

Anaplasmosis Diagnostics

Anaplasmosis Symposium
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DIAGNOSTIC LABORATORY

Presentation outline

“Gold standard”

Blood microscopic examination

Serum ELISA

Polymerase chain reaction (PCR)

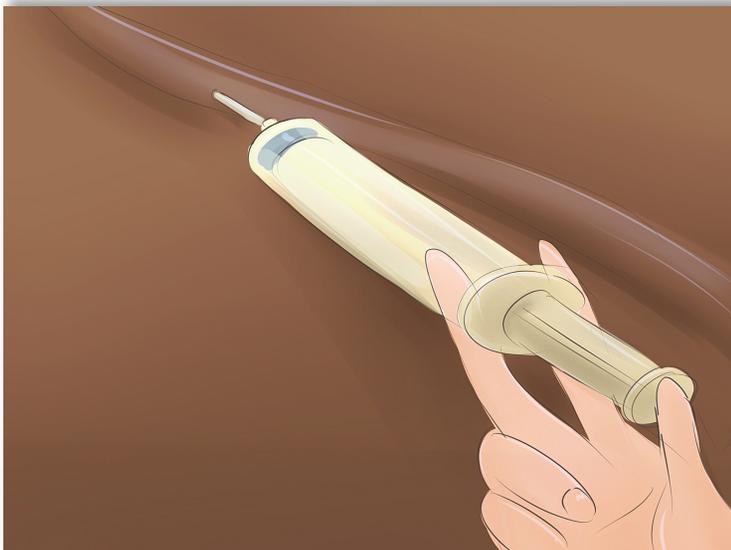


“The Gold Standard”

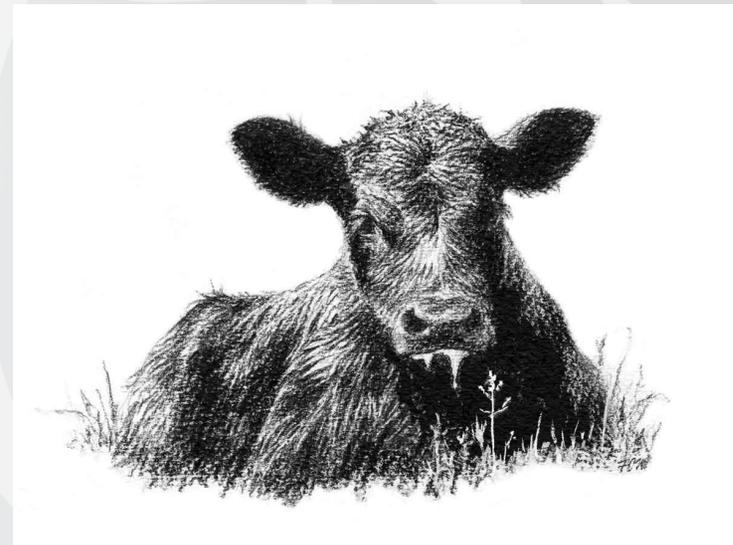
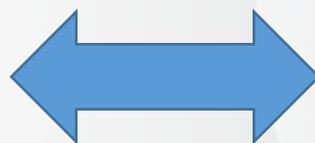
**Splenectomized (spleen
removed) calf injection**



