

M. bovis in Belgium :

Yesterday, today and tomorrow challenges



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Agenda

- ✓ Yesterday
- ✓ General aspect & Diagnostic
- ✓ Prevalence of *Mycoplasma bovis* in Belgium
- ✓ Challenges

01

M. bovis in Belgium : Yesterday

The begining of the story in Belgium



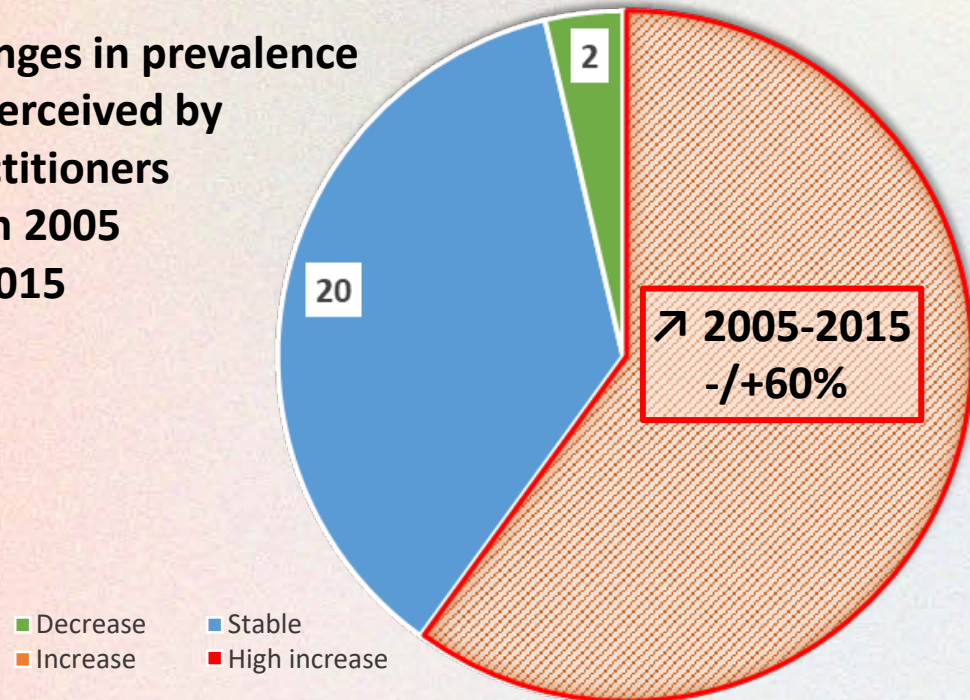
M. bovis = a new disease ?

In 2015, we had the feeling that we were being contacted more and more by vets/farmers facing severe problems with Mycoplasma bovis

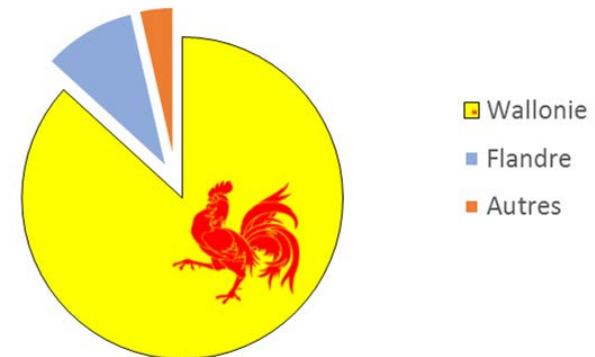
→ **Online questionnaire to practitioners**



Changes in prevalence as perceived by practitioners from 2005 to 2015



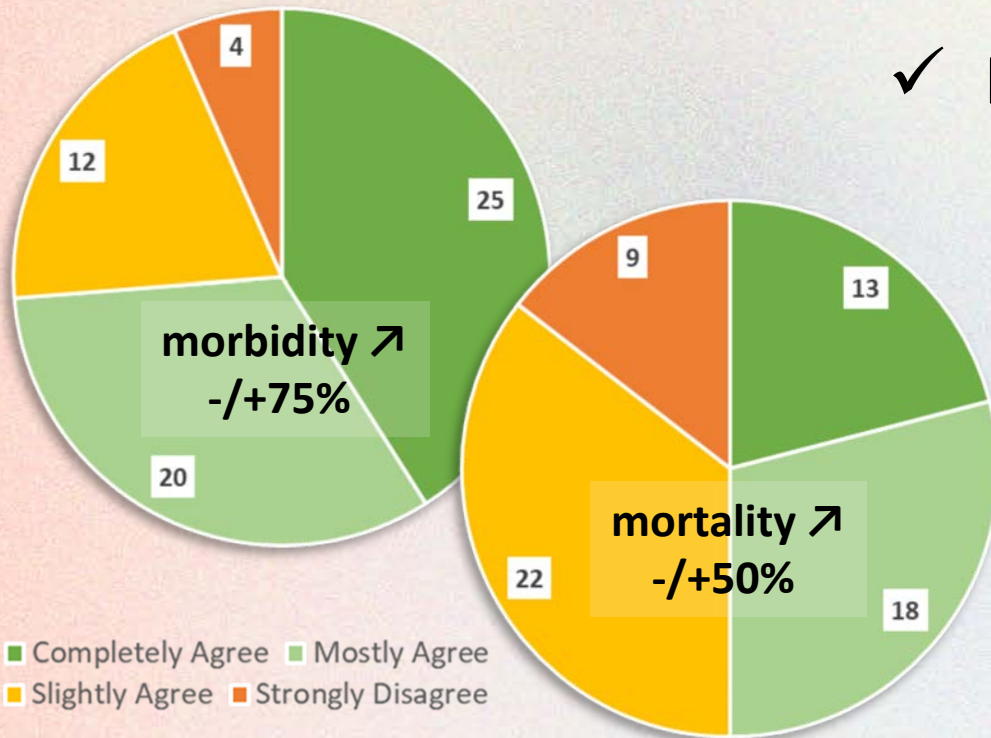
Distribution des origines des vétérinaires participants à l'enquête



M. bovis = a new disease ?

In 2015, we had the feeling that we were being contacted more and more by vets/farmers facing severe problems with Mycoplasma bovis

→ Online questionnaire to practitioners



✓ More severe (morbidity ↗ ↗, mortality ↗)

✓ More difficult to treat

✓ Disease not well known

M. bovis = a new disease ?

... retrospective serological study

Work package A

1. Historical collection of **tankmilk**
2. Selection of **250 ad random herds** for which a tankmilk was found each year **between 2012 and 2016**

| | % troupeaux | Nombre TRP sélectionnés |
|------------|-------------|-------------------------|
| BW | 5 | 13 |
| Hainaut | 29 | 72 |
| Liège | 26 | 65 |
| Luxembourg | 22 | 55 |
| Namur | 18 | 45 |
| Total | | 250 |

→ 1250 samples

3. Bio-X Mycoplasma bovis **ELISA kit**. (BIO K 162, Belgium)
Estimated detection threshold: 15% seropositive individuals



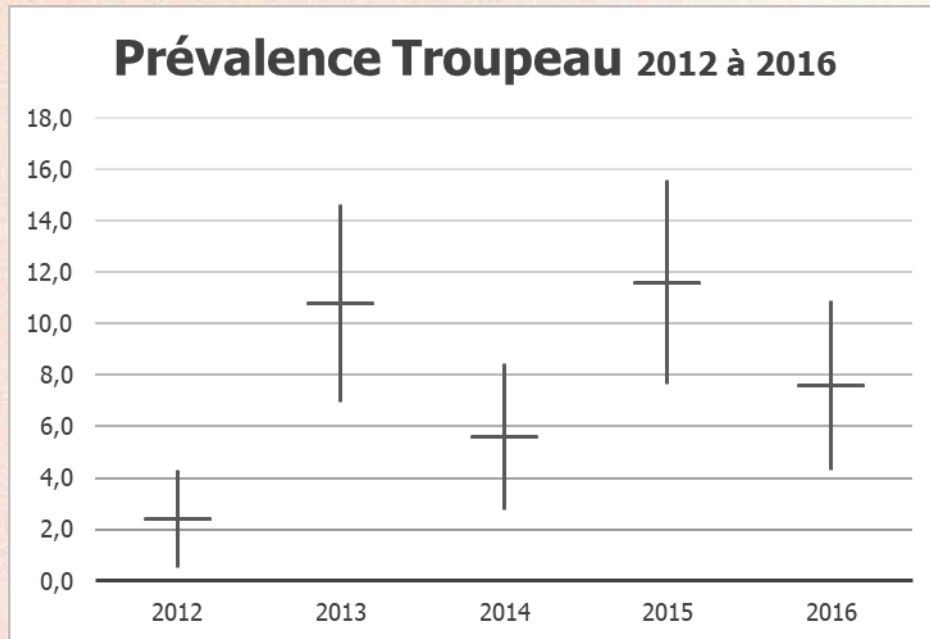
Work package B

1. Historic collection of **serums** (WSA 2010 and 2016)
2. Selection of **150 herds/year** (2010 ≠ 2016)
3. **max 10 animals/herds** (12-24 months)
4. Bio-X M. bovis **ELISA kit**. (BIO K 162, Belgium)



M. bovis = a new disease ?

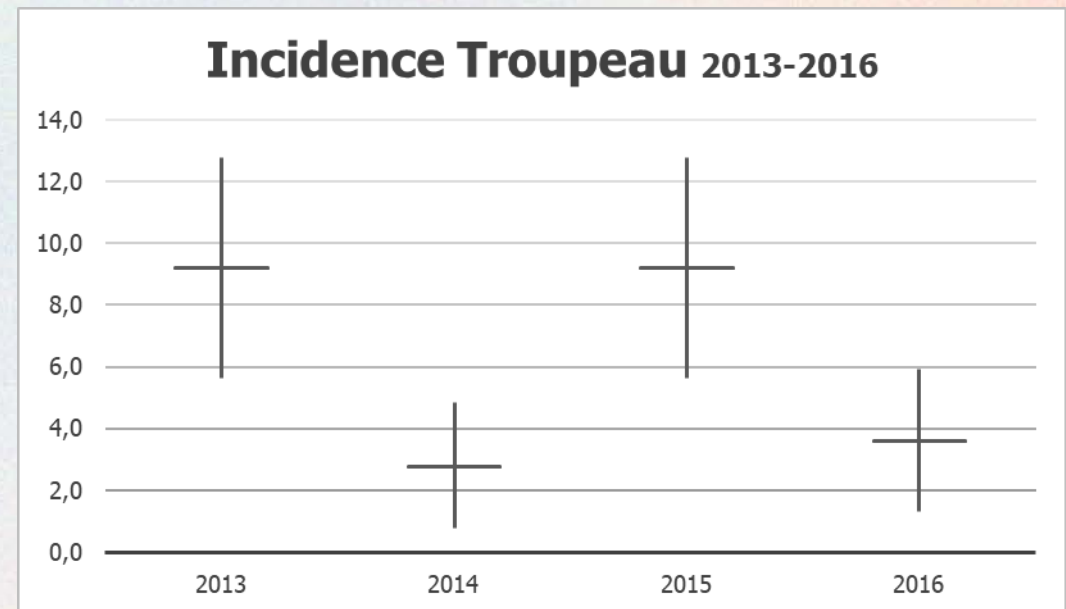
... retrospective serological study



Apparent herd prevalence in Wallonia in the 5 years : 23,6%

Work package A

1. Prevalence varies greatly from year to year (2,4 à 11,6%)
2. Not clear progressive increase of seroprevalence over time.



