

Unexpected strangles in imported horses: Molecular identification of *Streptococcus equi* subsp. *equi*

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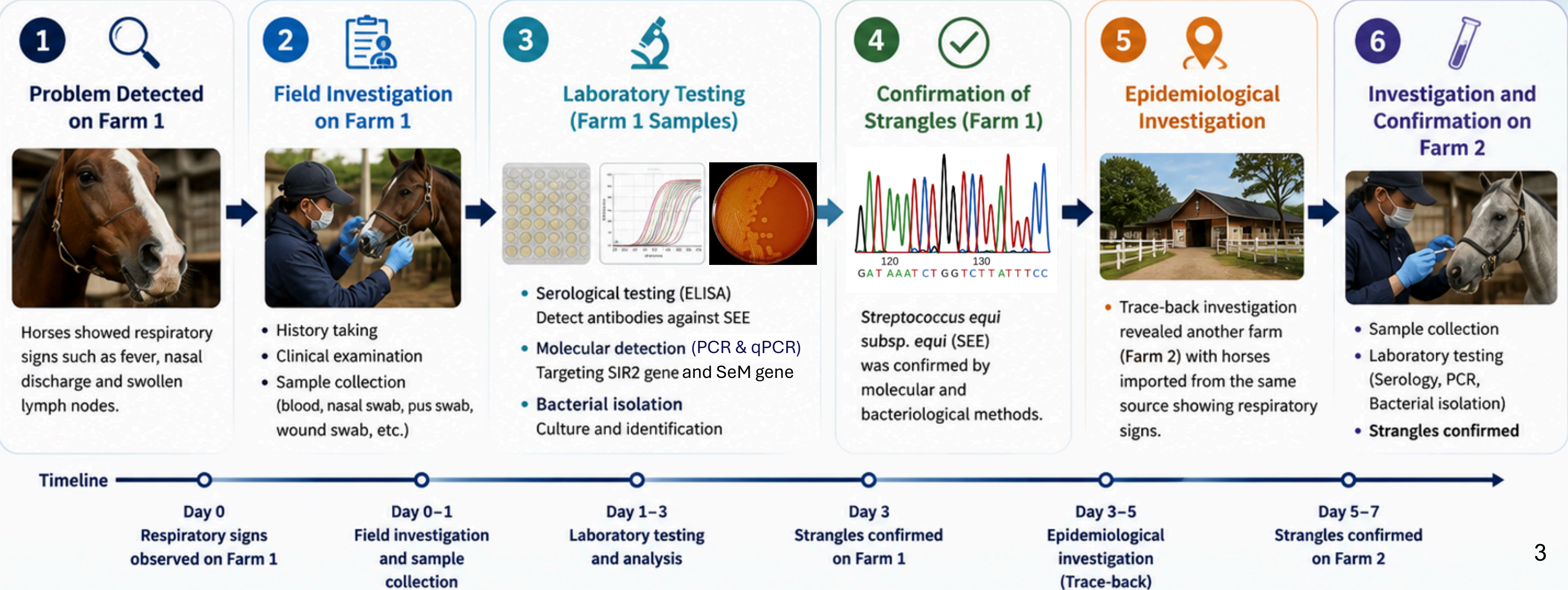
What is Strangles?

- *Streptococcus equi* subsp. *equi*
- Meaning: to kill or choke someone by compressing their throat to stop them from breathing.
- Young horses 1–5 years of age are the most susceptible, particularly when exposing to stressors such as transportation, mixing with unfamiliar horses, or overcrowding.
- The incubation period is typically 3–14 days after exposure.
- In Thailand, an imported case of strangles from the United Kingdom was reported in 2016 (Tonpitak et al., 2016)



OUTBREAK INVESTIGATION FLOW CHART

From the first case to confirmation in an additional farm



Clinical signs



Fever



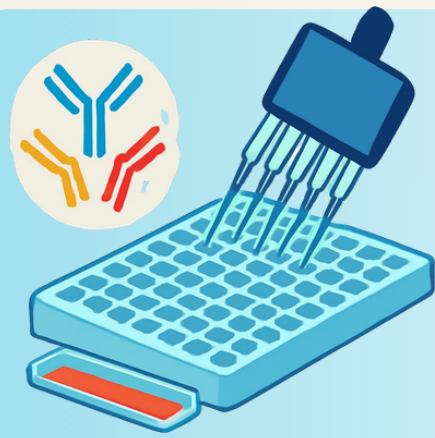
Abscess formation

(Submandibular lymph nodes or retropharyngeal lymph nodes swelling)



