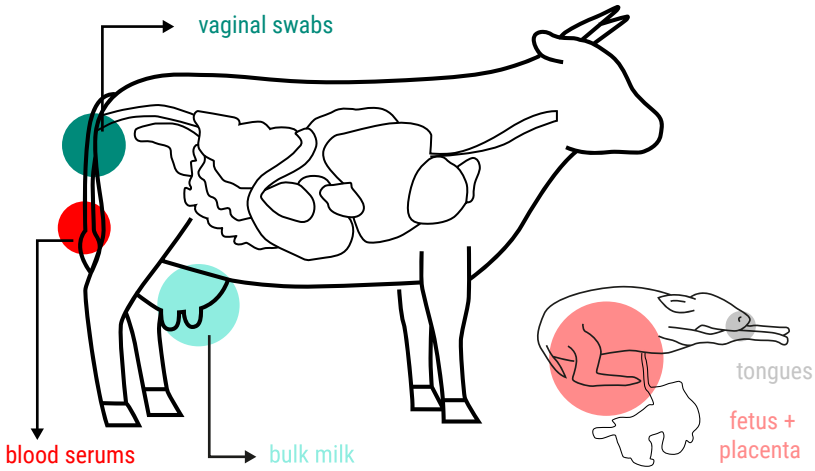
% of autovaccines including the different agents



Escherichia coli strains (even in digestive autovaccines) are selected based on the virulence factors they present

• reproductive processes

• sampling



• diagnostic panels

Abortion:

qPCR: Pathogenic Leptospira, Coxiella burnetii, Chlamydia abortus, Histophilus somni, IBR, Pestivirus, Neospora caninum

Infertility:

qPCR: Campylobacter fetus venerealis, Pathogenic Leptospira, Coxiella burnetii, Chlamydia abortus, Ureaplasma diversum, IBR, Pestivirus, Tritrichomonas foetus

Infertility (artificial insemination):

qPCR: Pathogenic Leptospira, Coxiella burnetii, Chlamydia abortus, Pestivirus, IBR, Ureaplasma diversum

Infertility (natural breeding):

qPCR: Campylobacter fetus venerealis, Tritrichomonas foetus

Metritis:

Microbiology: Bacteria isolation and identification, Anaerobic culture

Reproductive (bulk milk):

qPCR: Pathogenic Leptospira, Coxiella burnetii, Chlamydia abortus, Pestivirus, IBR

Reproductive - Serology:

Serology: Leptospira hardjo, Coxiella burnetii, Chlamydia abortus, BVD p80/Border, IBR gE, Neospora caninum

BVD (Bovela) - Vaccine strain differentiation:

qPCR: BVDV1 Bovela, BVDV2 Bovela

Pestivirus - Differentiation:

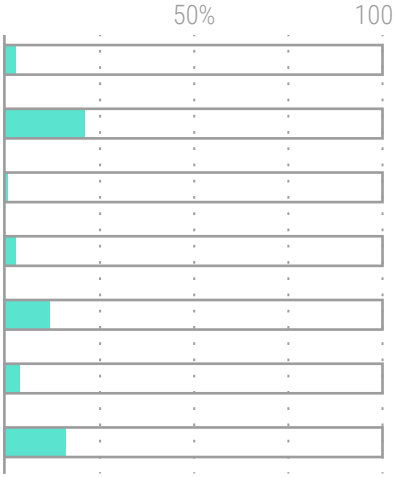
qPCR: BVDV1, BVDV2, BVDV3 (Hobi-like), Border Disease