Gold standard



Inject blood from suspect
Anaplasmosis animal

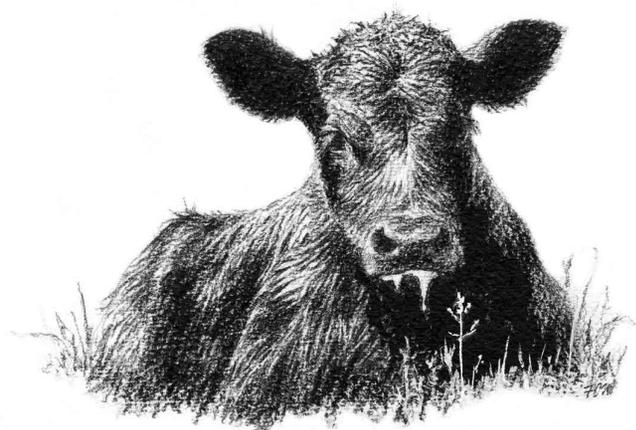


Calf with spleen removed



Clinical signs and/or blood test

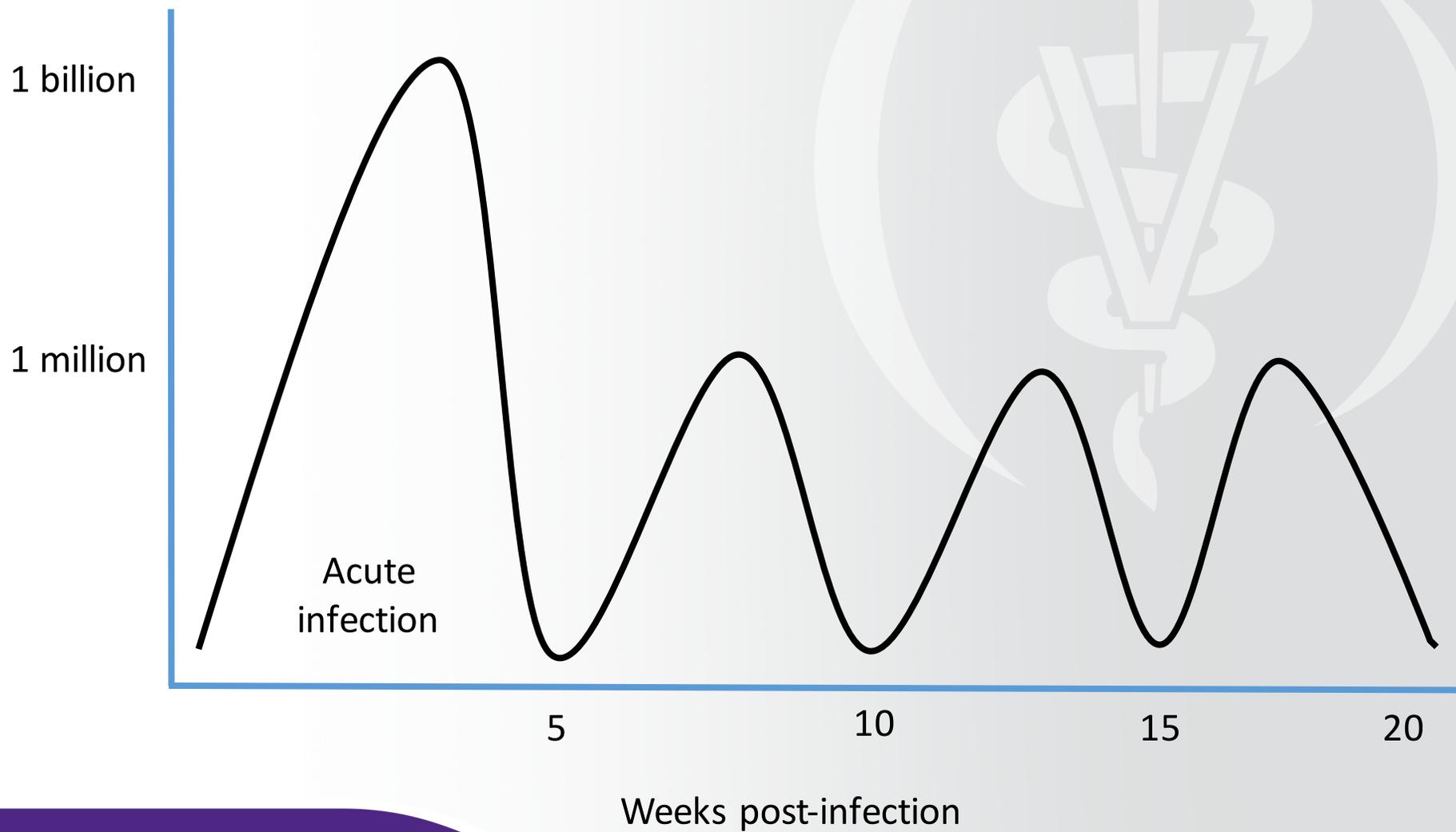
Gold standard



Not practical

Not ethical except
in some specific
research trials

A. marginale per ml blood





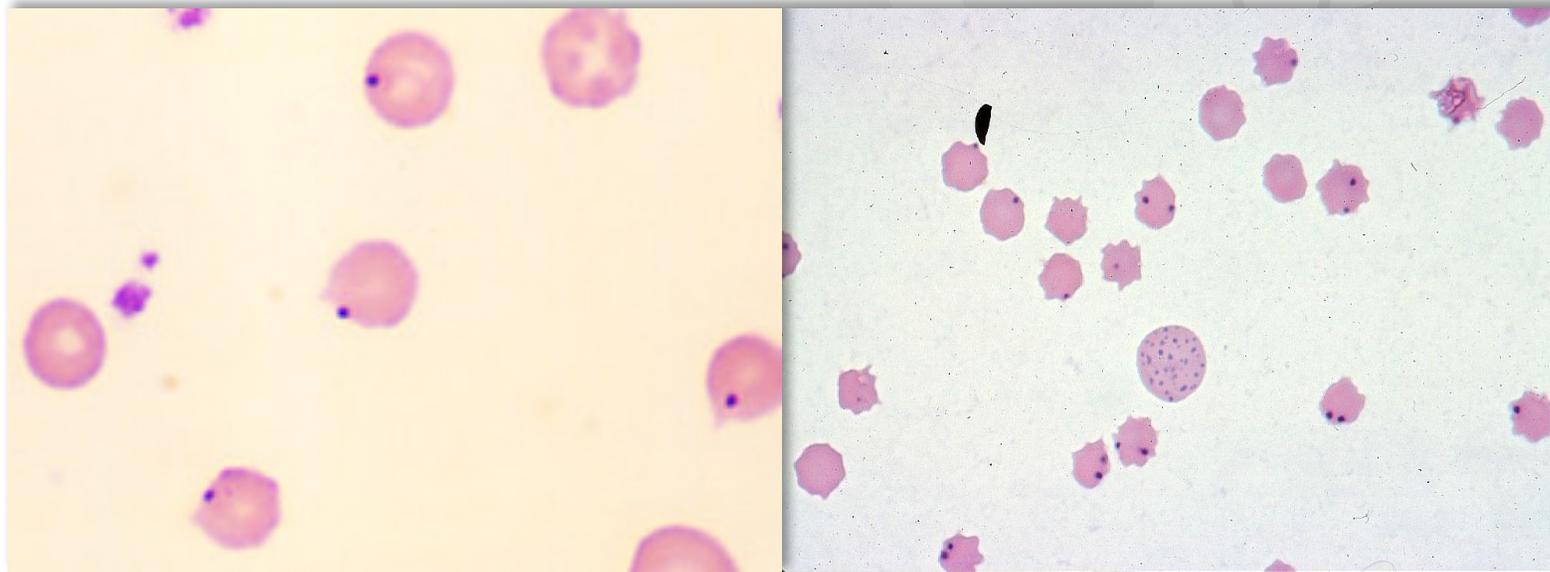