Mucopurulent nasal discharge

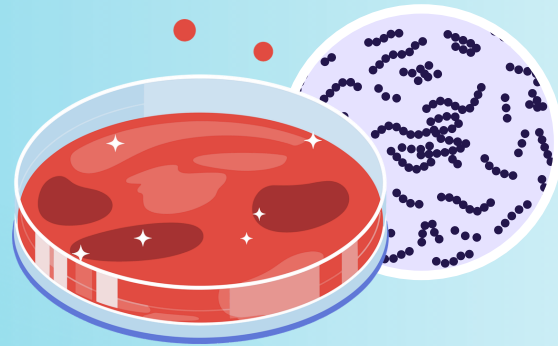
Diagnostic methods



ELISA



Conventional PCR



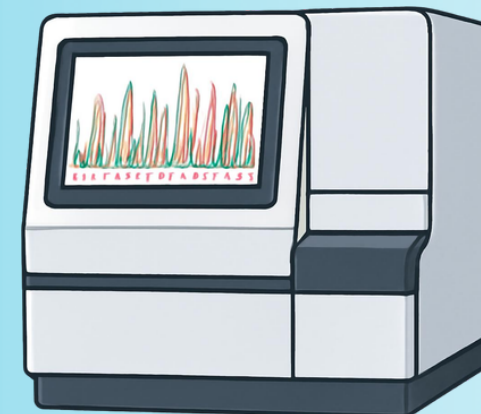
Bacterial Culture



Real-time PCR



Biochemical test



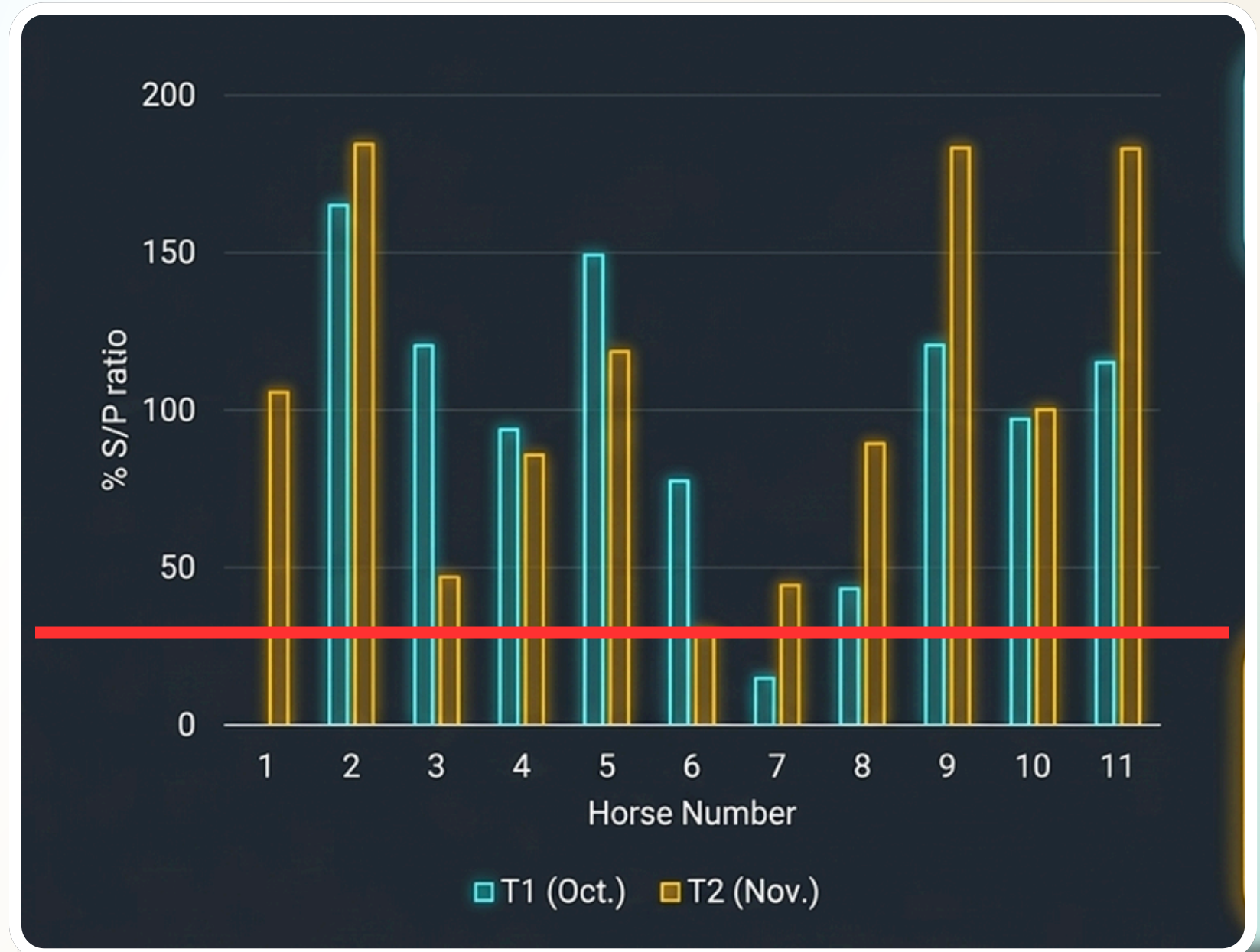
**Sanger
Sequencing**

ELISA

- Detect SEQ2190 (antigen A) and SeM (antigen C)
- protein M (SeM)
- Seropositive 48.6% (17/35 samples)
- Pair serum - Seroconvert