Leptospira sp. - Typing

• statistical results: diagnosis

pathogens analyzed in abortion panel

% of positives analyzed via qPCR



3% Pathogenic Leptospira *(n=759)*

21% Coxiella burnetii *(n=760)*

1% IBR *(n=749)*

3% Chlamydia abortus *(n=762)*

12% Neospora caninum *(n=744)*

4% Pestivirus (BVD) *(n=766)*

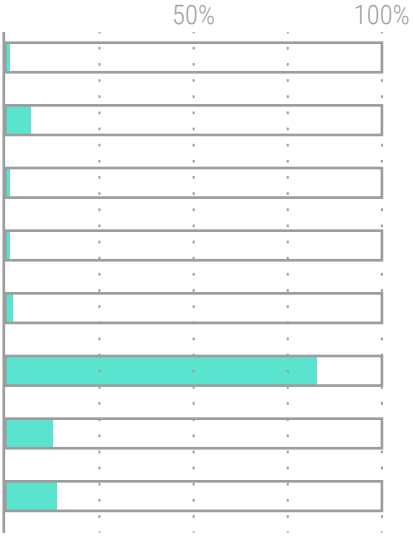
16% Histophilus somni *(n=196)*

Coxiella burnetii is the agent detected in the highest percentage. Its involvement in abortion processes is rare. More frequently, it causes infertility, metritis and decreased milk production.

Histophilus somni has been detected in reproductive processes, but it is essential to consider the detected concentration in order to evaluate its participation in the process.

pathogens involved in infertility processes

% of positives analyzed via qPCR



1% Pathogenic Leptospira *(n=159)*

7% Coxiella burnetii *(n=156)*

1% IBR *(n=160)*

1% Chlamydia abortus *(n=157)*

2% Pestivirus (BVD) *(n=162)*

80% Ureaplasma diversum *(n=123)*

13% Campylobacter f. venerealis *(n=662)*

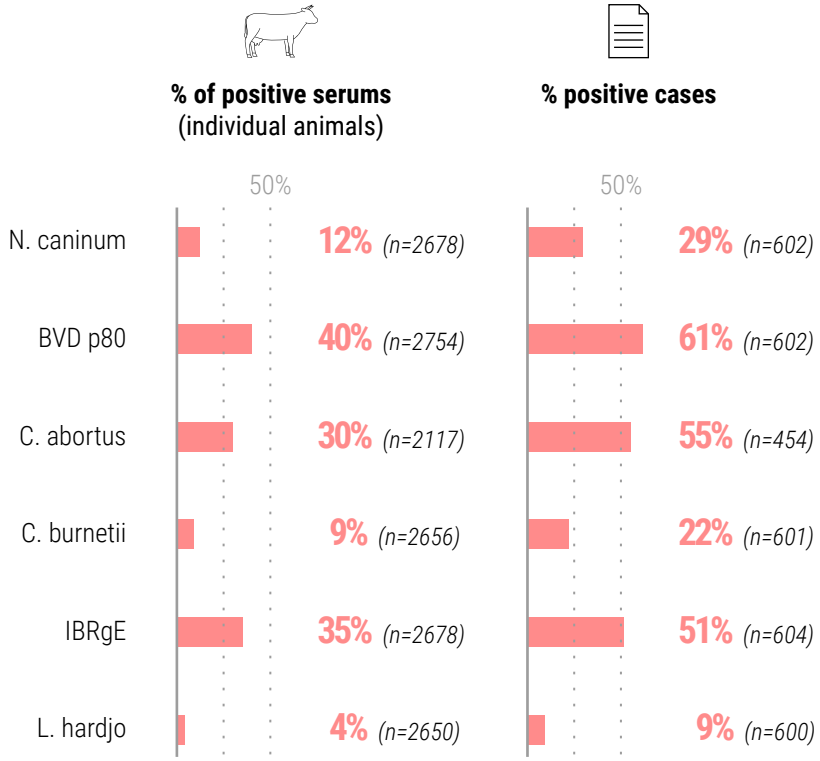
14% Tritrichomonas foetus *(n=704)*

Ureaplasma diversum, Campylobacter fetus venerealis and Tritrichomonas foetus are the three most detected agents. They are venereal transmitted reproductive pathogens.