Microscopic blood exam

Microscopic exam: Blood smear

Looking for infected red blood cells

Requires expertise to accurately identify

Can be confused with other red blood cell structures

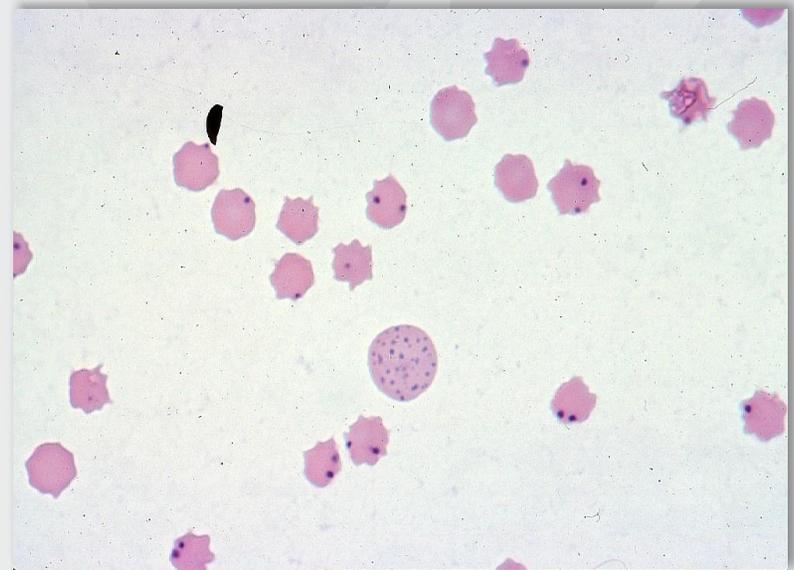


Anaplasma marginale

Microscopic exam: Blood smear

Useful to confirm clinical signs (acute infection stage)