M. bovis = a new disease ?

... retrospective serological study

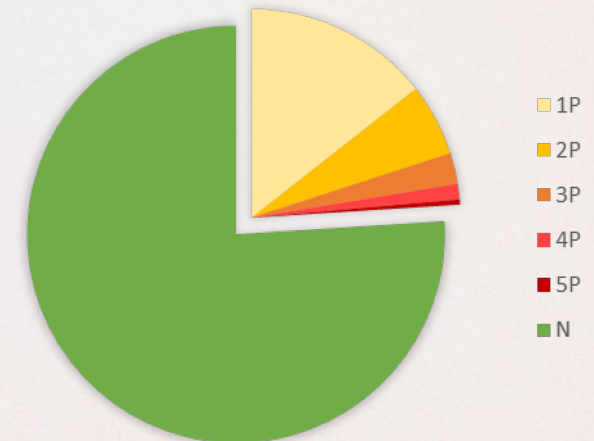
Work package A

Distribution of the profiles of *M. bovis* in the followed dairy herds over time

| | 2012 | 2013 | 2014 | 2015 | 2016 | Total | Total/catégorie |
|-----|------|------|------|------|------|-------|-----------------|
| Neg | N | N | N | N | N | 190 | 190 |
| 1P | P | N | N | N | N | 2 | 36 |
| | N | P | N | N | N | 13 | |
| | N | N | P | N | N | 3 | |
| | N | N | N | P | N | 13 | |
| | N | N | N | N | P | 5 | |
| 2P | N | N | N | P | P | 5 | 14 |
| | N | N | P | P | N | 3 | |
| | N | N | P | N | P | 1 | |
| | N | P | N | N | P | 1 | |
| | N | P | N | P | N | 2 | |
| | N | P | P | N | N | 2 | |
| 3P | P | P | N | N | P | 1 | 6 |
| | P | P | P | N | N | 1 | |
| | N | P | N | P | P | 2 | |
| | N | P | P | N | P | 1 | |
| | N | P | P | P | N | 1 | |
| 4P | P | P | N | P | P | 1 | 3 |
| | N | P | P | P | P | 2 | |
| 5P | P | P | P | P | P | 1 | 1 |
| | | | | | | Total | 250 |

Apparent herd prevalence for the 5 years : **23,6%**

RÉPARTITION DES RÉSULTATS OBTENUS SUR LAITS DE TANK 2012 À 2016



Apparent herd prevalence des positifs **récurrents** sur les 5 années: **9,6%**

M. bovis = a new disease ?

... retrospective serological study

Work package B

Prevalence of *Mycoplasma bovis* based on winterscreening 2010 and 2016

- Historical collection of serums (WSA 2010 et 2016)
- 150 herds from 2010 and 150 herds ≠ from 2016
- 10 animals/herds (12-24 months)

| HERD level | POS | NEG |
|-------------|-------------|--------------|
| 2010 | 28,67% (43) | 71,33% (107) |
| 2016 | 36,67% (55) | 63,33% (95) |

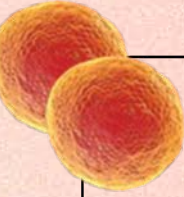
- No significant difference ($P = 0,176$ $\chi^2 = 1,834$)
- The mean of the within-herd prevalence : 17,8%

| Individual level | POS | NEG |
|------------------|------------|---------------|
| 2010 | 5,42% (62) | 94,58% (1082) |
| 2016 | 6,79% (97) | 93,21% (1332) |

- no significant difference ($P = 0,177$ $\chi^2 = 1,823$)

Prevalence is relatively close to the prevalence obtained in the 5-year follow-up of the tankmilk

M. bovis = a new disease ?



Conclusions...

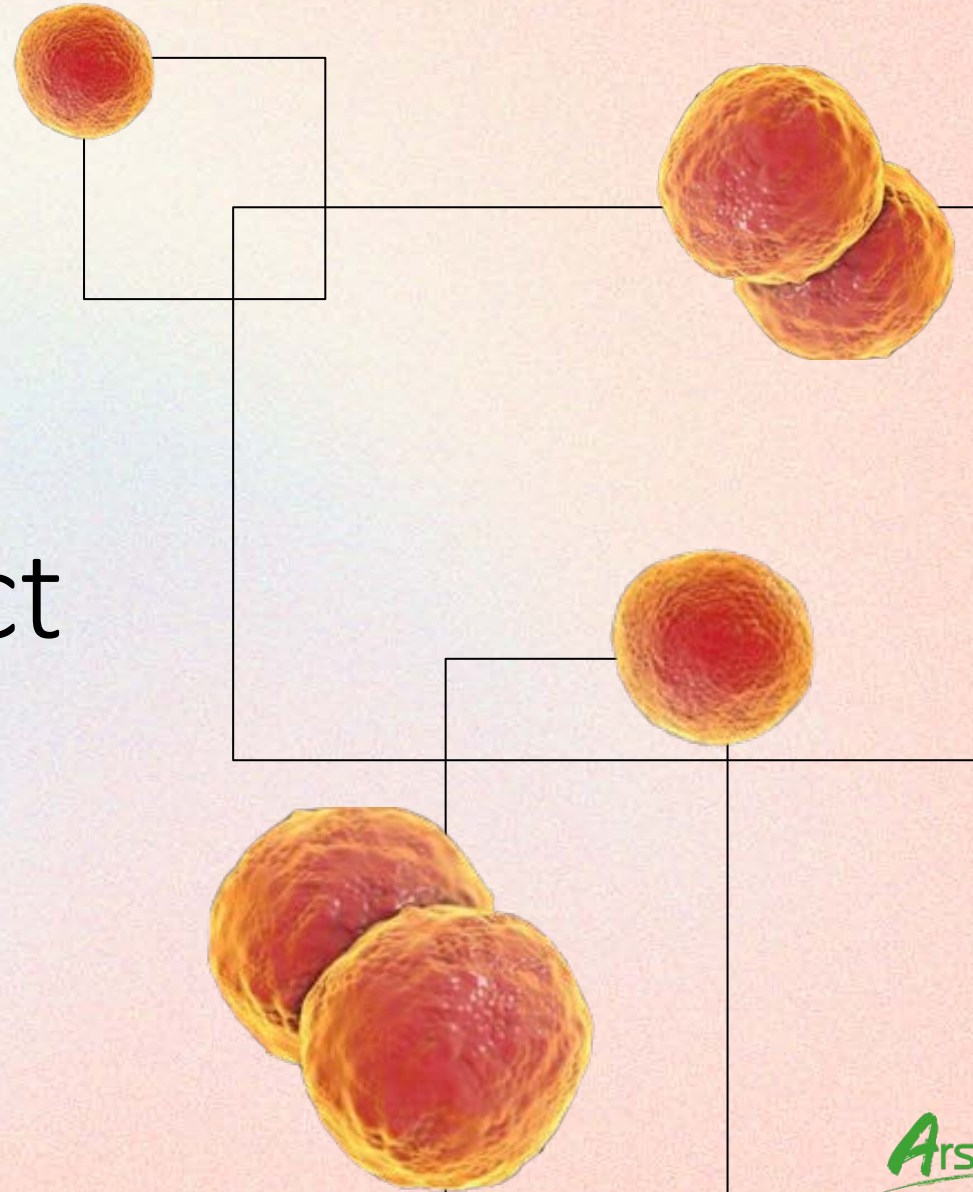
- *Mycoplasma bovis* is present in at **least 1 third** of Walloon herds.
- There are considerable **annual fluctuations**. Its prevalence **seems to have increased** in recent years, as confirmed by other studies.
- **Pneumonia/Otitis/Arthritis** correlated with *M.bovis* status ≠ Mastitis/seroma*
- Herd size, Meat and Mixt > Milk, Purchase, Purchase protocole non-use, Density, Bad ventilation are risk factors *

* <http://www.arsia-asbl.be/wp-content/uploads/documents-telechargeables/RA-2017-light-Quality.pdf>

02

M. bovis : General aspect

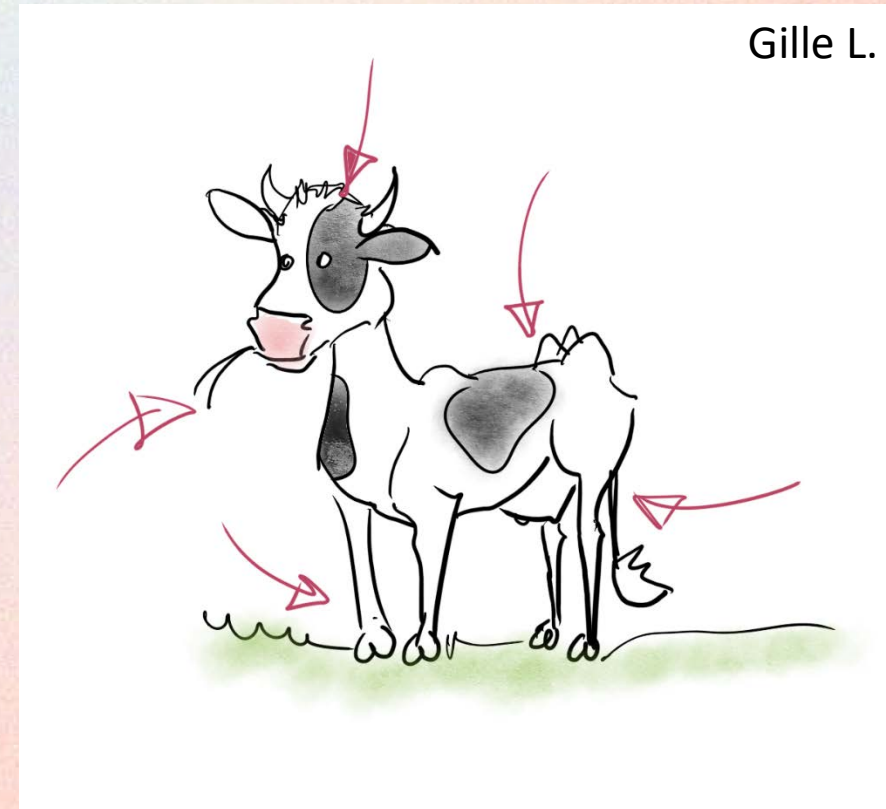
Bacteria, Disease & Diagnostic in Belgium



Mycoplasmataceae in Belgium

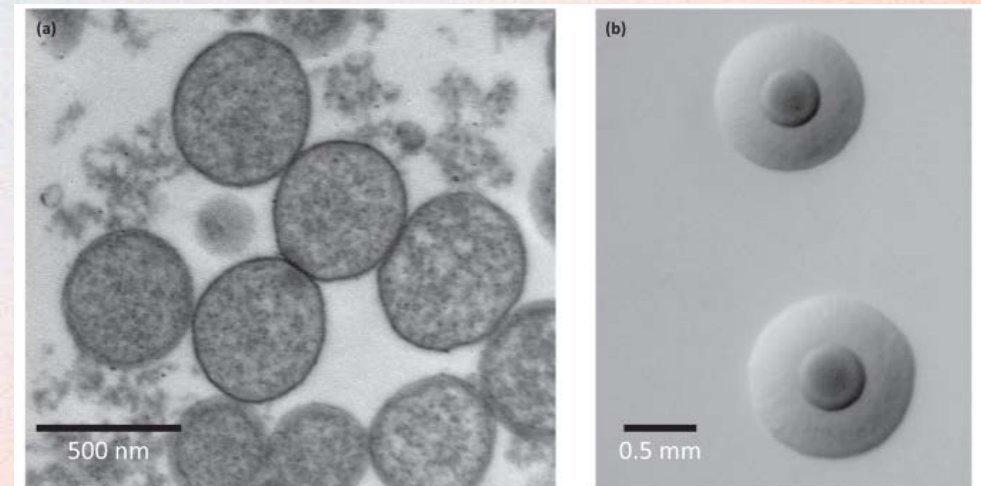
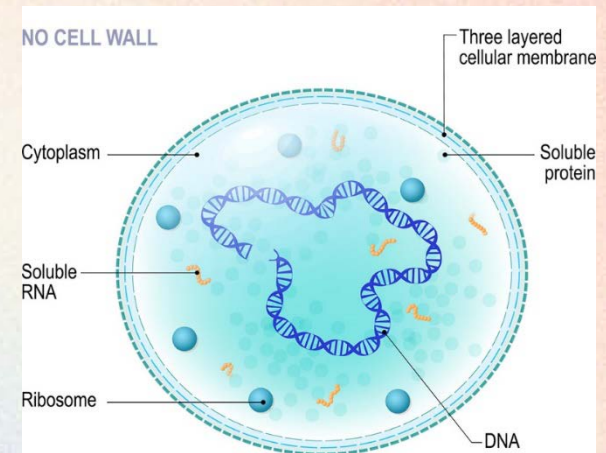
- *Mycoplasma bovis*
- *Mycoplasma wenyonii* → 2015
- *M. bovirhinis* → Commensal, sometimes pneumonia
- *M. alkalescens*
- *M. arginini*
- *M. canis*
- *M. dispar*
- *U. diversum*

Presents, relevance ?