Ureaplasma diversum causes infertility and genital lesions in females and males, although it is also detected in the reproductive tract of healthy animals. Coxiella burnetii is the most detected non-venereal agent in the case of infertility problems.

pathogens analyzed in serological-reproductive panel

seropositivity study using 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 not associated with the current reproductive process.

serological tests for BVD

persistently infected animal (PI)	antibodies: negative antigens: positive

if you have suspicions of the presence of PIs in the farm

1. presence of antibodies in the animals will be high
   antibody test (BVD p80)
2. on negative animals
   BVD antigen test

farm without suspicion of PI with low incidence of BVD

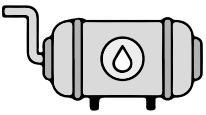
1. **BVD antigen test**
   via qPCR in pools of serums and unfolding positives
2. on positive animals
   antibody test (BVD p80)
3. newly infected animals may not yet have generated antibodies at the time of testing (antibody negative + antigen positive) to differentiate them from PIs
   repeat antibody test (BVD p80) in 15 days

analysis of antibodies for BVD protein 80
key enzyme in virus replication
(infection with field strain or vaccines)

- **inactivated vaccines:** negative results
- **live vaccine and field infections:** positive results

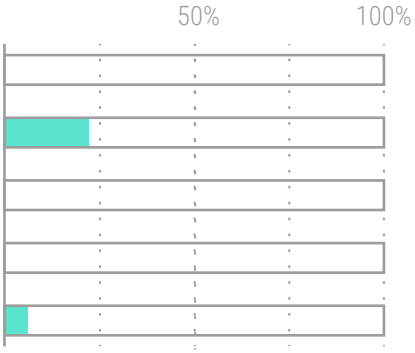
monitoring in bulk milk

- detection of infectious agents
- monitoring various diseases
- anticipating potential pathological processes



reproductive pathogens analyzed in bulk milk samples

% of positives analyzed via qPCR



0% Pathogenic Leptospira *(n=141)*

22% Coxiella burnetii *(n=141)*

0% IBR *(n=141)*

0% Chlamydia abortus *(n=141)*

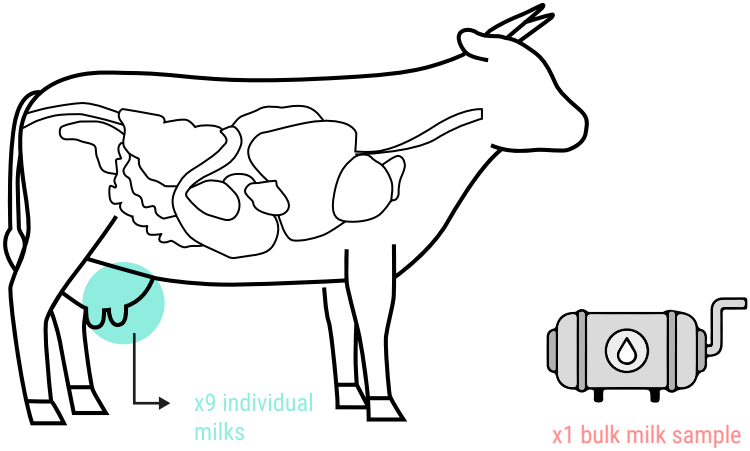
6% Pestivirus *(n=141)*

Coxiella burnetii is the most detected agent. Elimination via milk is not continuous, so a negative tank result does not guarantee freedom from disease.