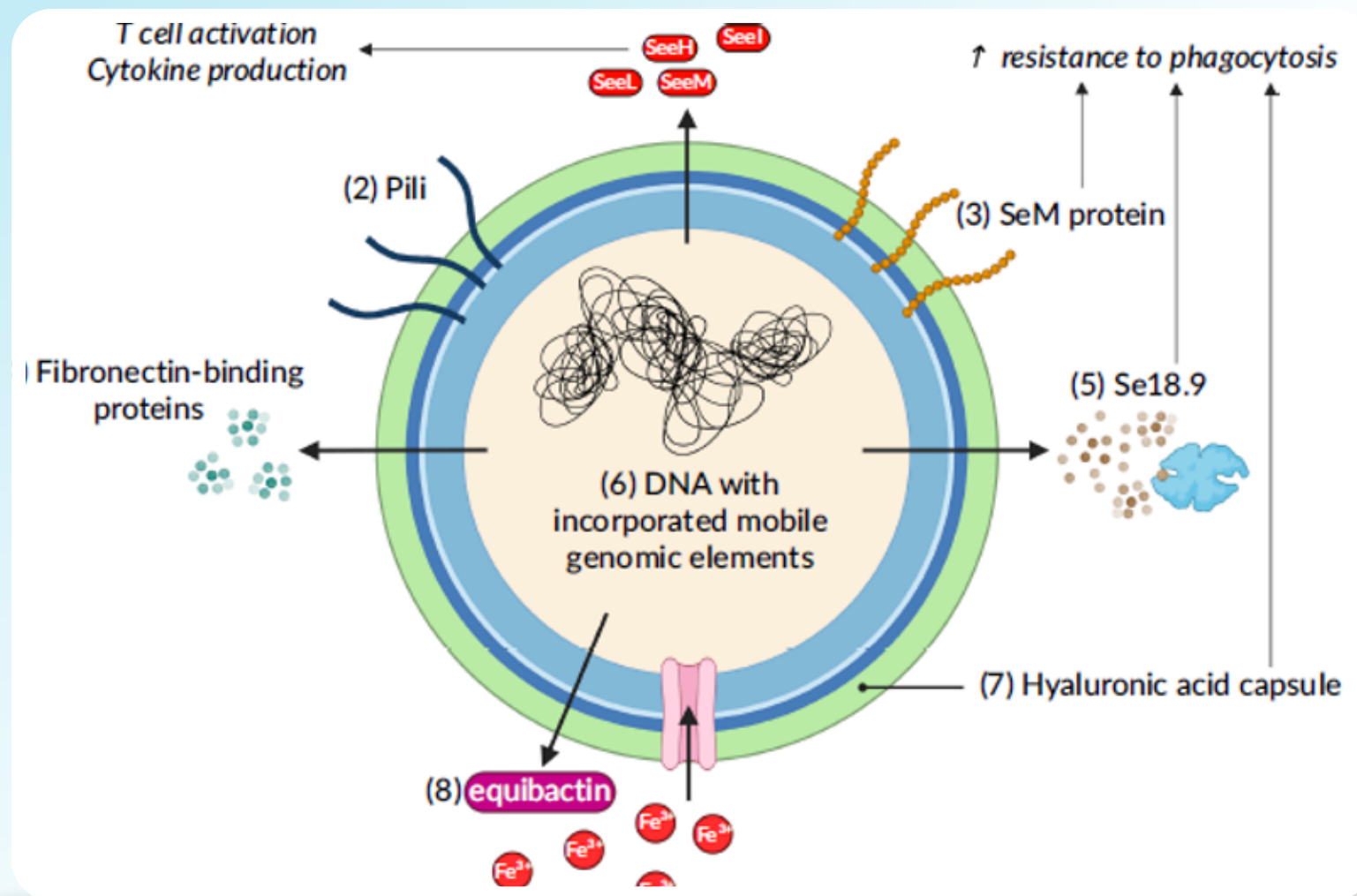


S/P RATIO
CUT-OFF 30%

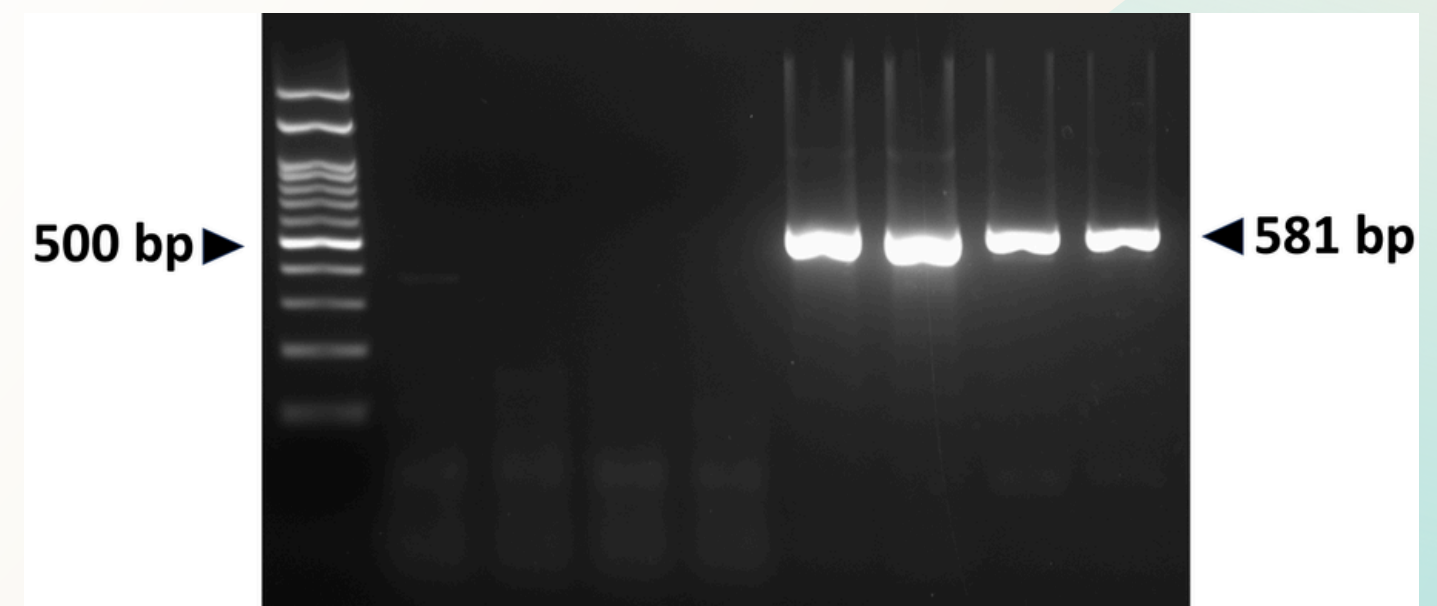
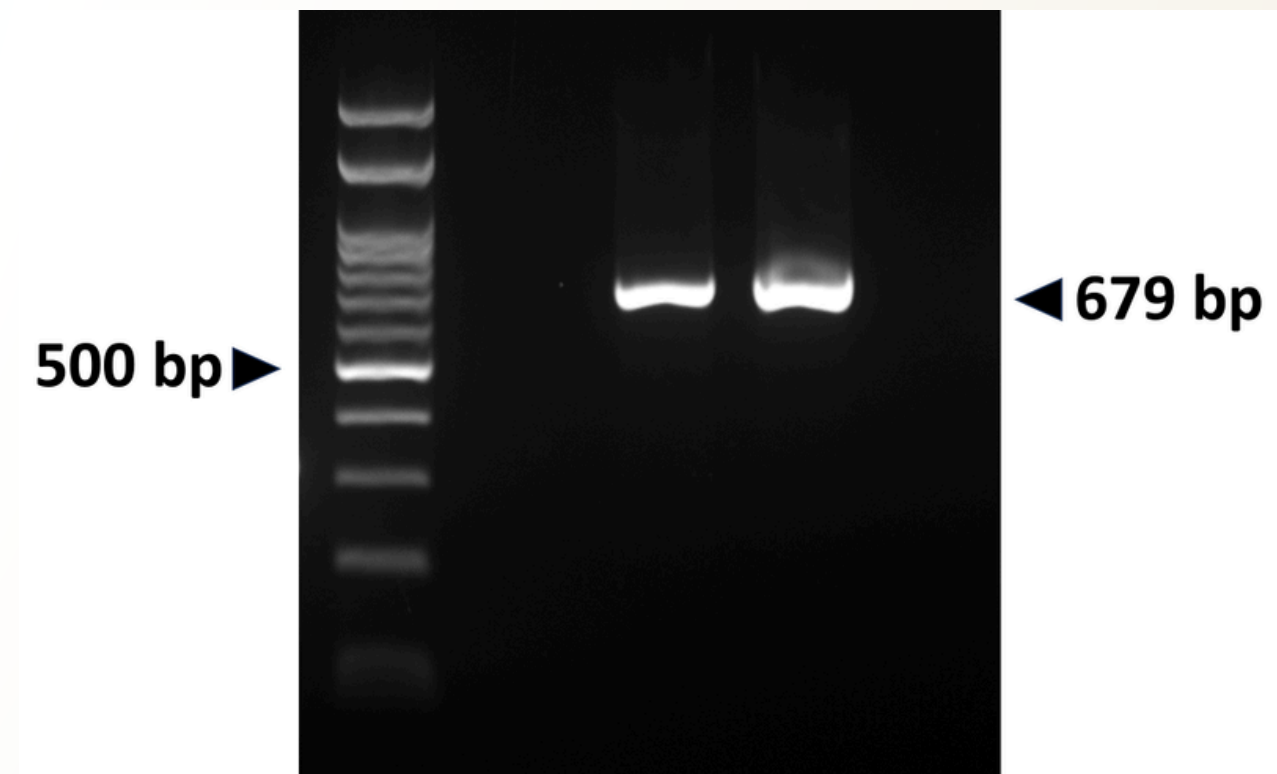


Conventional PCR

- *SeM* gene (PCR product 679 bp)
- *SIR2* gene (PCR product 581 bp)

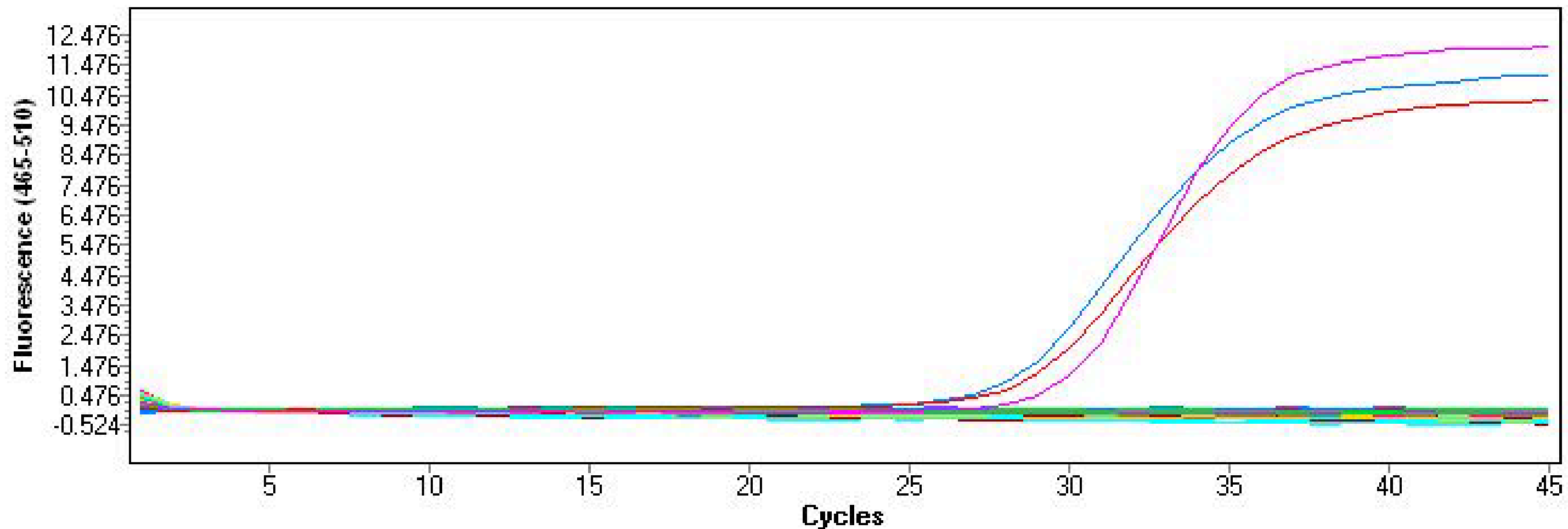


(3) SEM PROTEIN: BIND TO FIBRINOGEN AND IMMUNOGLOBULIN, INHIBITING C3B COMPLEMENT DEPOSITION. TARGETED BY THE SINGLE AND DUAL ELISA SEROLOGICAL TESTS



Real-time PCR

Target *SIR2* gene (ct value cut-off = 36)



29.63 Positive control
28.59 Pus swab rep1
28.13 Pus swab rep2

Sequencing

Sanger sequencing target

- 16S rRNA
- *SeM* gene
- *SIR2* gene

Result:

- Blastn (100% sequence identity)

Description

None

Molecule type

dna

Query Length

396

Other reports

[Distance tree of results](#) [MSA viewer](#) [?](#)

Percent Identity

100

 to

100

E value

to

Query Coverage

to

Filter

Reset

Descriptions

Graphic Summary

Alignments

Taxonomy

Sequences producing significant alignments

Download

Select columns

Show

100

?

⚙

Your results are filtered to match records with **percent identity** between 100 and 100.