Mycoplasma bovis

- Cell-wall-less bacterium
 - » Natural resistance to antimicrobials that target the cell wall (β -lactams, sulfonamides)
- Virulence factors
 - Variable Surface Proteins « VSP »
 - Biofilm
 - Immuno-modulation



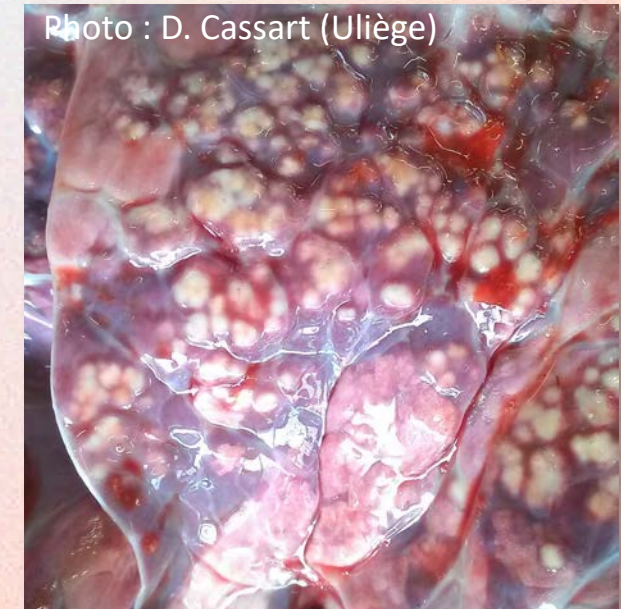
Symptômes



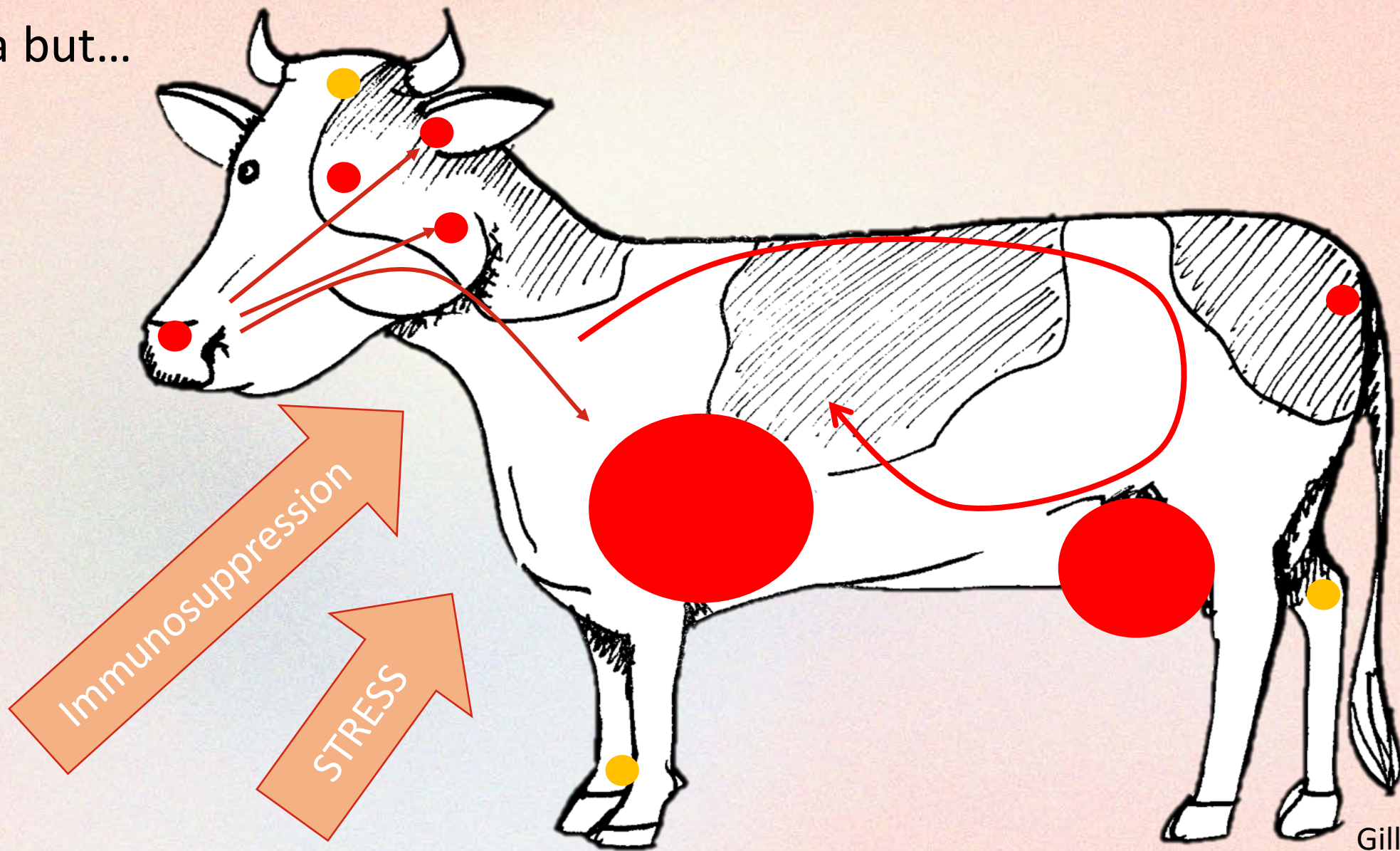
Gille L.

Pneumonia

- **Acute**
 - Mostly mild symptoms
 - Immunosuppression !
- **Chronic** : + others germs
 - Persistence
 - Weight loss
 - Pulmonary abscesses
- **M.bovis** in lungs
 - ➔ Significantly associated with pneumonia



Pneumonia but...
not only



Gille L.

Symptoms



Cows

Mastitis

Arthritis

Pneumonia

Keratoconjunctivitis

Genital infection

Aborption ?

Seroma



Calves

Pneumonia

Otitis

Arthritis

Keratoconjunctivitis

Meningitis

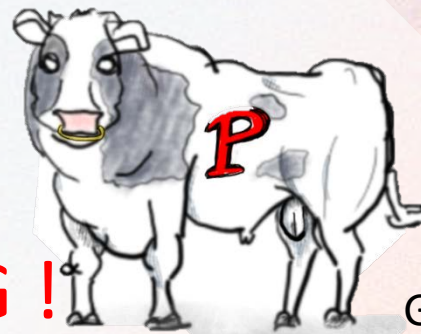
Polyserositis

Myocarditis

Abscess



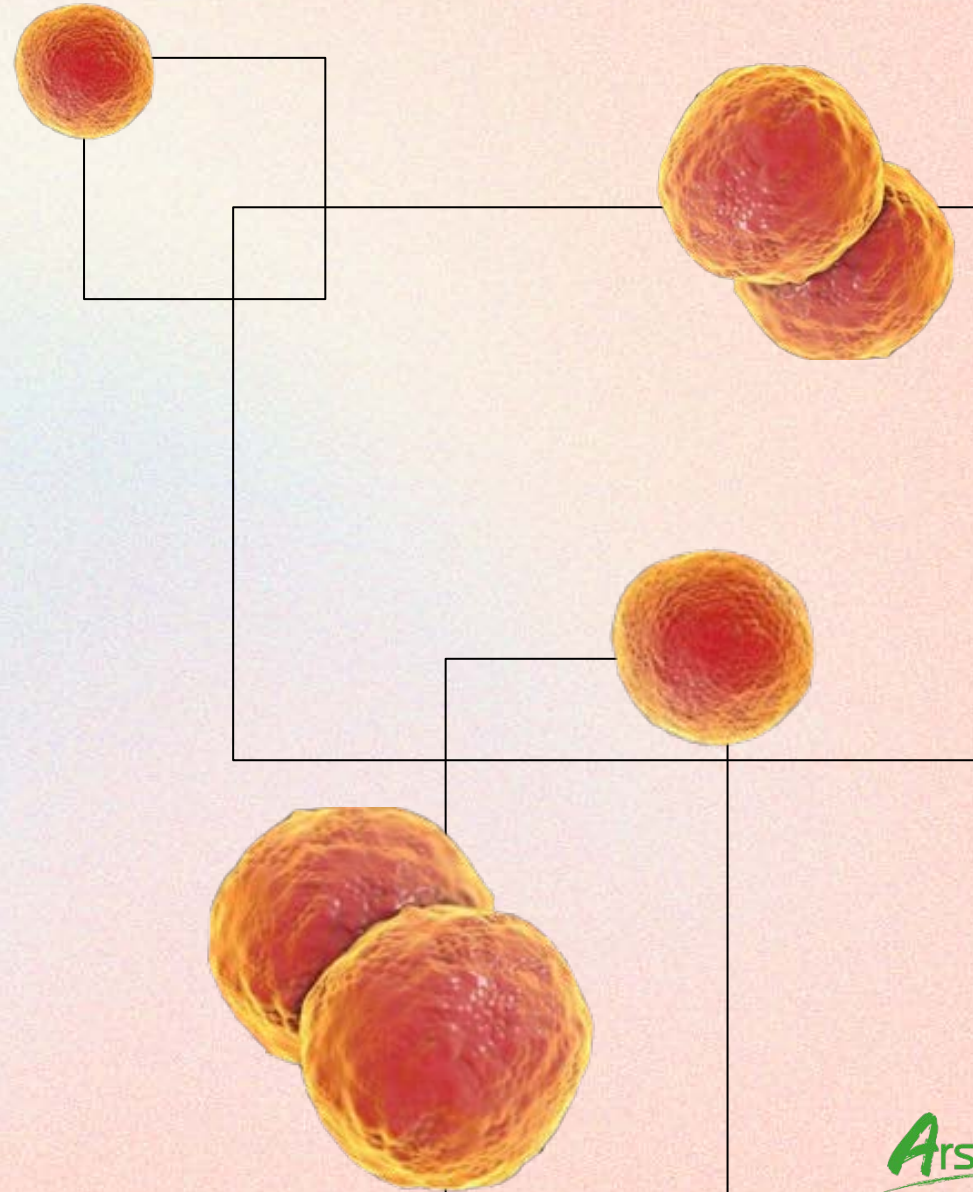
Or
NOTHING !



Gille L.