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

• mastitis

• sampling



• diagnostic panels

Mastitis 9 + bulk:

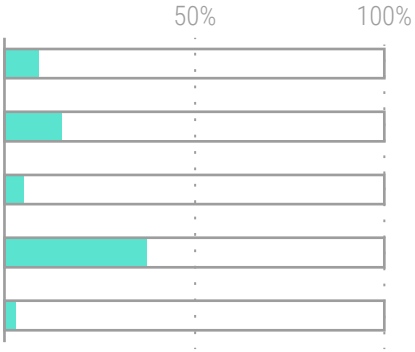
Microbiology: Bacteria isolation and identification, Antibiogram
qPCR: Mycoplasma bovis, Prototheca sp., Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis

Mastitis bulk:

qPCR: Mycoplasma bovis, Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Prototheca sp.

pathogens analyzed in mastitis panel in bulk milk

% of positives analyzed via qPCR



9% Mycoplasma bovis *(n=111)*

15% Staphylococcus aureus *(n=102)*

5% Streptococcus agalactiae *(n=95)*

37% Streptococcus uberis *(n=95)*

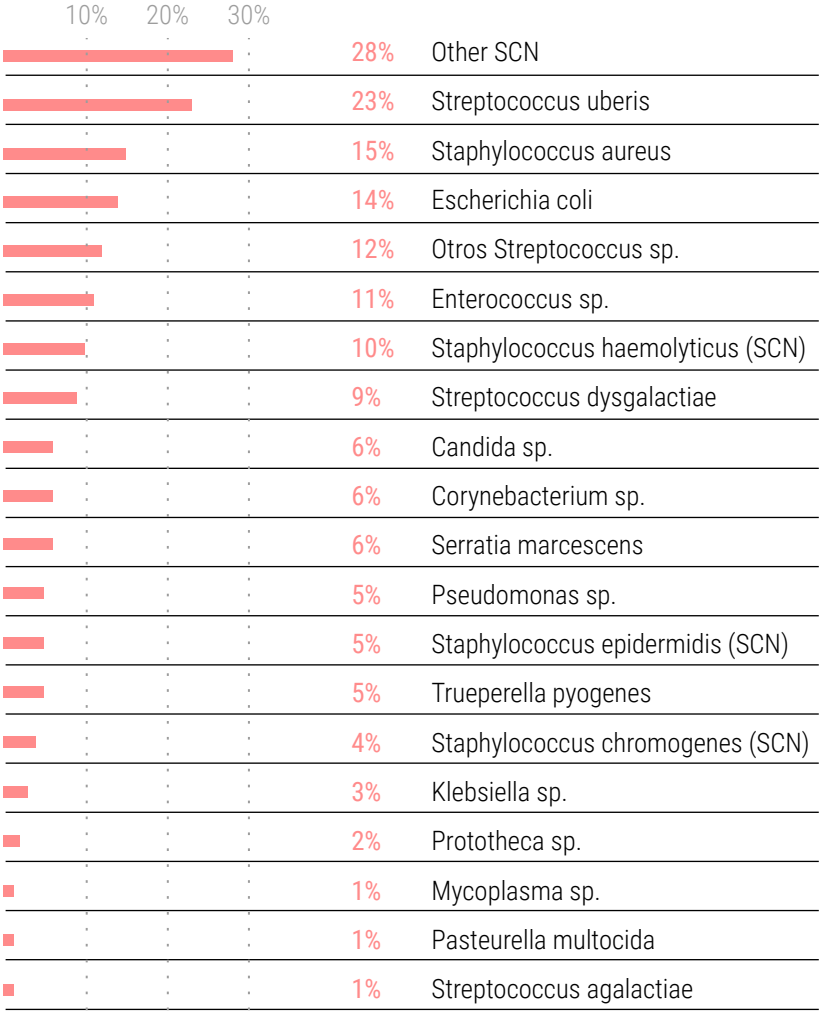
3% Prototheca sp. *(n=59)*

Streptococcus uberis is the most frequently detected pathogen, followed by Staphylococcus aureus.

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

When Mycoplasma bovis or Prototheca sp. are detected in tank samples, carrier animals should be located, since these are two contagious pathogens that do not respond well to antibiotic treatment, so the detection and elimination of carrier animals is necessary to control the infection at flock level.

pathogens isolated in microbiological culture of 189 cases of mastitis



As in bulk monitoring, mainly Streptococcus uberis and Staphylococcus aureus bacteria have been isolated in microbiological cultures of individual milks. In addition, various species of Coagulase-negative staphylococci (CoNS) have also been isolated.