Open mouth breathing
Staggering
Aggression.....



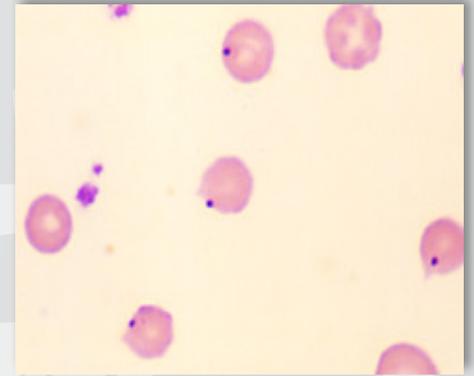
Anaplasma marginale

Microscopic exam: Blood smear

Not useful before clinical signs

Number of infected cells too low to observe

Blood smear



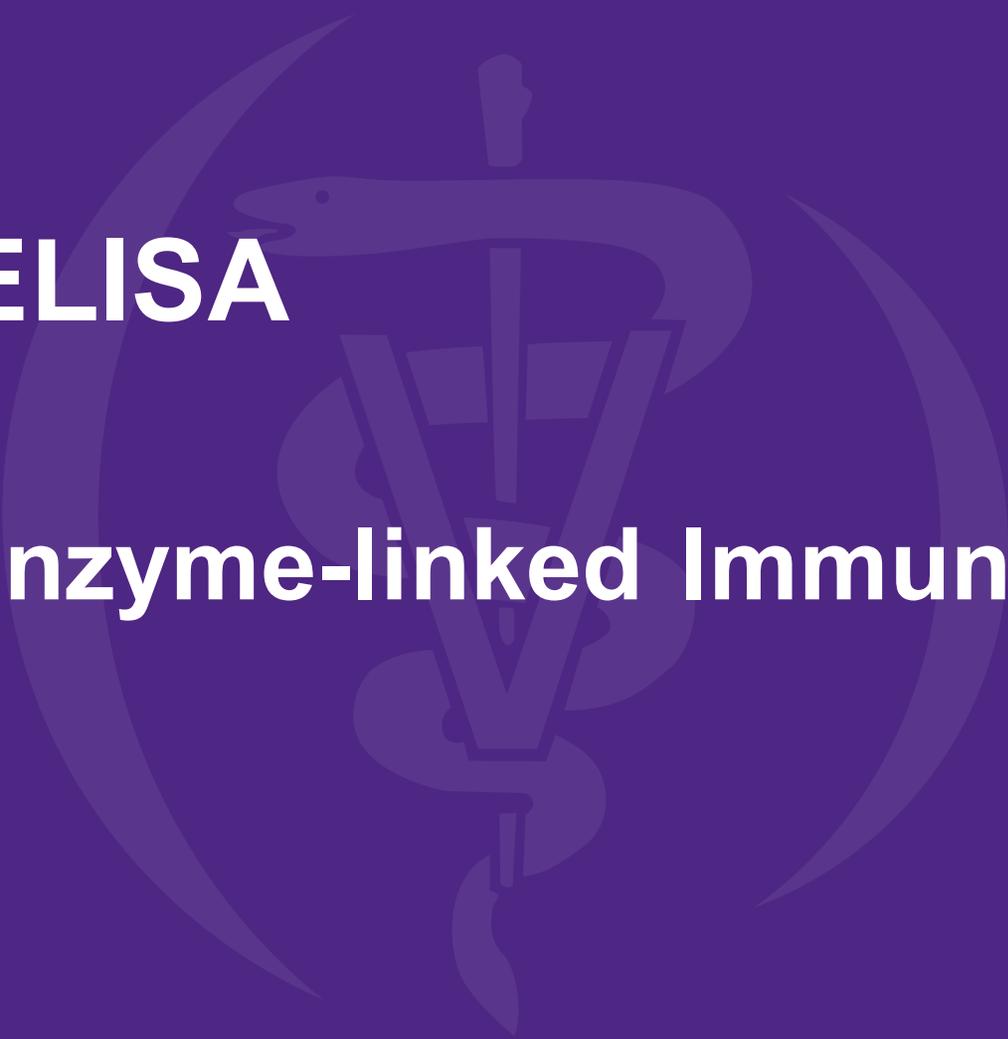
Not useful to identify persistently infected animals