02

Diagnostic



Tests

| | ELISA | PCR | Culture |
|--------------------------|---|---|---|
| Type | Antibodies | DNA/RNA | Living bacteria |
| Samples | Blood, milk | Every samples (blood less appropriated) | Every samples (blood less appropriated) |
| Duration | Hours | Hours | 2 days - 1 week |
| Moment of contact | Recently (2 weeks - 6 months) | Currently | Currently |
| Cost | + | +++ | + |
| Sensibility | + | +++ / + | ++ |

Elisa

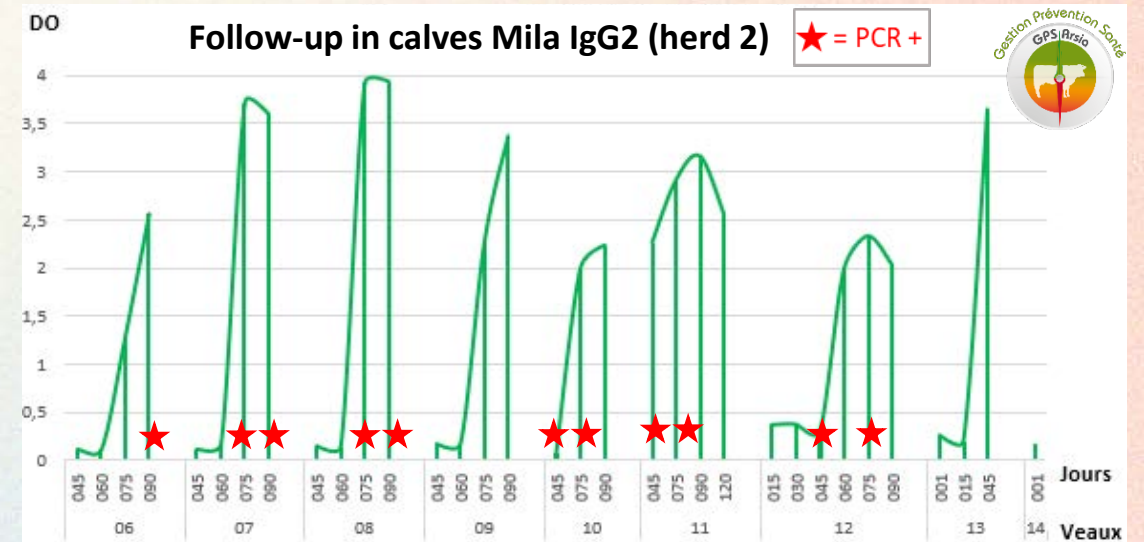
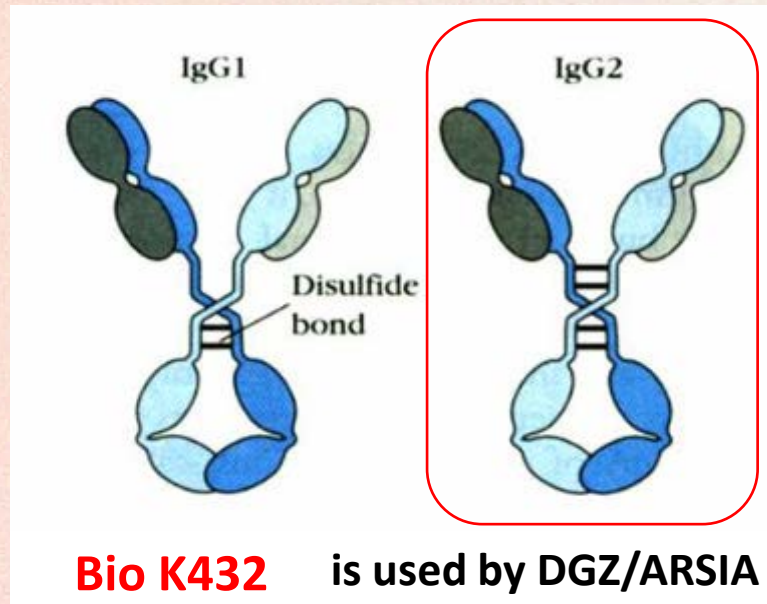
> [Vet Rec.](#) 2022 Nov;191(9):e2179. doi: 10.1002/vetr.2179. Epub 2022 Sep 6.

Comparison and optimisation of screening cutoff values for *Mycoplasma bovis* antibody ELISAs using serum from youngstock

Jade Bokma ¹, Sabrina Stuyvaert, Bart Pardon

ID-screen > **Bio K432** > Bio K302

Elisa



Early Positive PCR : day45
Seroconversion : day45 – day60

Advantages of IgG2 : No colostral interference

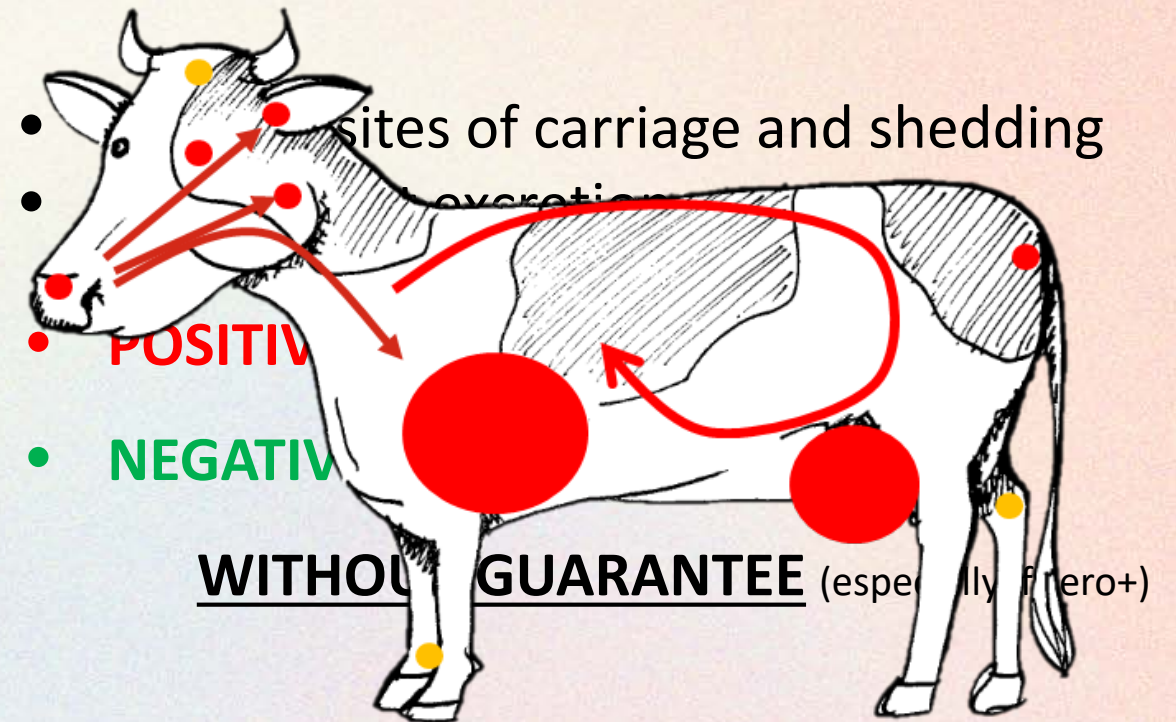
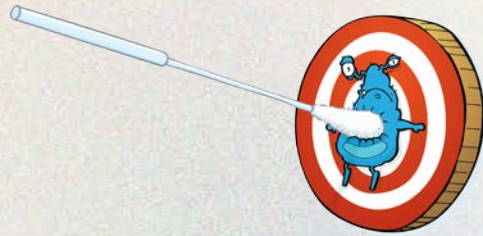
2 cut-offs (S/P):

- > 6 months : 80
- < 6 months : 30

PCR

Advantages of PCR / culture :

DIRECT search of the pathogen



In practice : Best (1) 1 swab of the 2 nostrils pooled with
(2) 1 genital swab (vagina ou sheath)



Prevalence of *Mycoplasma bovis* in Belgium and its evolution through the last years

Willem Van Praet | DGZ Vlaanderen | willem.vanpraet@dgz.be |





Studies on the prevalence of *Mycoplasma bovis*



Initial surveys/studies

Between-herd prevalence of *Mycoplasma bovis* in bulk milk in Flanders, Belgium (Passchyn et al., 2012)

- › Sampling of 3 BTM samples over period of 4 weeks on 200 Flemish dairy herds (2009)
- › Bacteriological analysis of BTM samples
- › *Mycoplasma bovis* detected in 1,5% of sampled herds

Seroepidemiology of respiratory infections in white veal calves under antimicrobial coverage and associations with respiratory disease and carcass traits (Pardon et al., 2012)

- › Serological status of white veal calves tested at arrival on commercial veal herd (2007-2009)
- › Antibody ELISA
- › 10,7% of calves had antibodies against *Mycoplasma bovis*



Veepeiler project '*Mycoplasma bovis* in Vlaanderen'

Use of a breeding bull and absence of a calving pen as risk factors for the presence of *Mycoplasma bovis* in dairy herds (Gille et al., 2016)

- › Cross-sectional study on 100 dairy farms with collection of BTM samples (2016)
- › Real-time PCR and antibody ELISA
- › Apparent prevalence on BTM 7% and 17% for PCR and antibody ELISA, respectively

No overlap between PCR and ELISA results => 24% of herds with positive results

Part 2 of the project focused on beef herds

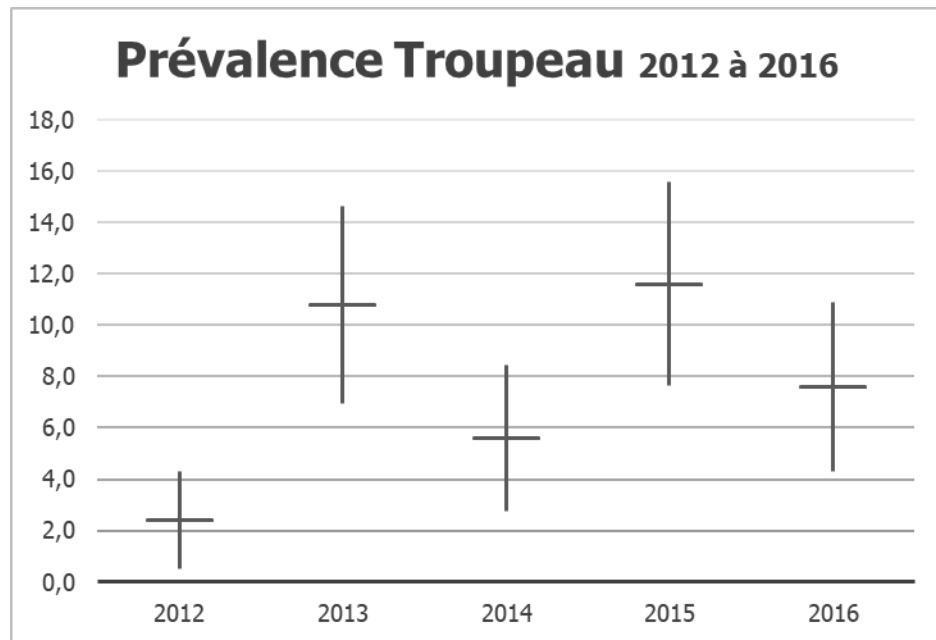
- › Collection of 5 serum samples from 100 beef herds (winterscreening 2014)
- › Antibody ELISA
- › At least one sample positive on 22% of selected herds and 7,2% of all individual samples tested were positive



GPS project on *Mycoplasma bovis*

Retrospective serological study

- › BTM (2012 – 2016) and serum samples (winterscreening 2010, 2016)
- › Antibody ELISA
- › Apparent seroprevalence on BTM over the 5 years 23,6%



Apparent seroprevalence on serum

| HERD level | POS | NEG |
|-------------|-------------|--------------|
| 2010 | 28,67% (43) | 71,33% (107) |
| 2016 | 36,67% (55) | 63,33% (95) |