Reset

☒ select all 17 sequences selected

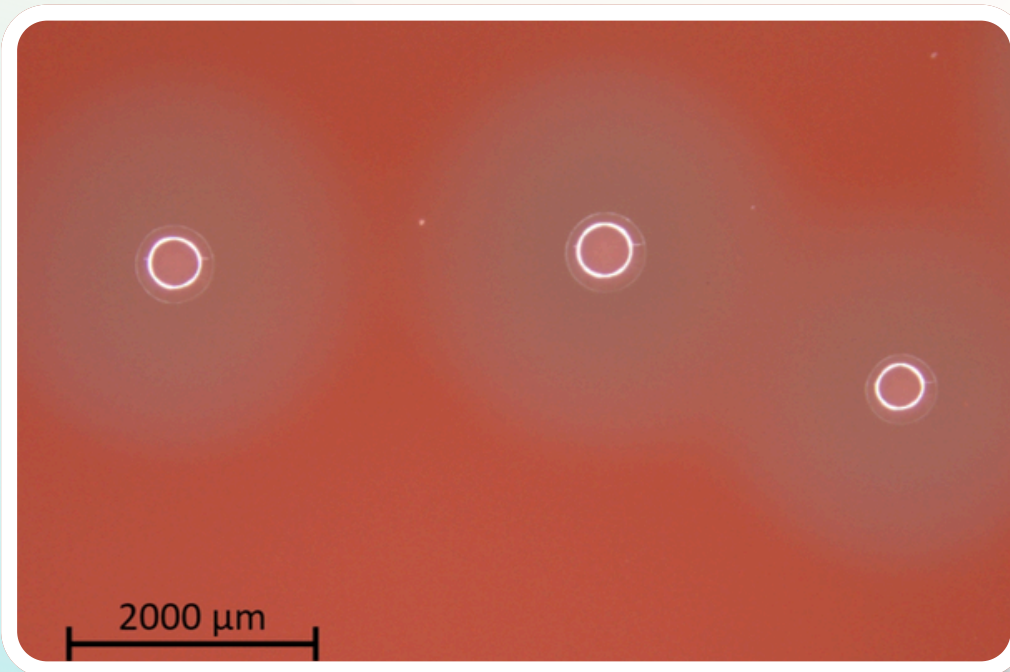
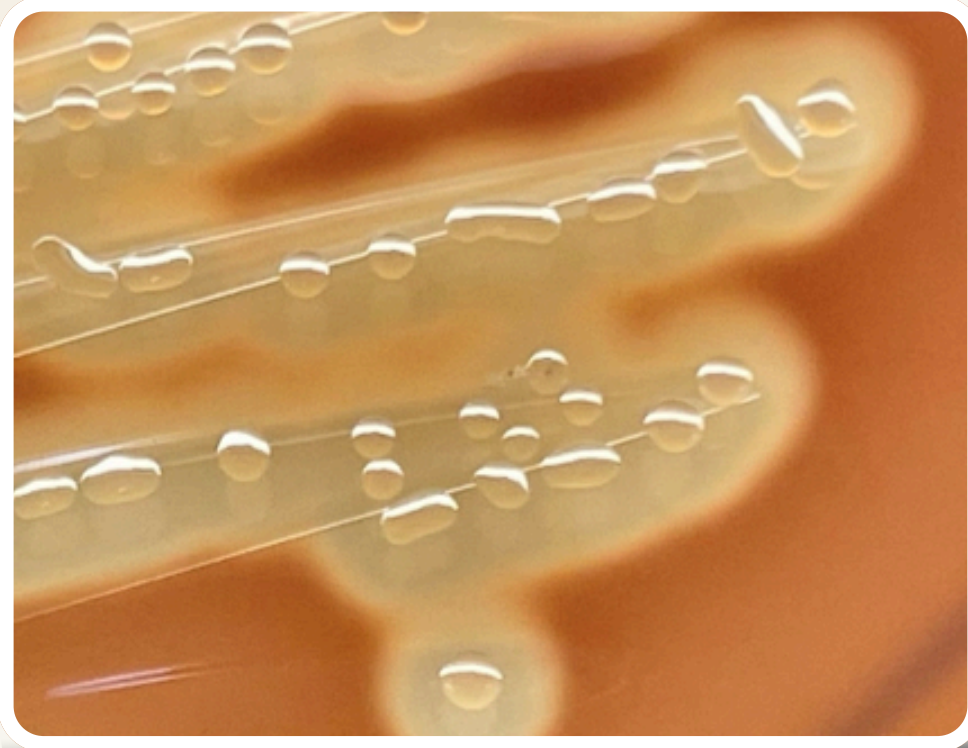
[GenBank](#) [Graphics](#) [Distance tree of results](#) [MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	Streptococcus equi subsp. equi strain NCTC9682 genome assembly, chromosome: 1	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2253416	LR134389.1
☒	Streptococcus equi subsp. equi strain 470_001 chromosome, complete genome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2263309	CP071148.1
☒	Streptococcus equi subsp. equi strain HTP232 chromosome, complete genome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2191031	CP133956.1
☒	Streptococcus equi subsp. equi 4047, complete genome	Streptococcus equi subsp. equi 4047	732	732	100%	0.0	100.00%	2253793	FM204883.1
☒	Streptococcus equi subsp. equi strain 470_007 chromosome, complete genome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2265767	CP071145.1
☒	Streptococcus equi subsp. equi strain 489_005 chromosome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2272870	CP071141.1
☒	Streptococcus equi subsp. equi strain HTP133 chromosome, complete genome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2150351	CP133955.1
☒	Streptococcus equi subsp. equi strain 489_002 chromosome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2263263	CP071143.1
☒	Streptococcus equi subsp. equi strain ATCC 39506, complete genome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2272071	CP021972.1
☒	Streptococcus equi subsp. equi strain 489_004 chromosome, complete genome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2265147	CP071142.1
☒	Streptococcus equi subsp. equi strain 489_010 chromosome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2252366	CP071140.1
☒	Streptococcus equi subsp. equi strain 470_008 chromosome, complete genome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2266249	CP071144.1
☒	Streptococcus equi strain Kargaly-22 chromosome, complete genome	Streptococcus equi	732	732	100%	0.0	100.00%	2253418	CP119575.1
☒	Streptococcus equi subsp. equi strain 470_006 chromosome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2265538	CP071146.1
☒	Streptococcus equi subsp. equi strain HT1112 chromosome, complete genome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2156526	CP133957.1
☒	Streptococcus equi subsp. equi strain 470_003 chromosome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2266254	CP071147.1
☒	Streptococcus equi subsp. equi strain XJ5012 chromosome, complete genome	Streptococcus equi subsp. equi	732	732	100%	0.0	100.00%	2196100	CP134538.1