Sensitivity = 19.5% - 25.8%

74% to 80% false negatives

ELISA

Enzyme-linked Immunosorbent Assay



ELISA

Detects anaplasma ANTIBODY in serum

Specific for *A. marginale*....(*ovis*, *centrale*)

Not found in cattle

Not found in U.S.

Sample: serum (red top blood tube)

Cost: ~ \$8.00/sample

ELISA

Not extremely useful: very early infections (acute infection)

Experimentally infected calves

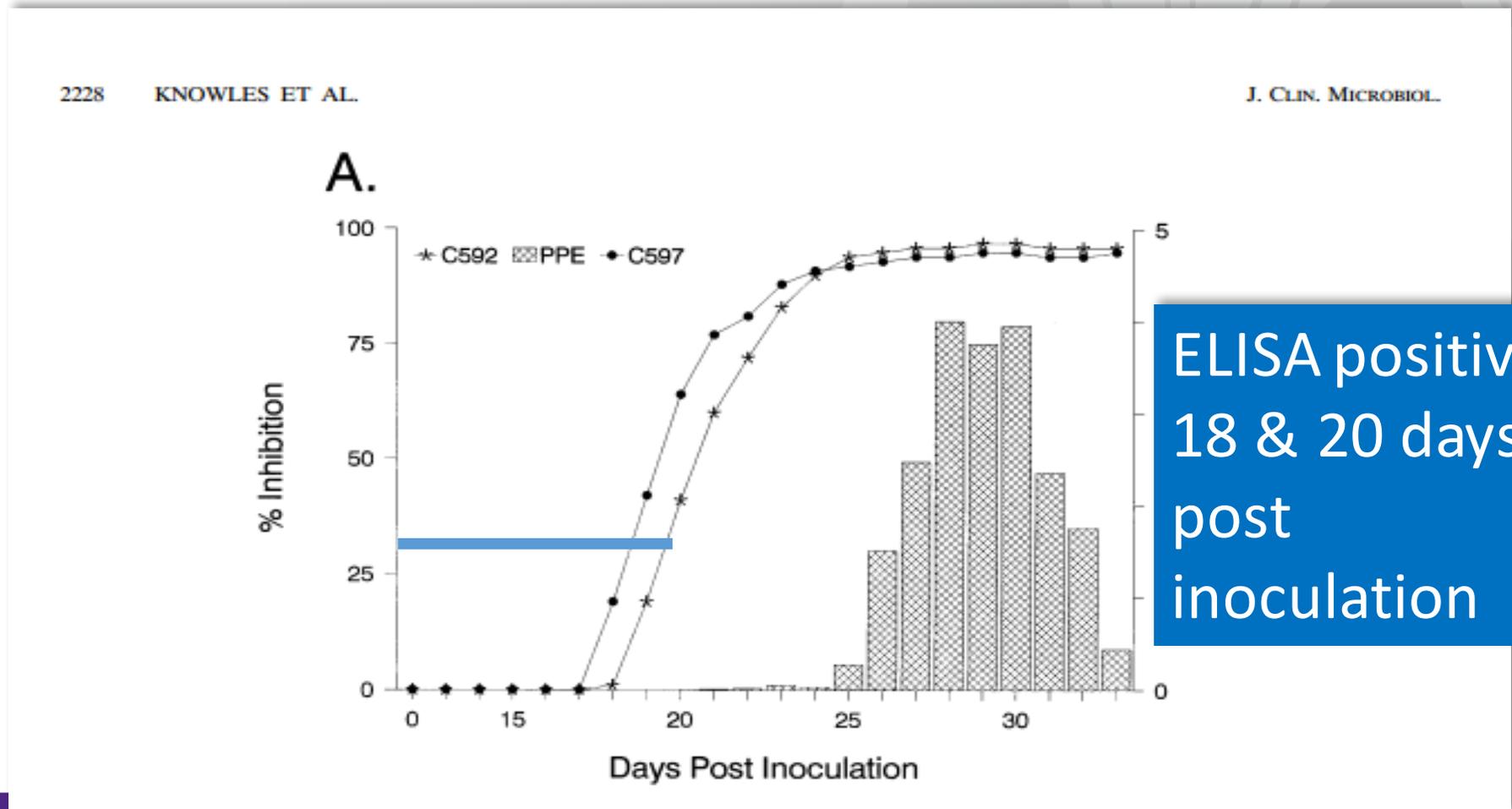
Sensitivity = 50.0% before day 10

Sensitivity = 99.9% after day 13 through day 156

Able to identify positive animals before 1% of RBC were infected

ELISA

After exposure to infected *D. andersonii* ticks

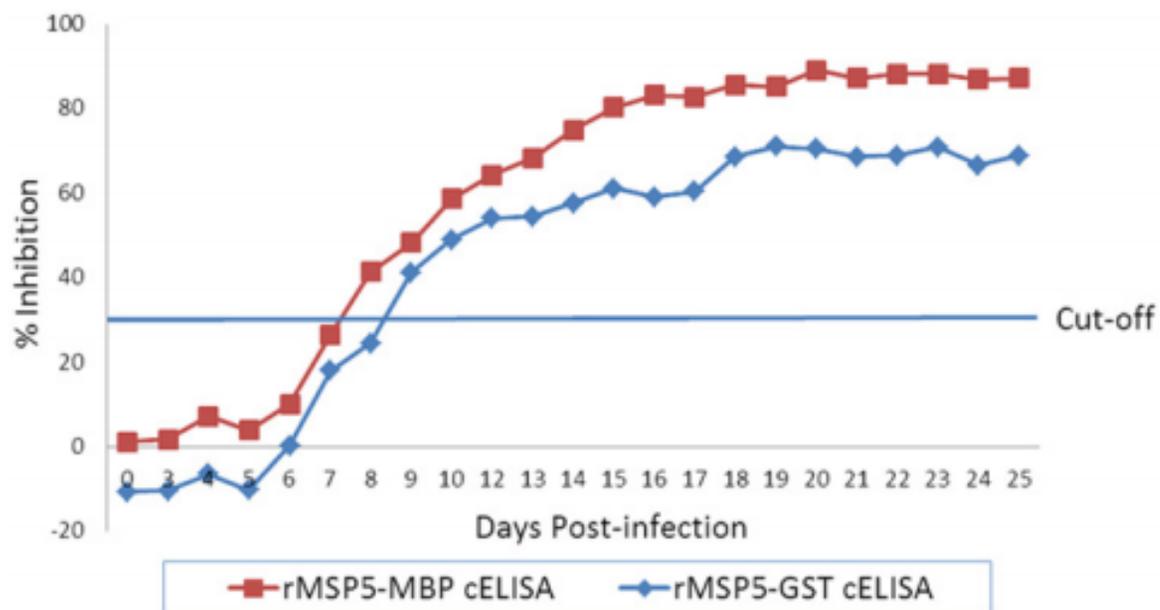


ELISA positive
18 & 20 days
post
inoculation

Serum ELISA

Improved performance of *Anaplasma* antibody cELISA

67