As a general rule, a decrease in clinical cases related to contagious agents such as Streptococcus agalactiae has been observed. This is due to the improvements in management and hygiene carried out in the livestock sector.

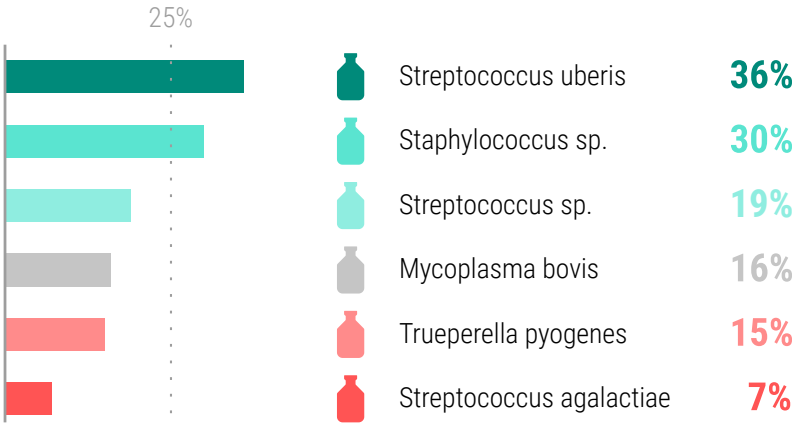
The isolation of Mycoplasma and Prototheca requires specific conditions and a specific culture. Also, the culture of pure strains is more difficult. For this reason, combining both techniques (qPCR and microbiological cultures) is important in the diagnosis of both pathogens to increase diagnostic sensitivity.

• statistical results: autovaccines

Most autovaccines contain several strains, often of different bacterial species, depending on what has the diagnoses have shown. Therefore, there is a great diversity of different combinations. The graph represents the percentage of vaccines that contain the different agents.

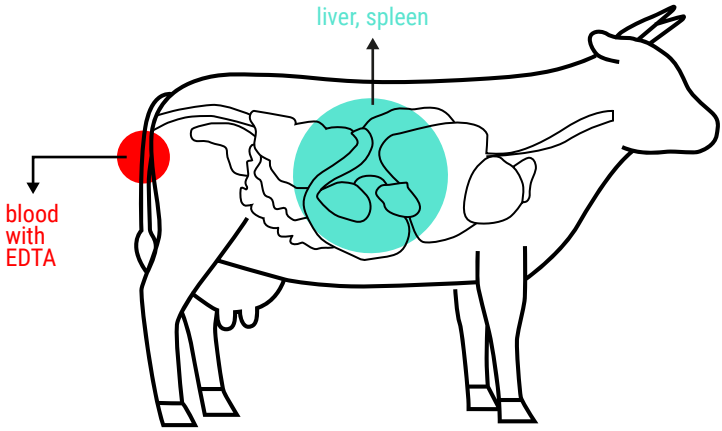
autovaccines produced for mastitis processes:

% of autovaccines including the different agents



• hemoparasites

• sampling



• diagnostic panels

Hemoparasites:

qPCR: Piroplasmas, Babesia bigemina, Babesia bovis, Theileria annulata, Anaplasma sp., Anaplasma marginale, Mycoplasma wenyonii

it is important to differentiate the hemoparasites involved, since some species are more pathogenic than others

Piroplasms: Babesia and Theileria

⚠ Babesia bigemina*, Babesia bovis*, Theileria annulata*

Anaplasmosis

⚠ Anaplasma marginale*

*species present in the Iberian Peninsula considered more pathogenic. There are many more non-pathogenic species and/or species present in other territories.