Bacterial Culture

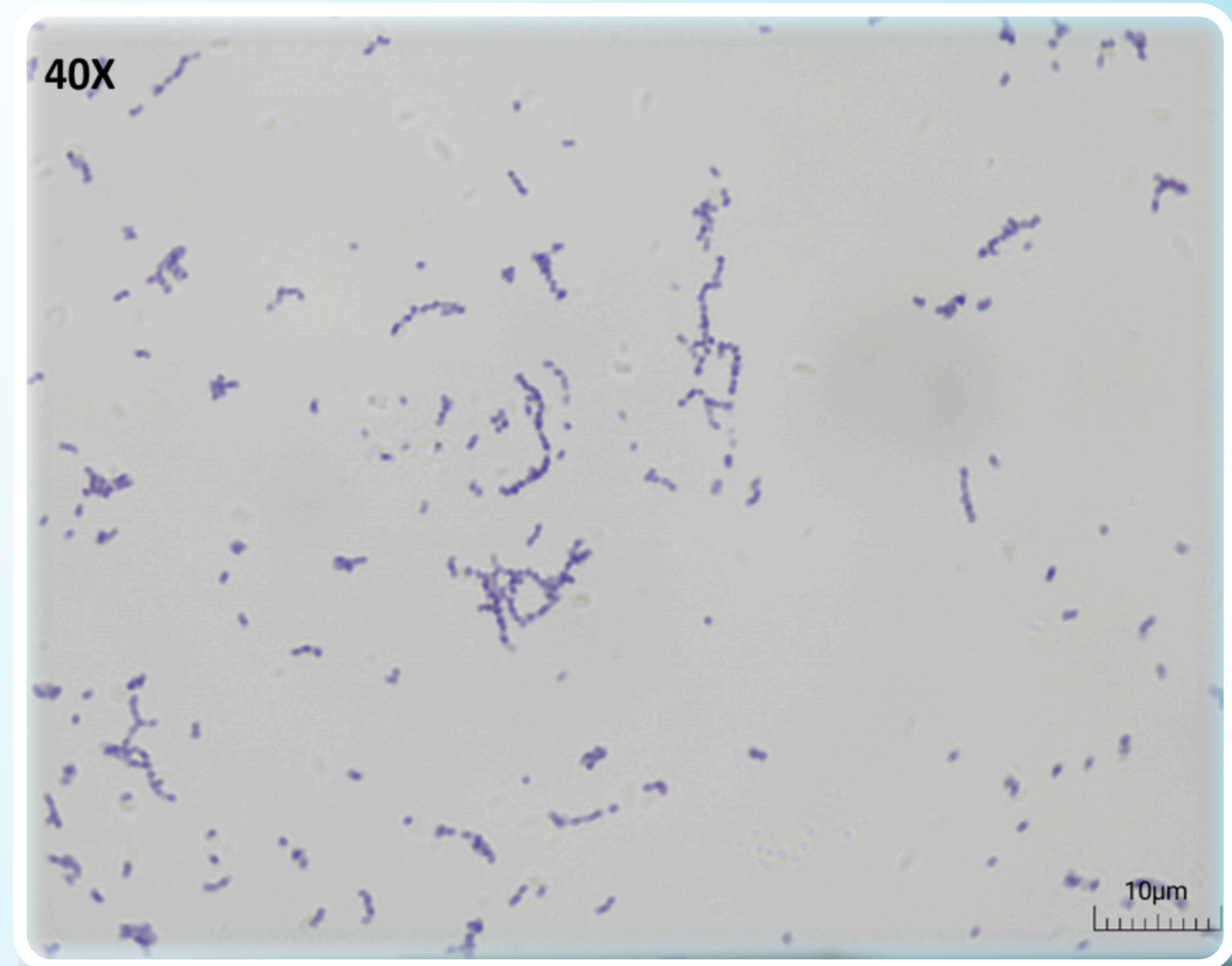
Colonies morphology

- Small (about 1 mm in diameter)
- Translucent colonies with beta-hemolysis on blood agar.
- No growth on MacConkey agar

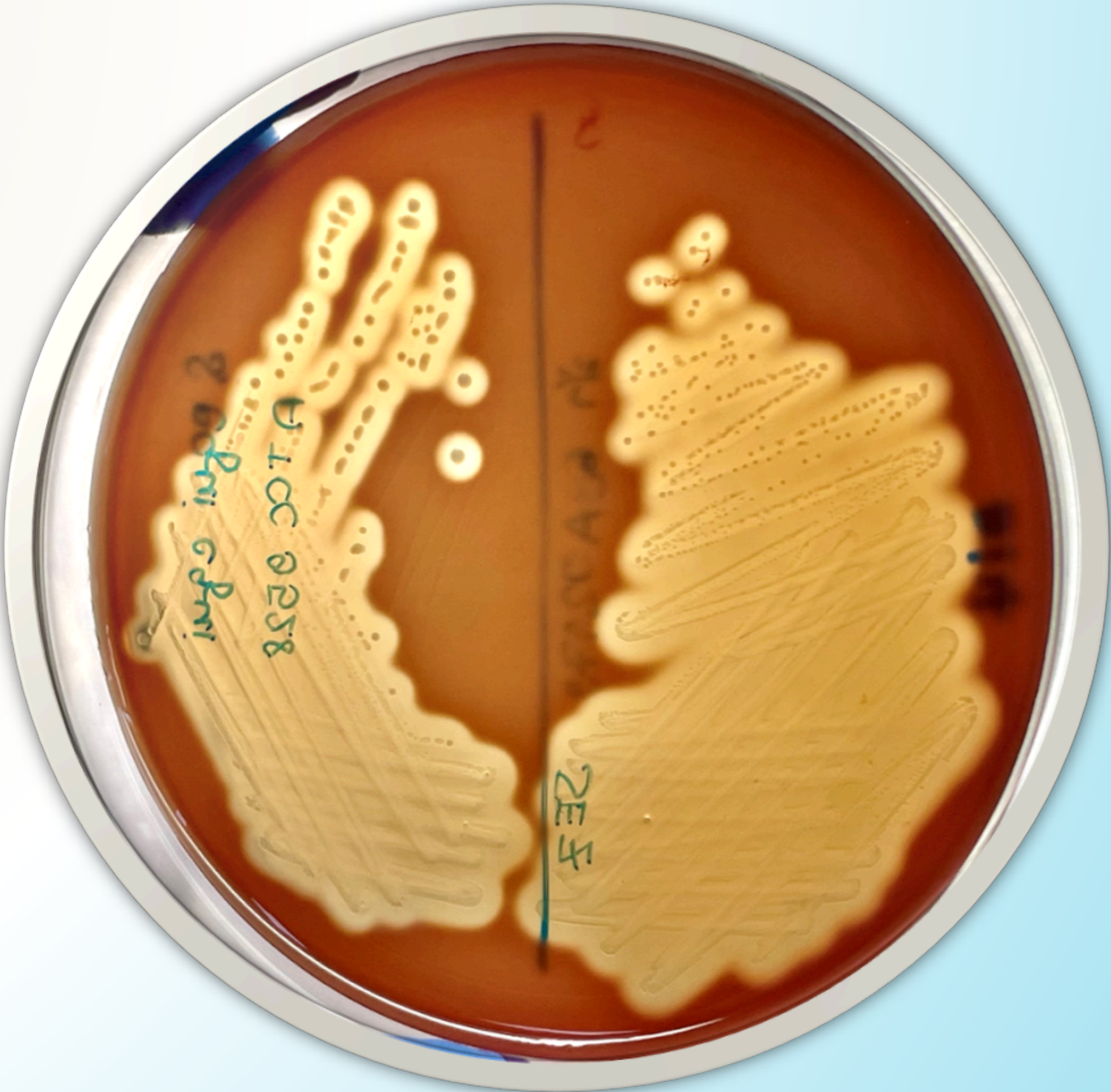


Gram strain

- Gram Positive
- Cocci shape
- Size around 1 μm



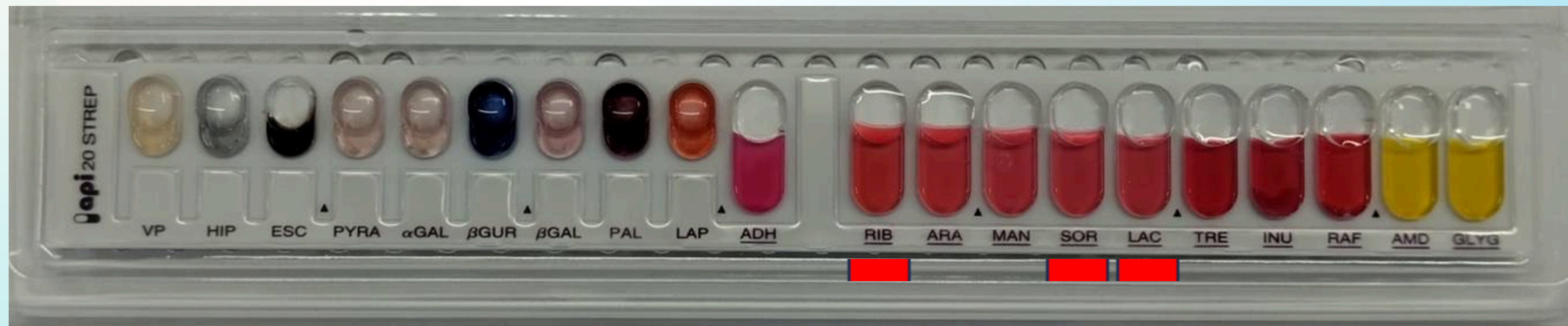
SEE VS SEZ



Biochemical test

- Catalase test: Negative
- Oxidase test: Negative
- Commercial biochemical test (api® 20 STREP) identification *S.equi* subsp. *equi* 99.6%
RIB - / SOR - / LAC -

VERY GOOD IDENTIFICATION						
Strip	API 20 STREP V8.0					
Profile	4 4 6 1 0 0 7					
Note	ID. NOT VALID BEFORE 24 HOURS					
Significant taxa	% ID	T	Tests against			
Streptococcus equi ssp equi	99.6	0.67	ESC	1%		
Next taxon	% ID	T	Tests against			
Streptococcus dysgalactiae ssp equisimilis	0.3	0.38	ESC	25%	RIB	97% TRE 99%