1 calf, naturally infected: ELISA positive on day 9

ELISA

Useful to identify carriers

In persistent (carriers), naturally infected animals

Sensitivity = 99.9%

0.1% false negative

Specificity = 99.7%

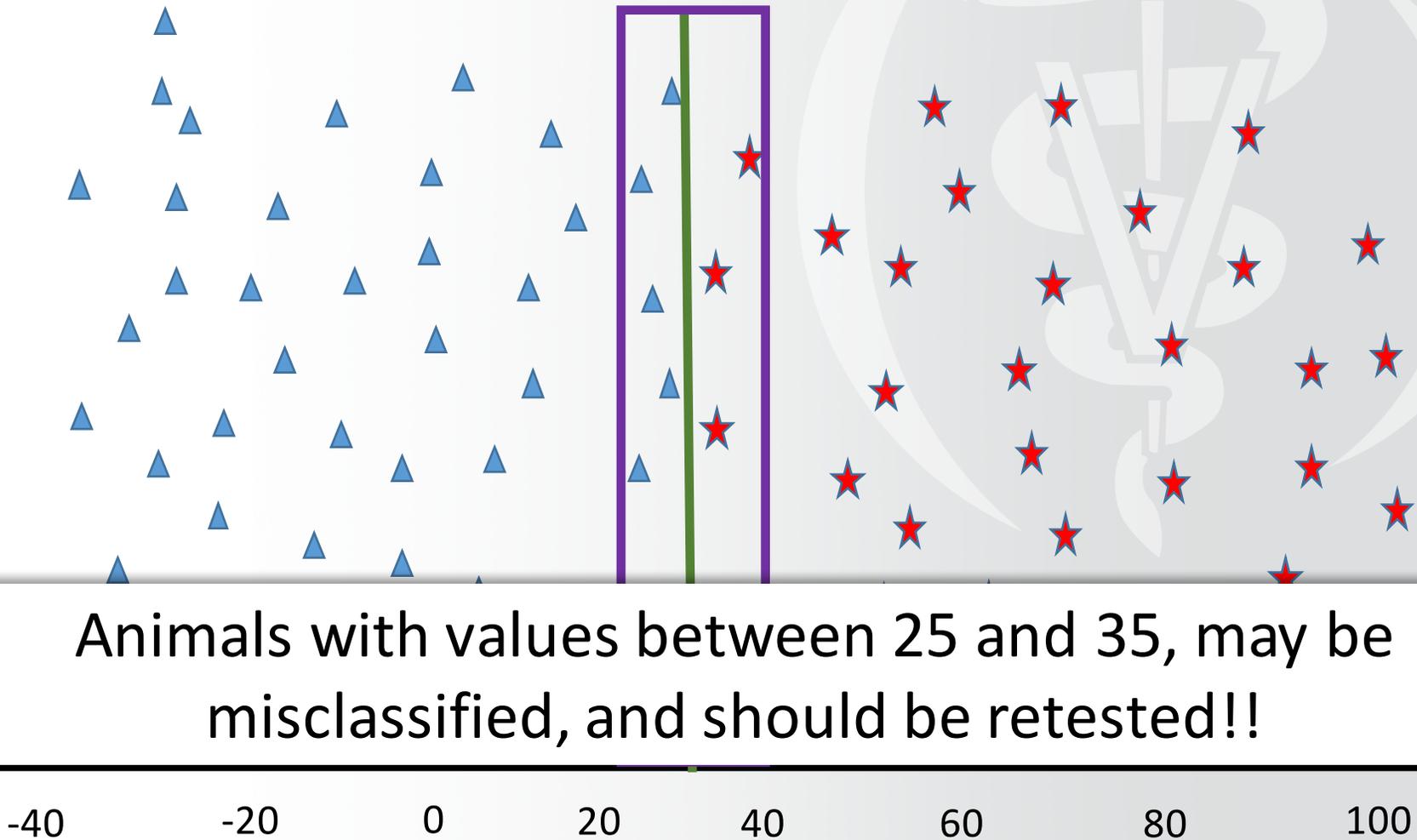
0.3% false positive

ELISA result reporting

Negative

Cut-off = 30% I

Positive



Animals with values between 25 and 35, may be misclassified, and should be retested!!