| Individual level | POS | NEG |
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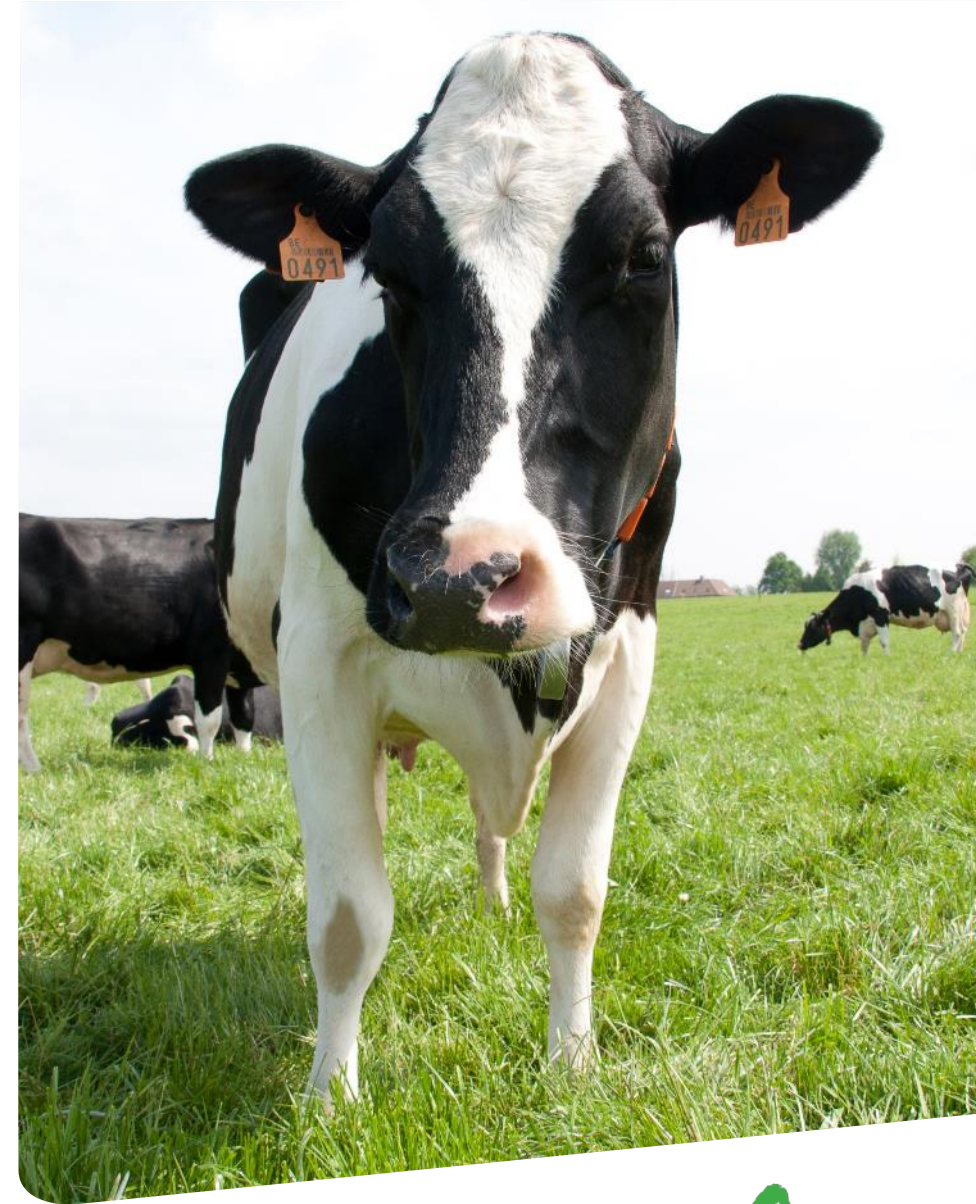
Veepeiler project 'MycoScreen'

Project started end of 2023 with the aim of developing a testing protocol to reliably determine the herd status for *Mycoplasma bovis*

First phase of the project to identify possible negative farms

- › BTM samples of 50 dairy herds collected
- › Antibody ELISA
- › Apparent seroprevalence on BTM of 62,0%

Second phase consist of extensive sampling on a limited number of farms tested negative on BTM.



PathoProof™ mastitis assay (detection of multiple pathogens)

- › In case of mastitis on farm
- › Periodic screening of BTM

| | 2022 | 2023 | 2024 |
|-------------------------|------|------|------|
| <i>Mycoplasma bovis</i> | 2,3% | 2,1% | 0,7% |
| <i>Mycoplasma spp.</i> | 5,8% | 4,5% | 6,3% |

Also PCR analyses performed on quarter milk, but data not included here

Mycoplasma bovis in the 'Griepbarometer'

The 'Griepbarometer' at DGZ

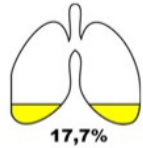
PERIODE: 4 februari tot 3 maart 2024

AANTAL GEVALLEN: 79

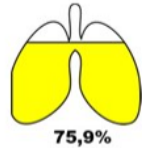
Bacteriën



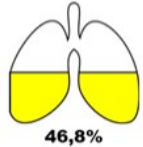
Mycoplasma bovis



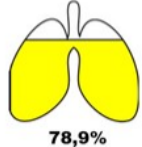
Histophilus somnus



Mannheimia haemolytica

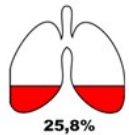


Pasteurella multocida

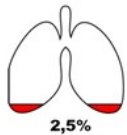


Virussen

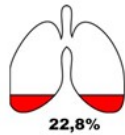
BRSV



PI3-virus



Coronavirus



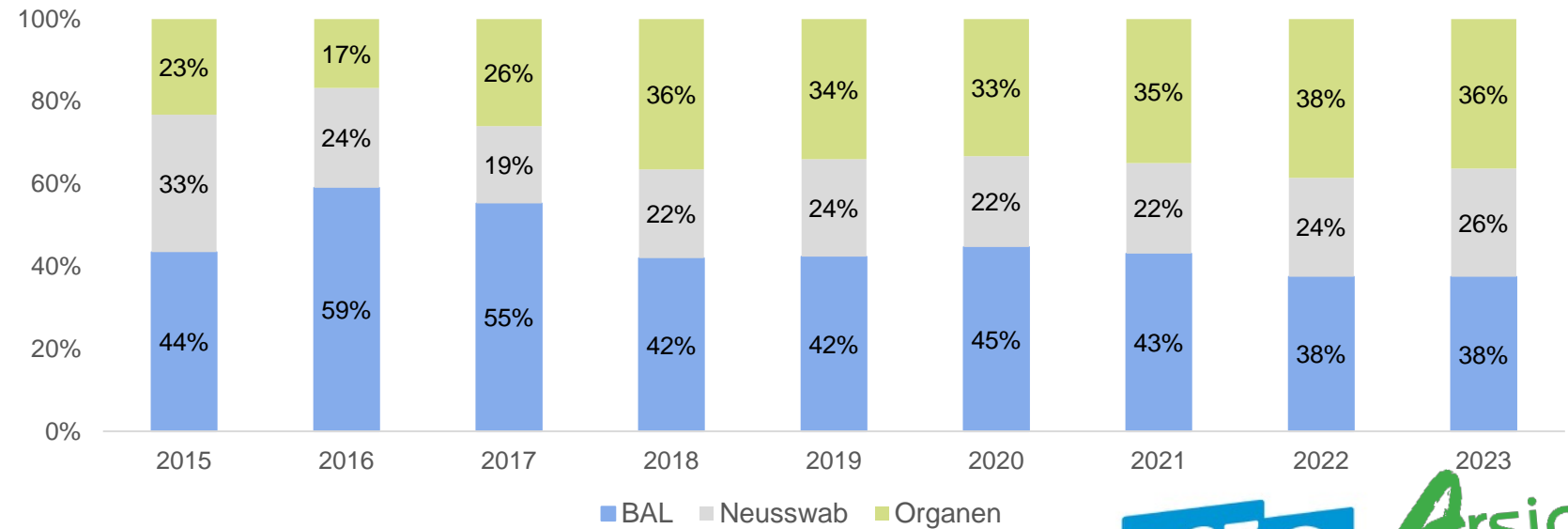
1.800 farms



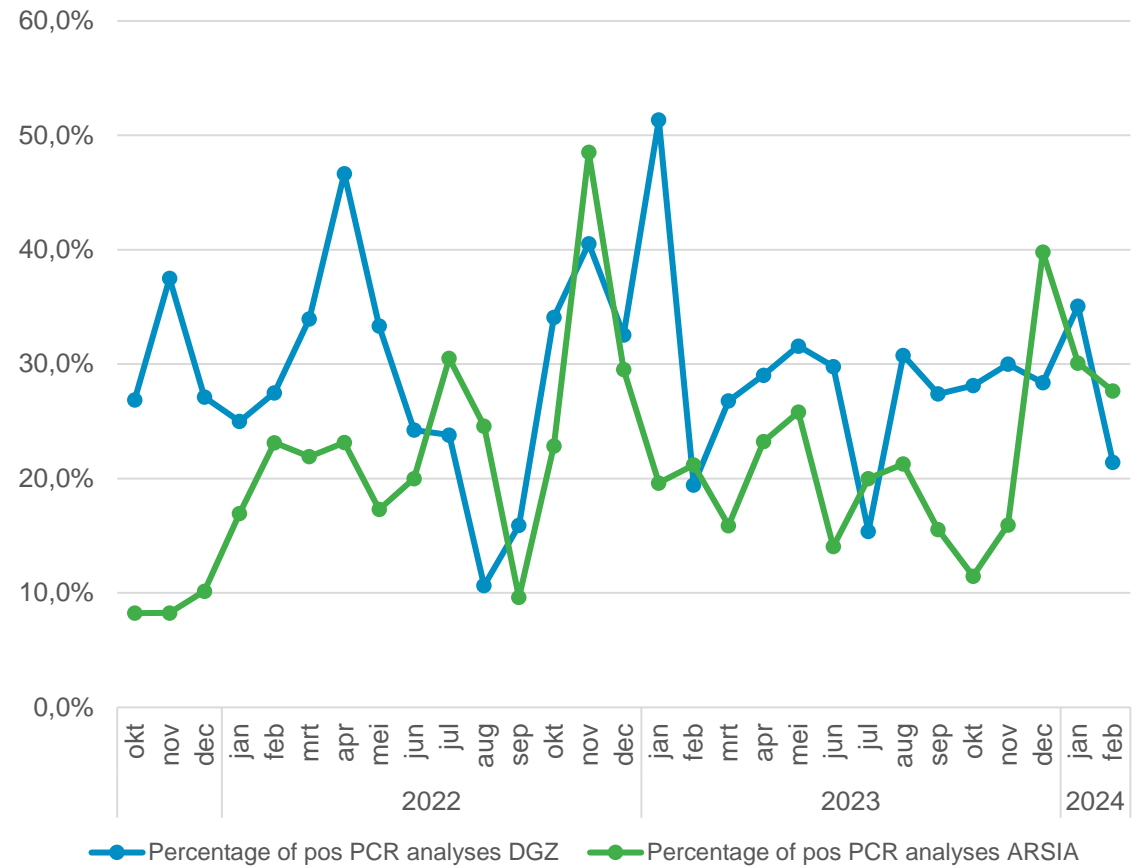
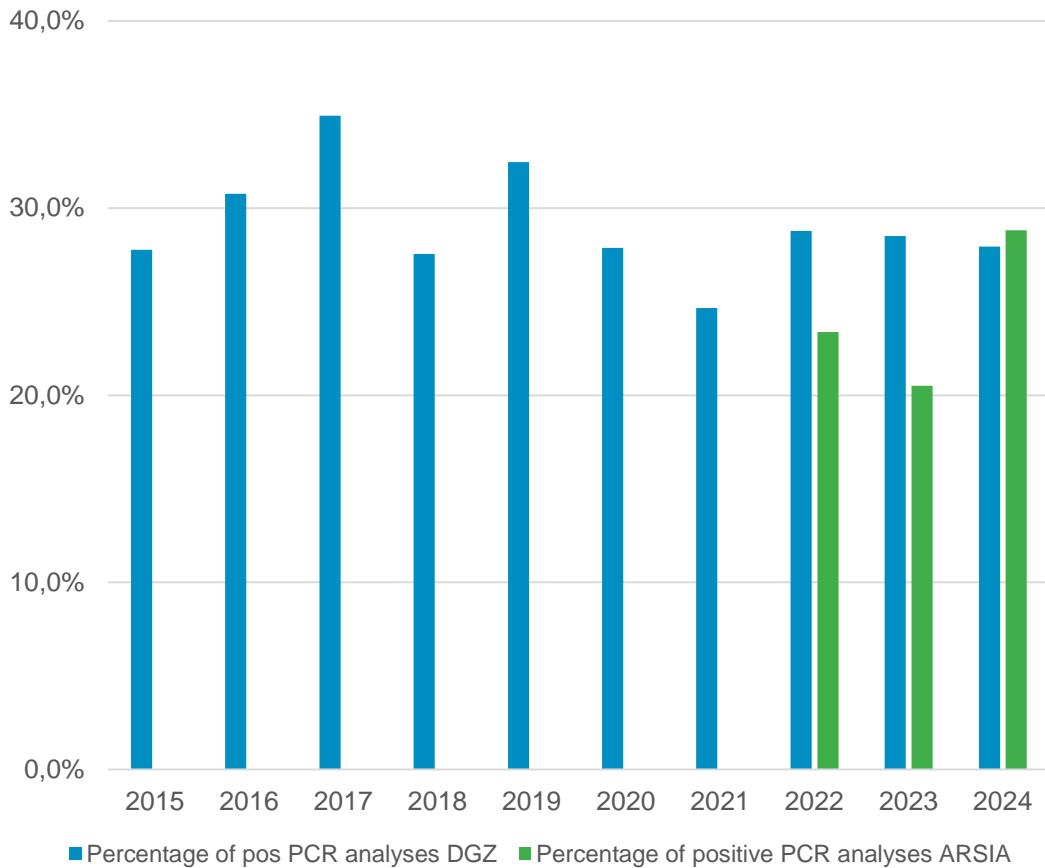
400 veterinarians