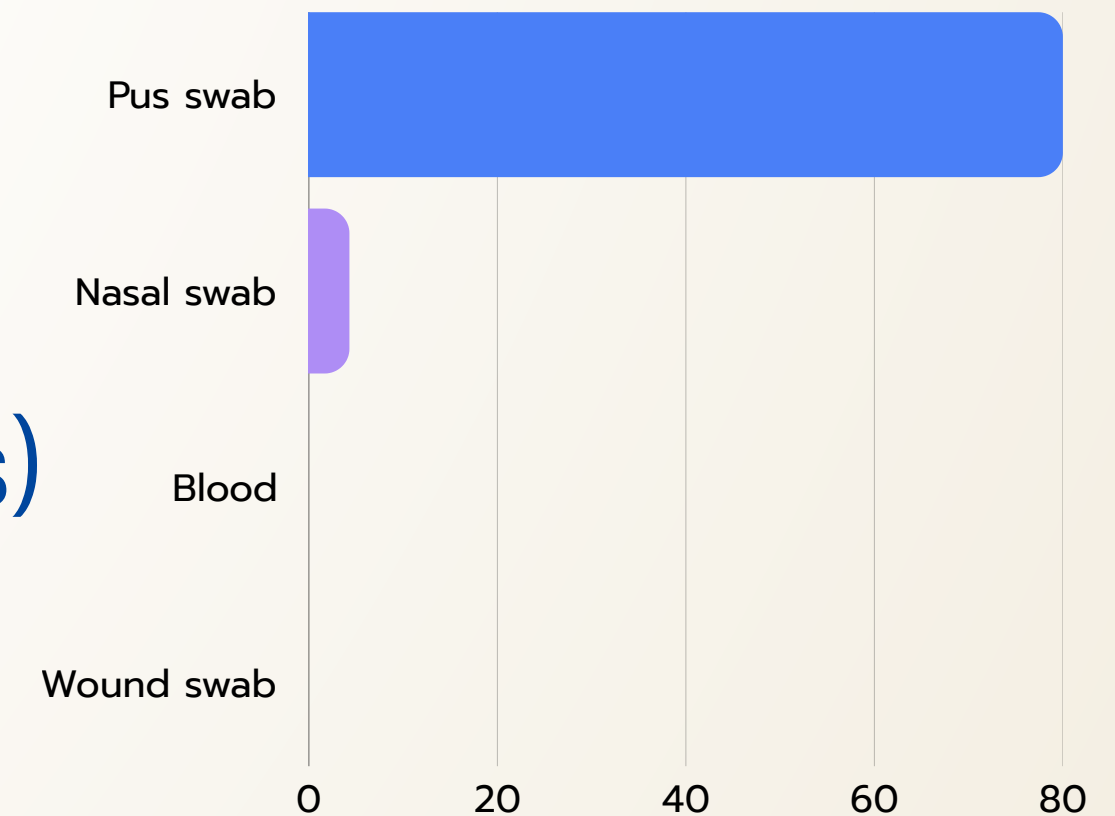
Results summary

ELISA

- Serum positive 48.6% (17/35 samples)

PCR and real-time PCR

- Pus swabs positive 80% (4/5 samples)
- Nasal swabs positive 4.3% (1/23 samples)
- Blood samples positive 0% (0/52 samples)
- Wound swabs positive 0% (0/3 samples)



Discussion & Conclusion

Strict Post-Arrival Quarantine Protocols for Horses

- Import horses only from reliable sources with health certification and testing records.
- Maintain a 30-day post-arrival quarantine period.
- Conduct close routine monitoring for clinical signs throughout quarantine.
- Perform disease testing before release from quarantine.

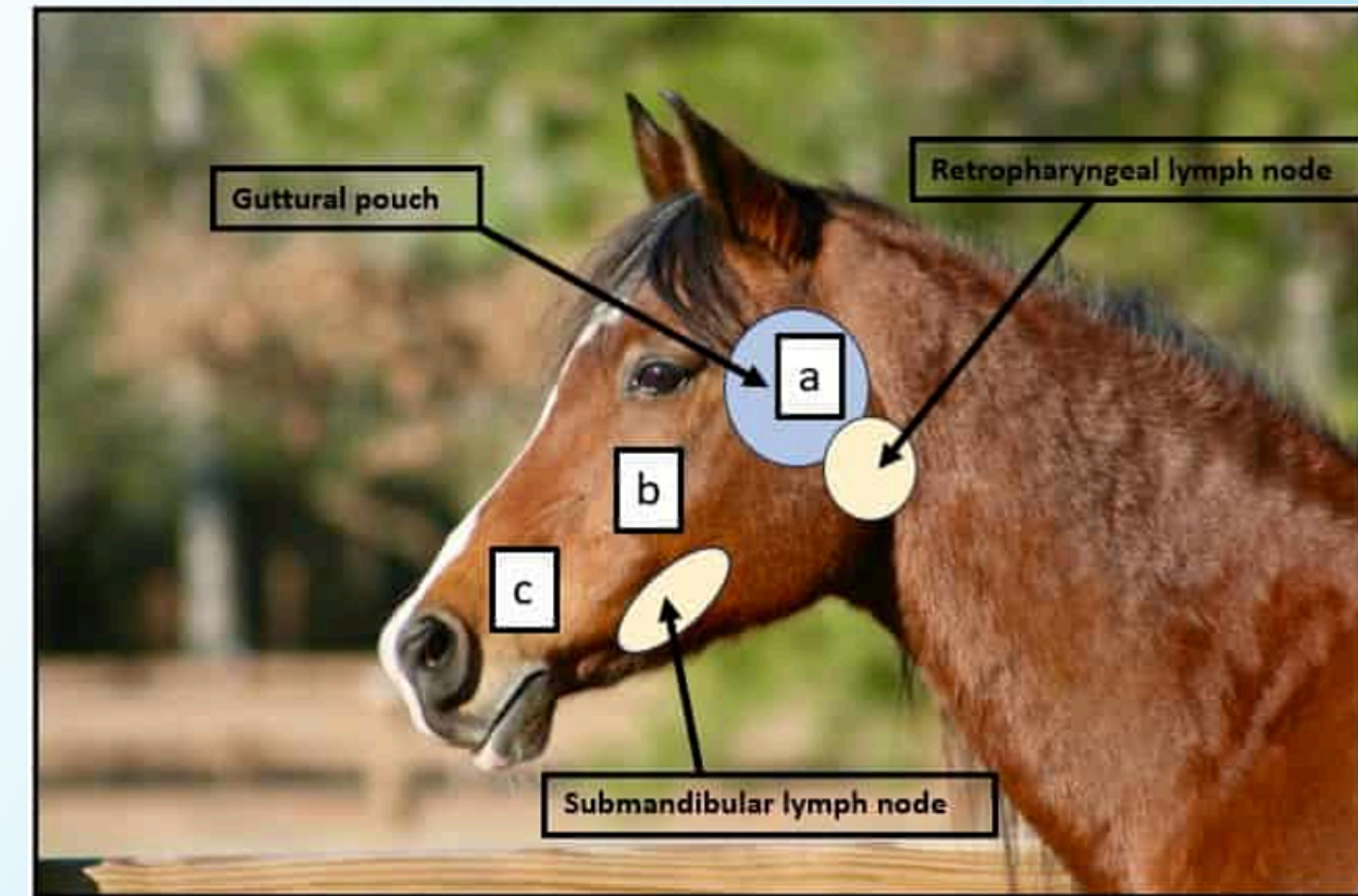
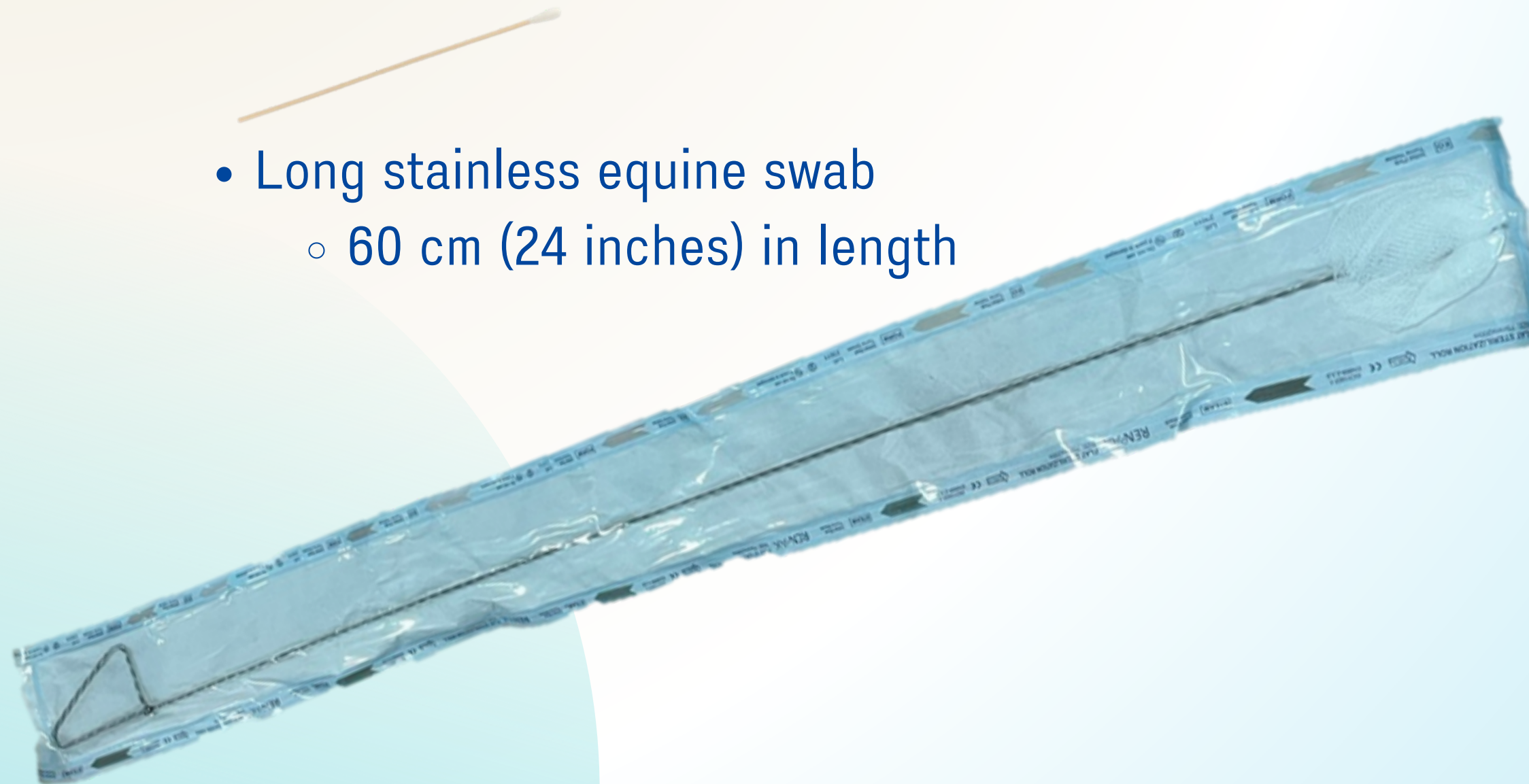
Zoonosis Case Reports in Strangles