PCR

Polymerase Chain Reaction



PCR

Detects the ORGANISM (alive or dead) : (rRNA)

A. marginale and *A. phagocytophilum*

A. phagocytophilum

does not cause disease in cattle in the U.S.

prevalence in U.S. cattle is believed to be low (unknown)

carried by many species including dogs, wildlife, etc.

causative organism of human anaplasmosis

PCR

Detects the ORGANISM (alive or dead) : (rRNA)

Sample : whole blood (purple top tube)
: fresh spleen

Cost: \$32.00/sample
(Pool up to 5 animals for \$32.00 total)

Would not want to use pooling in suspect positive groups
(unless prevalence is very, very low)

PCR

Better than ELISA for early (acute) infections

Experimentally infected calves (3)

Detected on day 5 to 7 post infection

(ELISA 14-17 days post infection)

Experimentally infected calves (8)

Detected on day 21 post infection

(ELISA 42 days post infection)

PCR

Comparable to ELISA in persistently infected animals

Experimentally infected, persistent animals

Sensitivity = very good

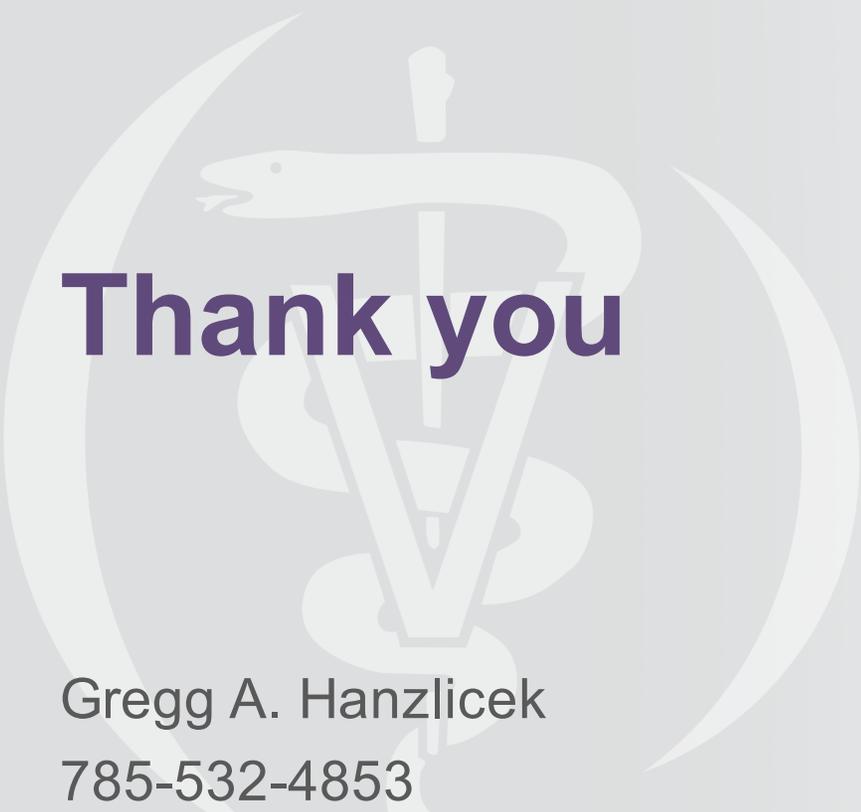
Positive = one organism in sample

Specificity = very good

Not any estimate in the literature to the diagnostic sensitivity of this PCR

Method	Freedom from infection: population	Individual animal: freedom from infection	Confirm clinical cases	Prevalence of infection: surveillance
Microscopic examination	-	+	+++	-
PCR	-	+++	+++	-
ELISA	+++	+	-	+++

Method	Freedom from infection: population	Individual animal: freedom from infection	Confirm clinical cases	Prevalence of infection: surveillance
Microscopic examination	-	+	+++	-
PCR	-	+++	+++	-
Wait until 3 weeks post exposure				
ELISA	+++	+	-	+++
Wait until 3-6 weeks post exposure				



Thank you

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