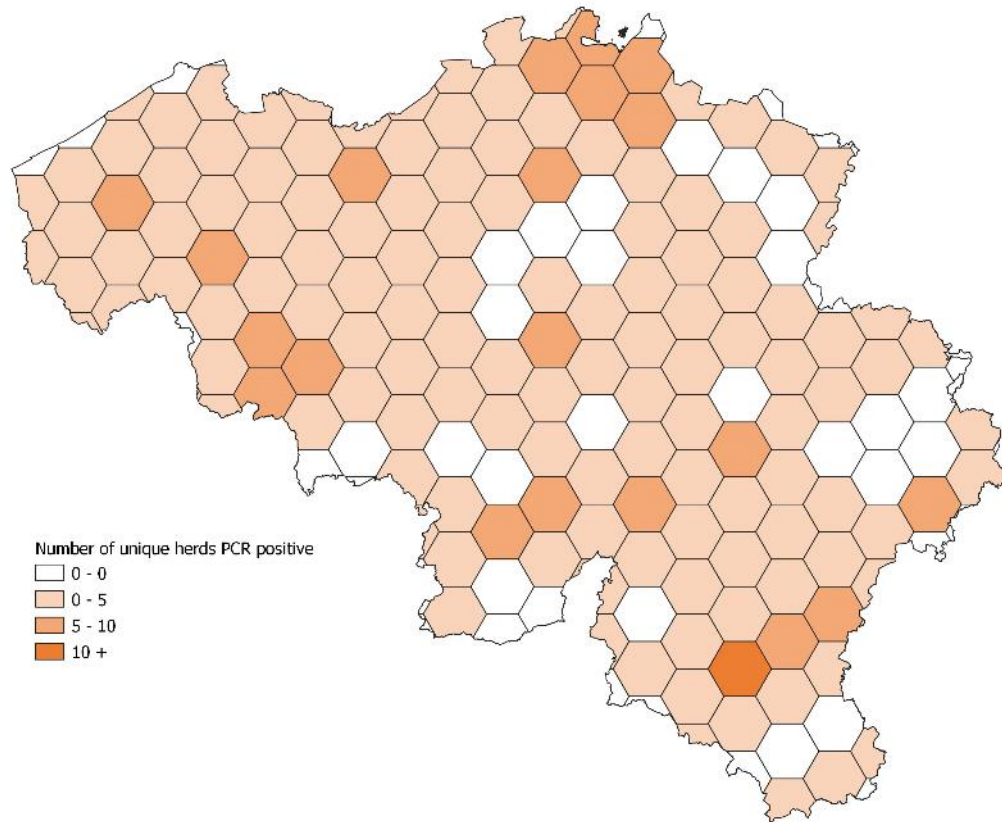
> 5.000 PCR analyses



Mycoplasma bovis detected in the 'Griepbarometer'



PCR positive herds in the 'Griepbarometer'



Results from the 'Griepbarometer' from 2022 onwards

Count of unique PCR positive herds !

Mycoplasma bovis detected across the country

Percentage of positive herds in the 'Griepbarometer'

| | |
|----------|-------|
| Belgium | 30,7% |
| Wallonia | 27,9% |
| Flanders | 34,4% |

Mycoplasma bovis PCR versus culture

| | | PCR | | Totaal |
|--------|-----|-----|-----|--------|
| | | NEG | POS | |
| BAC | NEG | 452 | 59 | 511 |
| | POS | 2 | 134 | 136 |
| Totaal | | 454 | 193 | 647 |

| | | PCR | | Totaal |
|--------|-----|-------|-------|--------|
| | | NEG | POS | |
| BAC | NEG | 69,9% | 9,1% | 79,0% |
| | POS | 0,3% | 20,7% | 21,0% |
| Totaal | | 70,2% | 29,8% | 100% |

| | | PCR | | Totaal |
|--------|-----|-------|-------|--------|
| | | NEG | POS | |
| BAC | NEG | 88,5% | 11,5% | 100% |
| | POS | 1,5% | 98,5 | 100% |
| Totaal | | 70,2% | 29,8% | 100% |

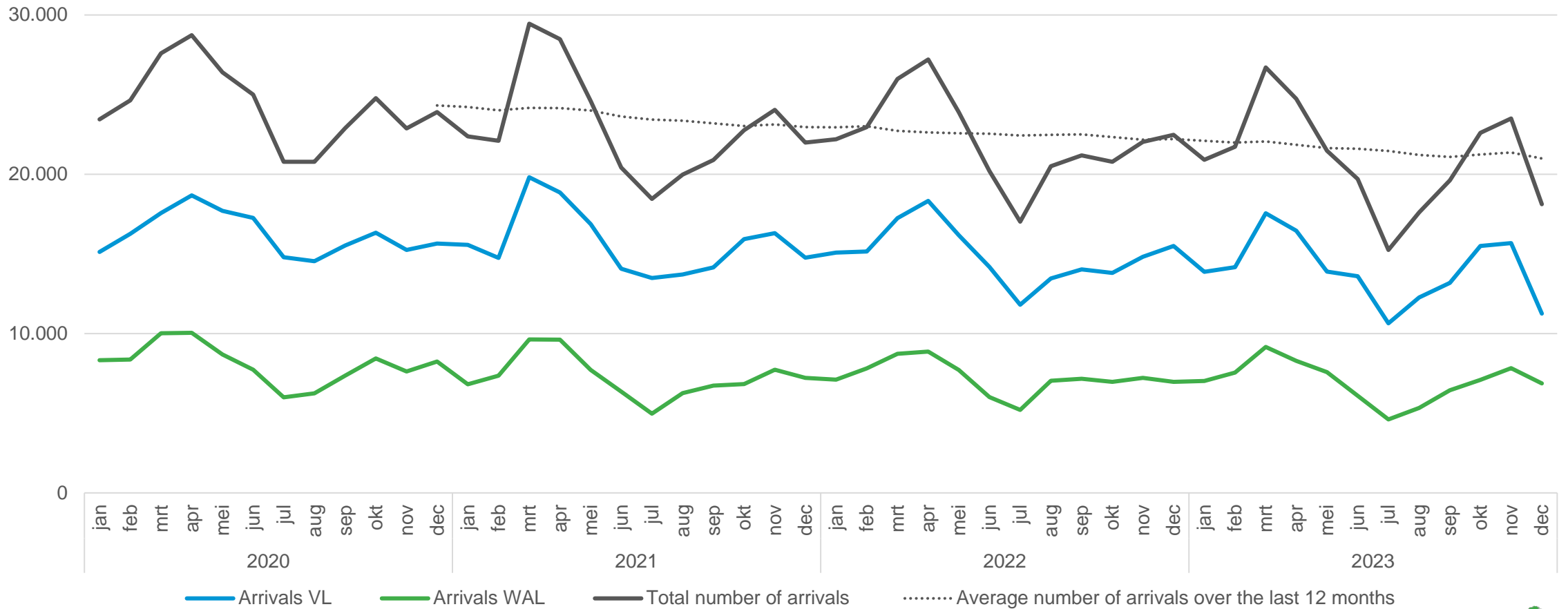
| | | PCR | | Totaal |
|--------|-----|-------|-------|--------|
| | | NEG | POS | |
| BAC | NEG | 99,6% | 30,6% | 79,0% |
| | POS | 0,4% | 69,4% | 21,0% |
| Totaal | | 100% | 100% | 100% |





Mycoplasma bovis in the purchase protocol

Number of cattle arrivals on farms in Belgium



Mycoplasma bovis in the purchase protocol

At the end of 2019 *Mycoplasma bovis* was added to the purchase protocol in Belgium

Use of Bio-X kit, at DGZ starting from October 2021, but...

Small difference in the reporting of the results in the next slides



Negative < 80 ≤ positive

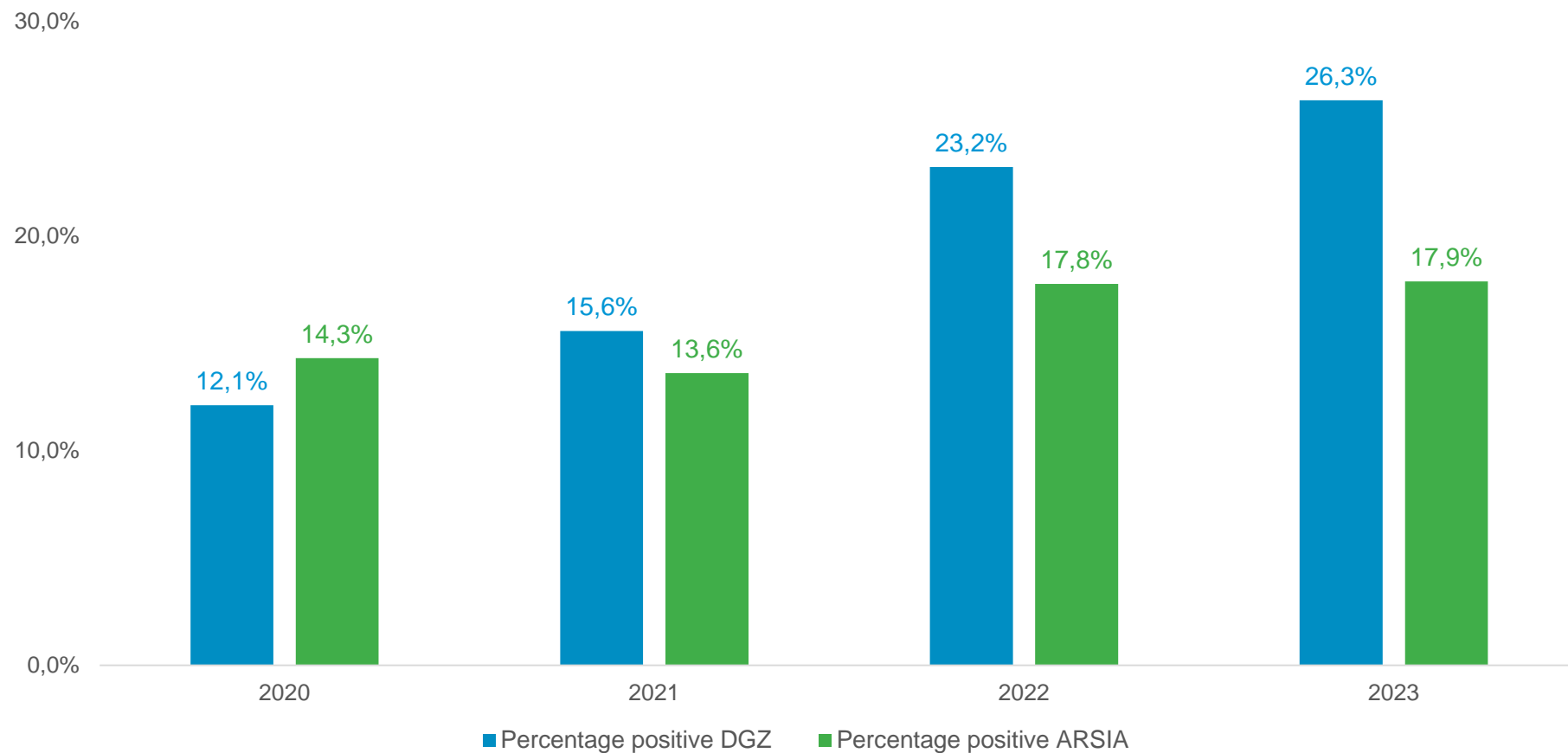


Negative < 30 ≤ positive (< 5months)
Negative < 80 ≤ positive (> 5months)