- *Streptococcus equi* subsp. *equi* in Retropharyngeal Abscess: Case Report and Review of Literature (Waśniewska-Włodarczyk et al., 2022)
- A case of bacteremia and pneumonia caused by *Streptococcus equi* subspecies *equi* infection in a 70-year-old female following horse exposure in rural Wyoming (Bohlman et al., 2023)
- *Streptococcus Equi*: A rare cause of cellulitis and bacteremia in human (Sharaf et al., 2026)

Discussion & Conclusion

Which swab should we use?

- Regular cotton swab
 - 15 cm (6 inches) in length
- Long stainless equine swab
 - 60 cm (24 inches) in length



- a) Guttural pouch is where the strangles bacteria is primarily harbored. Guttural pouch wash performed here – direct contact to the bacteria.
- b) Where a nasopharyngeal wash is performed. Does not enter pouch so can still miss bacteria harbored in there.
- c) Nasal swab. Highest false negatives due to not entering nasopharynx or guttural pouch – easy to miss the bacteria.

Table 1. Comparison of *S. equi* samples.

<i>S. equi</i> Sample	Pros	Cons
Aspirate of mature abscessed lymph node	High yield of bacterial organisms	Requires this stage of disease
Moistened rostral nasal swab ^a	Ease of sampling	Animal needs to have active mucopurulent discharge
Moistened nasopharyngeal swab ^a	Ease of sampling	False negatives possible in early febrile state (not shedding yet)
		False negatives possible due to intermittent shedding from guttural pouch
Nasopharyngeal wash	Ease of sampling	False negatives possible in early febrile state (not shedding yet)
	Sampling more surface area	False negatives possible due to intermittent shedding from guttural pouch
	Was found to be more sensitive than nasopharyngeal swab ²⁹	
Guttural Pouch lavage ^b	Best for detection of carrier animals	Special equipment needed
		Experience entering the guttural pouch
		More time consuming
		False negative if lymph nodes have not yet ruptured into the pouch

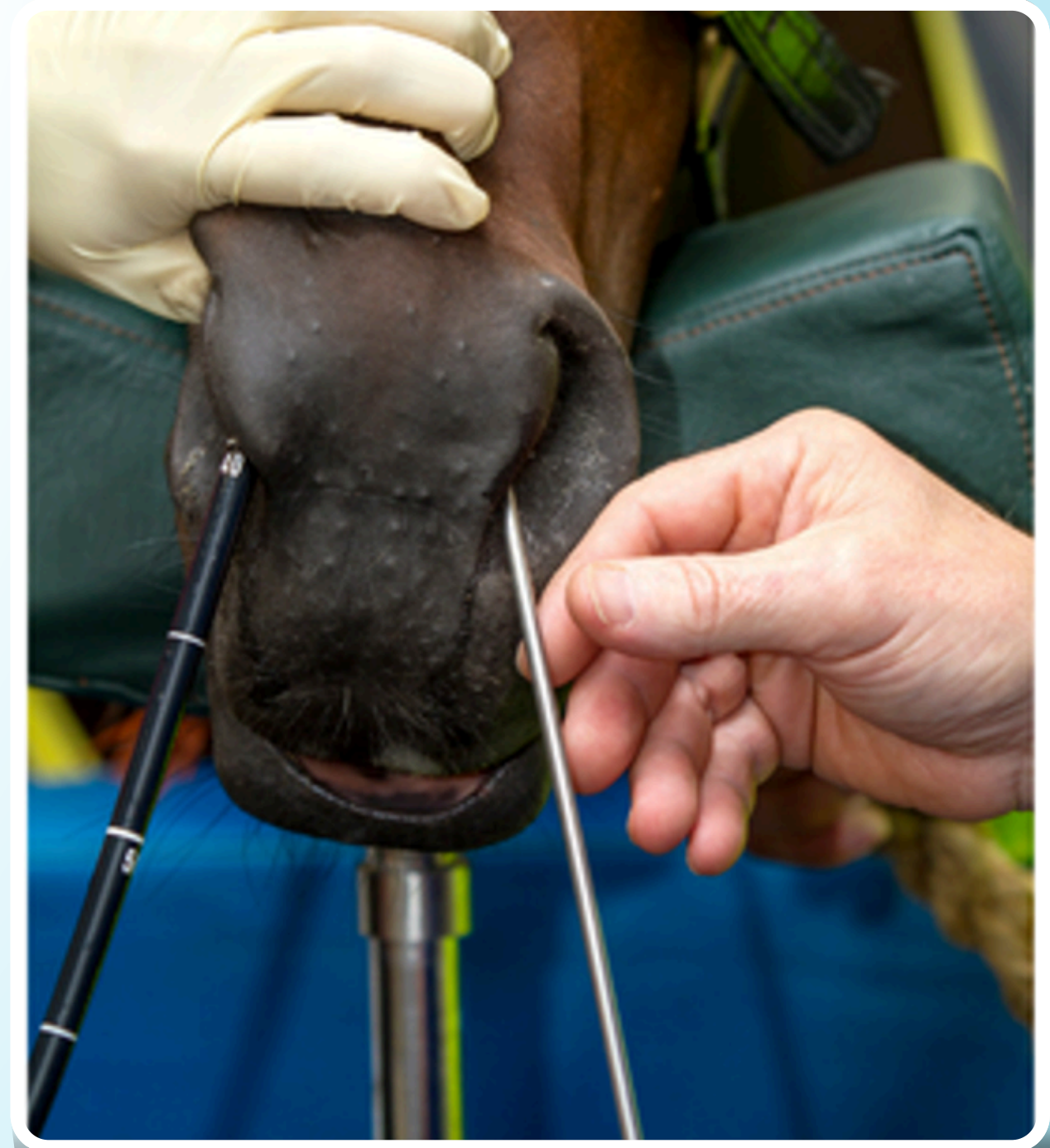
^aSynthetic microfiber flocked swabs have not shown increased detection rates over traditional rayon or cotton swabs.