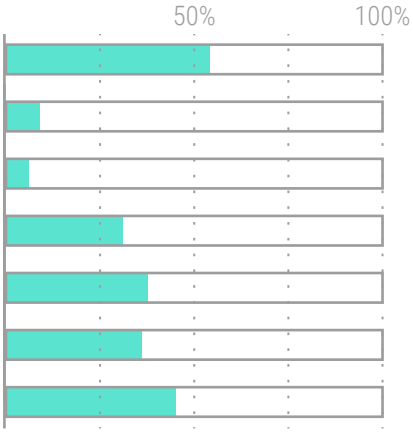
Mycoplasmas

⚠ Mycoplasma wenyonii: it can be present alone or in conjunction with other parasites, which worsens the clinical case.

• statistical results: diagnosis

pathogens analyzed in hemoparasite panel

% of positives analyzed via qPCR



54% Piroplasms *(n=200)*

9% Babesia bigemina *(n=200)*

6% Babesia bovis *(n=82)*

31% Theileria annulata *(n=200)*

38% Anaplasma sp. *(n=200)*

36% Anaplasma marginale *(n=200)*

45% Mycoplasma wenyonii *(n=190)*

Piroplasms were detected in 54% of cases, although not all of them contained a pathogenic species.

Theileria annulata is the piroplasm species which was detected in the highest percentage. It presents with serious systemic symptoms (such as jaundice, anemia, cachexia, sometimes bloody diarrhea) and there are no effective treatments.

ocular processes

sampling

conjunctival swabs

diagnostic panels

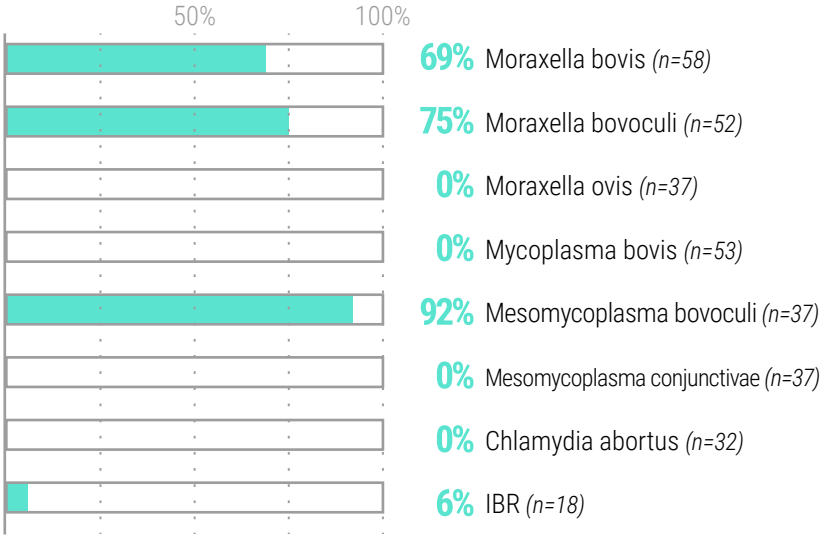
Ocular disease:

qPCR: Moraxella bovis, Moraxella bovoculi, Moraxella ovis, Mycoplasma bovis, Mesomycoplasma bovoculi, Mesomycoplasma conjunctivae, Chlamydia abortus, IBR

statistical results: diagnosis

pathogens analyzed in ocular panel

% of positives analyzed via qPCR



Moraxella bovis, Moraxella bovoculi and Mycoplasma bovoculi were detected in most of the analyzed cases.

Mycoplasma bovoculi is believed to act as an immunosuppressant and promotes the development of infectious bovine keratoconjunctivitis caused by the two Moraxella species.

The rest of the analyzed agents have been occasionally detected in ocular processes.

• statistical results: autovaccines

autovaccines produced for ocular processes

% of autovaccines containing the different agents

autovaccines against Moraxella bovis and Moraxella bovoculi

✓ effective for the control of **contagious bovine keratoconjunctivitis**, for which we do not have registered vaccines

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