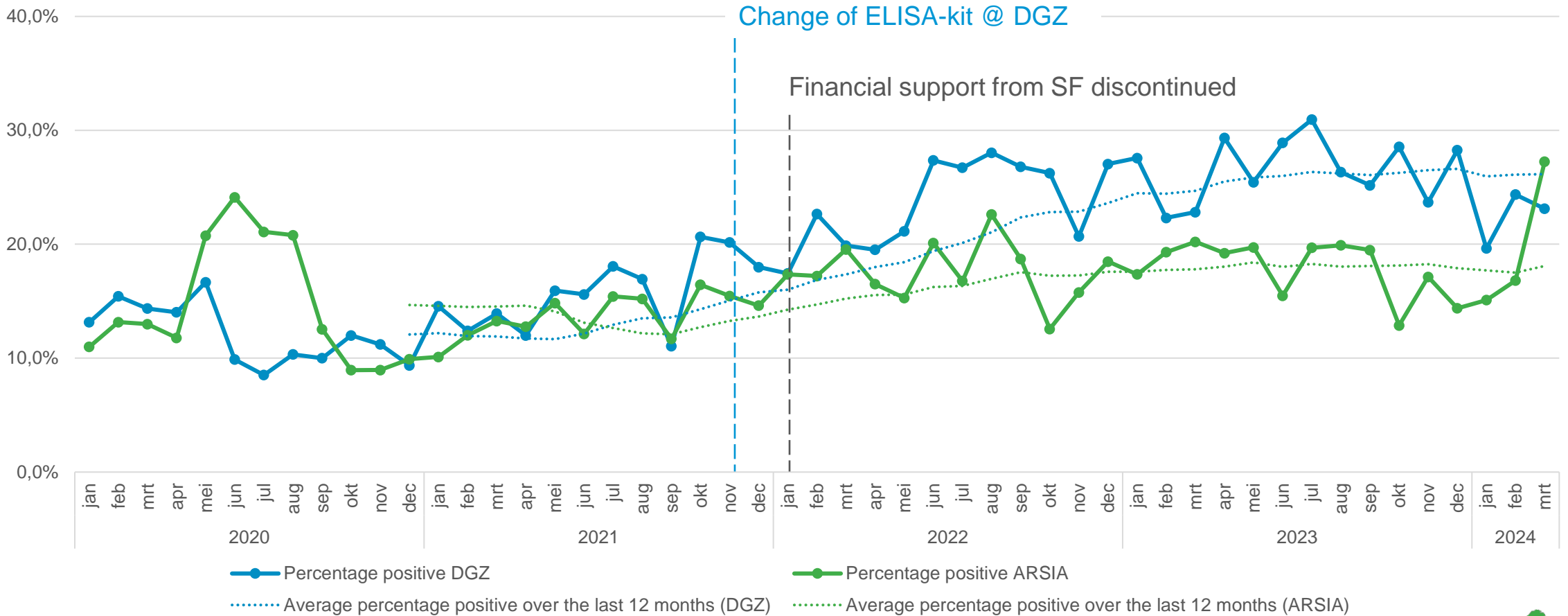




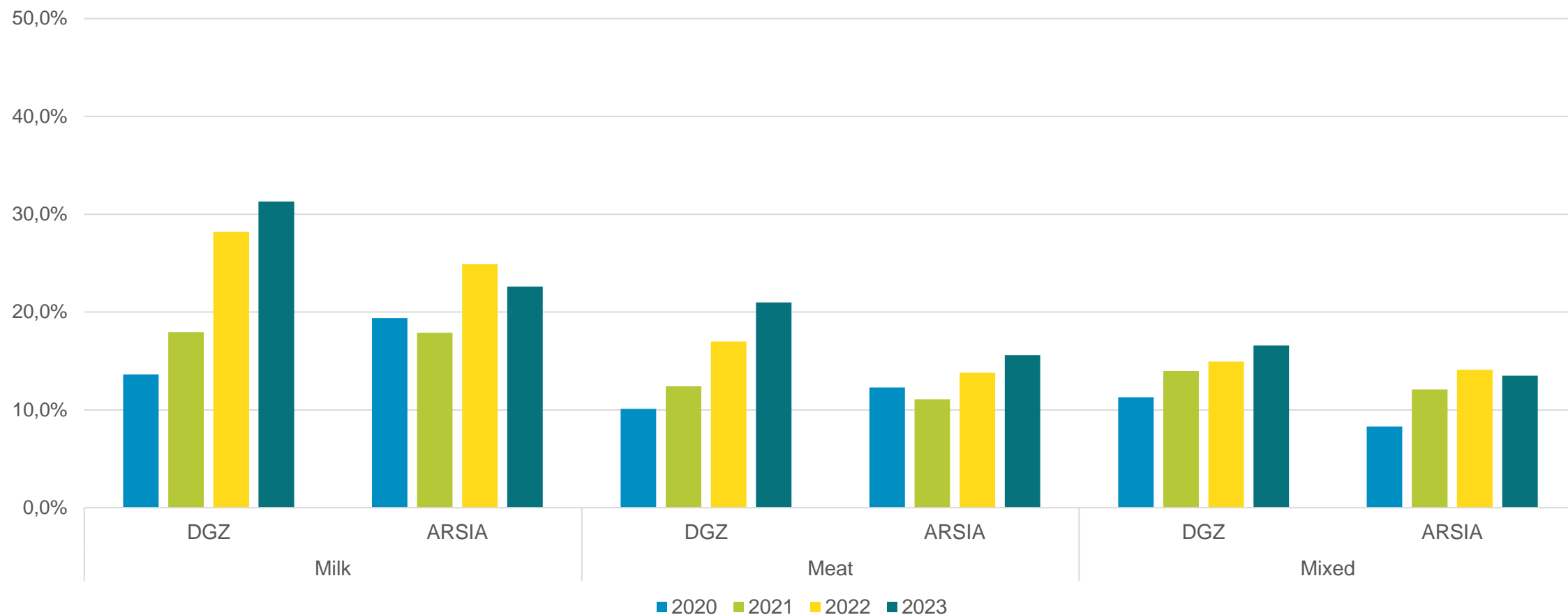
Positive results *Mycoplasma bovis* at purchase



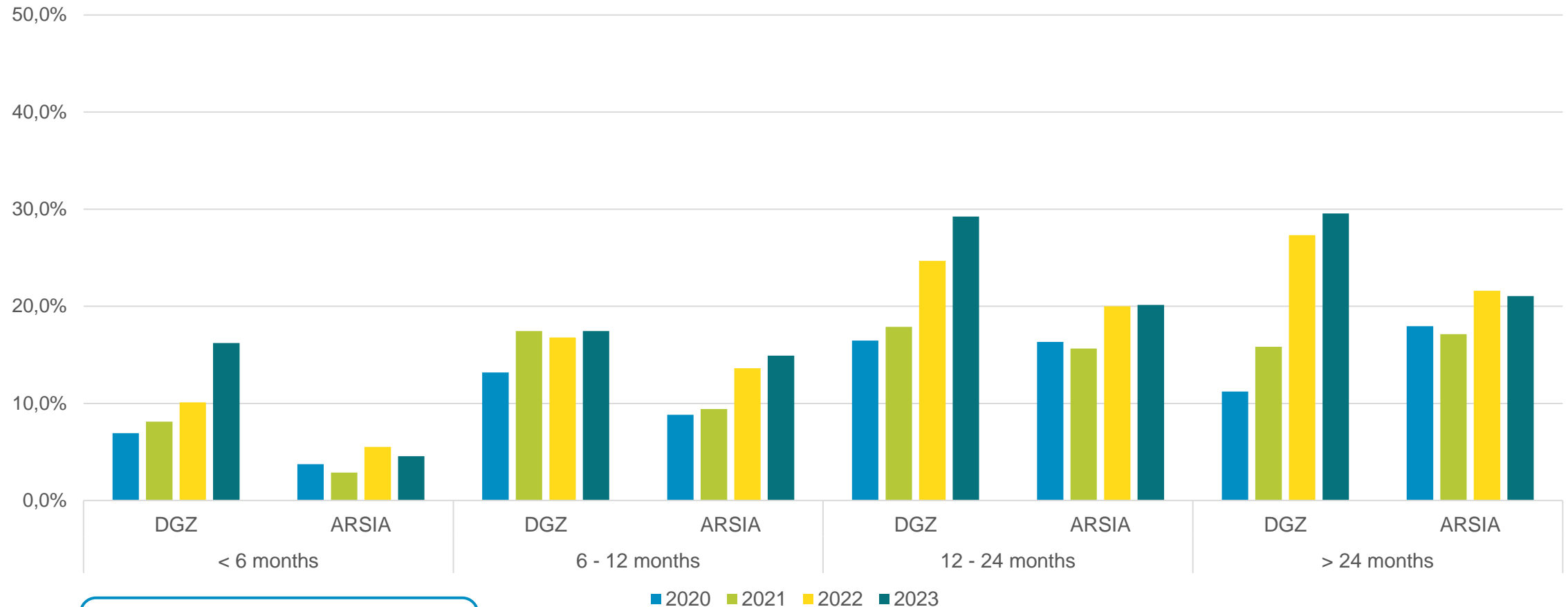
Positive results *Mycoplasma bovis* at purchase



Mycoplasma bovis antibodies at purchase by race type



Mycoplasma bovis antibodies at purchase by age group

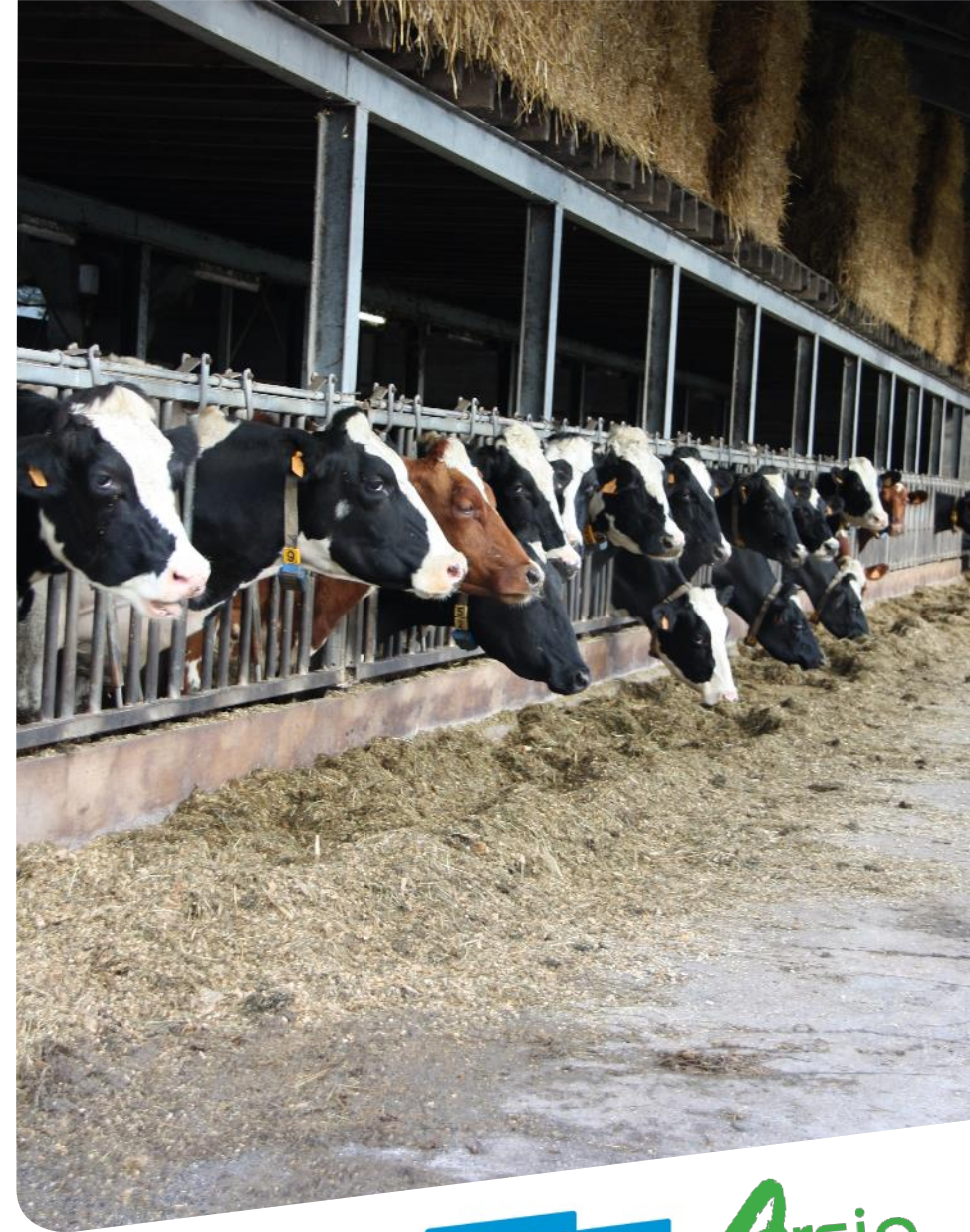


Negative < 30 ≤ positive (< 5months)
 Negative < 80 ≤ positive (> 5months)