^bThe committee recommends guttural pouch lavage qPCR for the detection of carriers with concurrent visual inspection of the guttural pouch via endoscopy. In order to limit the contamination of the environment and the veterinarians, we recommend collection directly from the guttural pouch, rather than free catch from the nasal passage.

Endoscopes in horse



[HTTPS://WWW.BURWASHEQUINE.CA/BLOG/2024/3/7/PERFORMANCE-HORSE-COMPETITION-PREPARATION-RESPIRATORY-HEALTH-AND-BALS](https://www.burwashequine.ca/blog/2024/3/7/performance-horse-competition-preparation-respiratory-health-and-bals)



[HTTPS://WWW.EQUINE-AMERICA.COM/BLOGS/NEWS/NOSE-BLEEDS-RHINOS-AND-ENDOSCOPES?](https://www.equine-america.com/blogs/news/nose-bleeds-rhinos-and-endoscopes?SRLTID=AFMB000FIZ9WZ_SPWM2WOLXXJ6IFVREUY0AURGKIT_4KWP0V9FRPQ8VI)
SRLTID=AFMB000FIZ9WZ_SPWM2WOLXXJ6IFVREUY0AURGKIT_4KWP0V9FRPQ8VI

Discussion & Conclusion

Coinfection

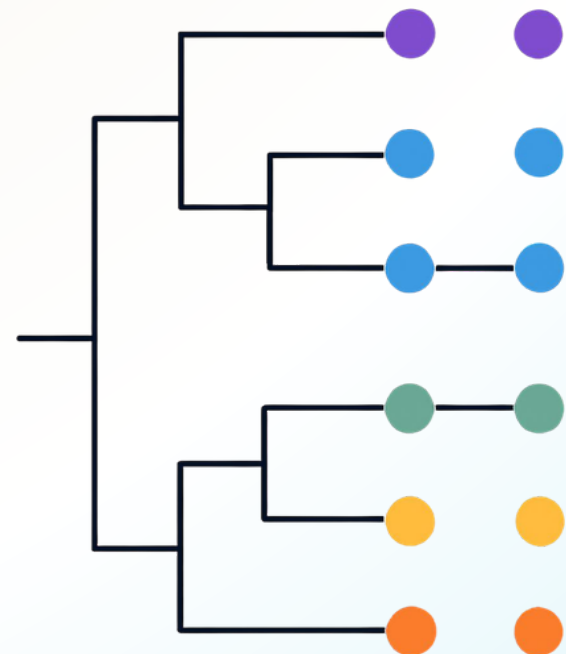
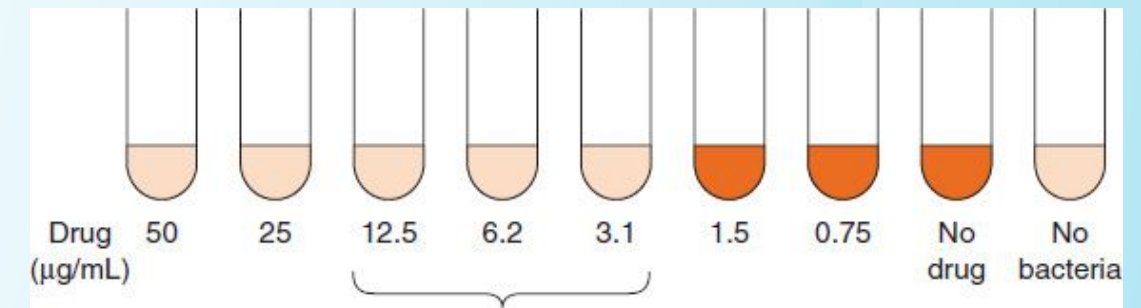
- Co-infections may have contributed to increased disease severity, although this has not yet been investigated.
- Strangles has a high morbidity (40–90%) but low mortality (1–5%).
- Most horses responded well to antimicrobial treatment, consistent with the antimicrobial susceptibility results.
- Several severely affected horses were euthanized due to poor welfare, despite strangles itself rarely being directly fatal.
- Misdiagnosis
 - The absence of *S. equi* and the frequent detection of *S. equisimilis* and *S. zooepidemicus* suggest that beta-haemolytic streptococci other than *S. equi* could be the causative agent of strangle-like disease. (Laus et al., 2007)

Drug	SEE	SEZ
Ampicillin	S	R
Ceftiofur	S	S
Erythromycin	S	I
Ofloxacin	S	S
Penicillin	S	S
Tetracycline	R	R

Disk diffusion testing

Future plan

- Minimum Inhibitory Concentration (MIC)
- SeM gene typing
- Multi-locus sequence typing (MLST)
- Whole Genome Sequencing
 - Virulence genes, AMR genes profiling
 - Phylogenetic tree

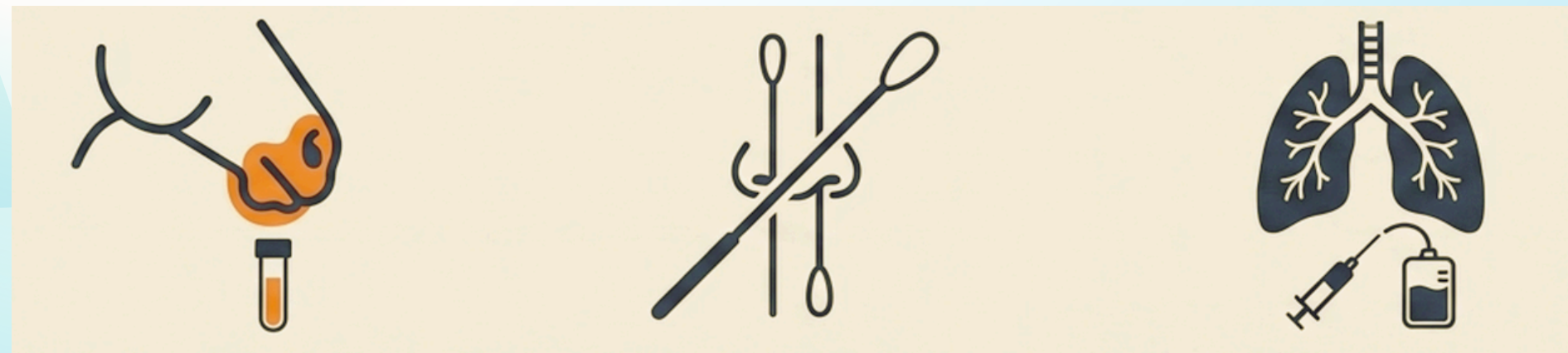


Take-home message

“เก็บคางหมอง

ป็นสองจุก

ดูหน้าหายใจ”



Acknowledgement

- **Department of Immunology**
- **Department of Pathology**
- **Department of Parasitology**
- **Department of Bacteriology and Mycology**
- **Bureau of Disease Control and Veterinary Services**

References

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Thank You

Q & A