In summary

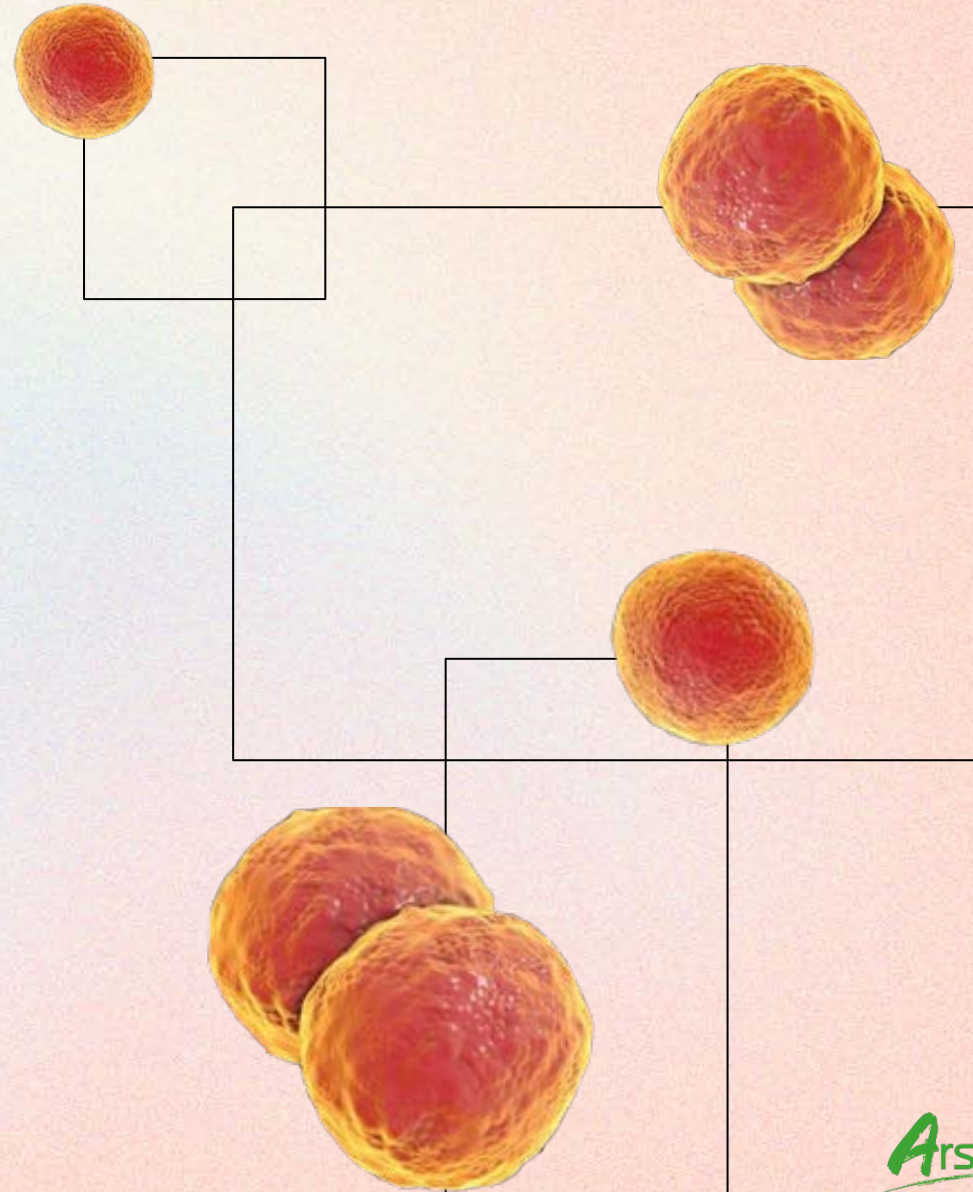
- › Evidence of increasing prevalence of *Mycoplasma bovis*
- › Between 20% and 30% of samples tested PCR positive in the 'Griepbarometer' every year and around 30% on herd level
- › Increasing presence of antibodies against *Mycoplasma bovis* in animals tested within the purchase protocol

Mycoplasma bovis is present in at least one third of all cattle herds in Belgium



03

Tomorrow's challenges



Challenges



Benefits of the auto-vaccine

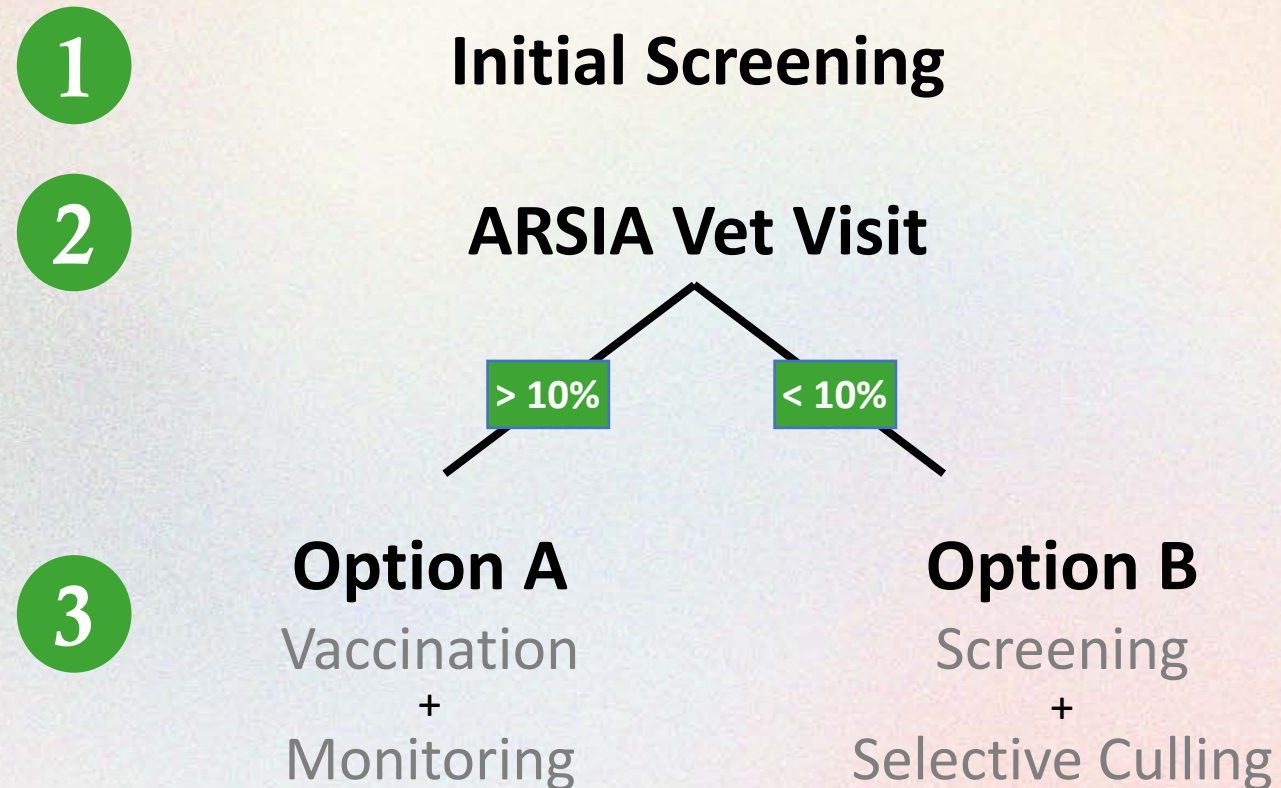
| Dec 2020 | PCR | | ELISA (IgG2) | |
|----------|----------|----------|--------------|----------|
| | Negative | Positive | Negative | Positive |
| 0-6m | 5 | 5 | 10 | 0 |
| 6-24m | 20 | 3 | 9 | 14 |
| 24m+ | 12 | 1 | 2 | 11 |
| Total | 37 | 9 | 21 | 25 |

Autovaccine
end of 2021



| Dec 2021 | PCR | | ELISA (IgG2) | |
|----------|----------|----------|--------------|----------|
| | Negative | Positive | Negative | Positive |
| 0-6m | 10 | 0 | 20 | 0 |
| 6-24m | 5 | 0 | 5 | 0 |
| 24m+ | 8 | 0 | 0 | 8 |
| Total | 23 | 0 | 25 | 8 |

Challenge : Individual Eradication Plan



Challenge : Individual Eradication Plan

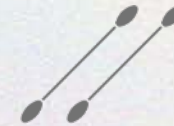
Initial Screening

Serological screening
(>6months)



1
OR

Bacteriological screening
(all)

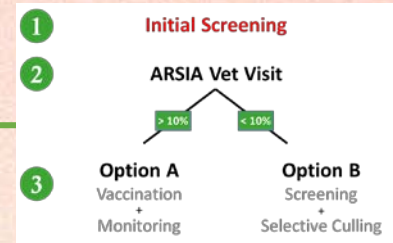


Serological screening
(9 calves < 3months)



2
AND

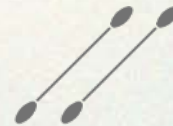
Serological screening
(9 calves 3-6 months)



Challenge : Individual Eradication Plan

Option A : Vaccination + Monitoring

YEARS 1-2

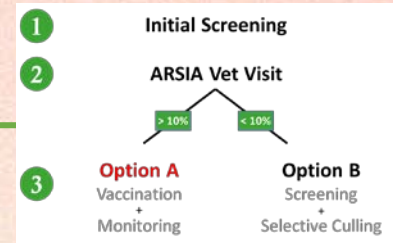


Nasals swabs performed ad random at different age categories

2 consecutive
negative
screenings

YEARS 3-5

- Degressive vaccination
- Vaccinated animals : nasal swab
- Non-vaccinated animals : serology



Challenge : Individual Eradication Plan

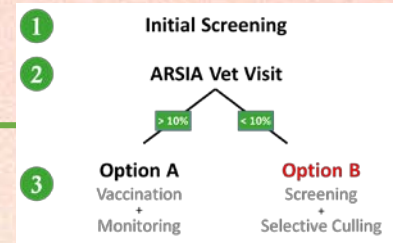
Option B : Screening + Selective Culling

YEARS 1-5

- Serological screening (> 6mois)
- ARSIA Visit → Advices
- Cullings when 2 consecutive seropositive result

2 consecutive
negative
screenings

HERD STATUS « A »



Challenges

Conclusions and for the future...

- *M.bovis* is not going away, **it's here** and we have to deal with this now in particular in the **fight antibiotic resistance**! A **national surveillance** could be interesting to put in place.
- The **control of *M. bovis*** improved these last few years but **research** could still help the understanding of the disease in order to facilitate the eradication.
- Evident **lack of motivation** from farmers
 - Others concerns (IBR, BVD...)
 - Resigned to live with *M.bovis* (speeches of traders...)

Arsia
ASBI

**ET VOUS,
COMMENT SOUHAITEZ-VOUS
GÉRER LE MYCOPLASME
DANS VOTRE TROUPEAU?**

OPTION 1

OPTION 2

**LES SOLUTIONS POUR RÉDUIRE
LA CIRCULATION DE MYCOPLASMA BOVIS
DANS VOTRE TROUPEAU EXISTENT!**

**OPTEZ POUR LE PLAN DE LUTTE
STRUCTURÉ PROPOSÉ PAR L'ARSIA!**

Thank you to :

My colleagues
Bovine Funds
Linde Gille

