Strangles

"The disease that will not go away"

BAYER

Science For A Better Life

THE UNIVERSITY OF
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Te Whare Wānanga o Waikato

N.Z. EQUINE
RESEARCH
FOUNDATION

Ray Cursons

Talk Outline

- **Strangles**-The Disease
- **Getting to grips with strangles**-The microbiology of *Streptococcus equi*
- **Vaccination** for the prevention of Strangles
- **New research** on other group C beta haemolytic streptococci (*Streptococcus zooepidemicus*)

What is Strangles

- Highly contagious upper respiratory tract infection of equids
- Referenced to in Europe since the 13th century
- Worldwide, most diagnosed infectious diseases in horses
- Posing major welfare and economic impacts

Symptoms

- First clinical signs appear 3-14 days
- Fever
- Mucopurulent Nasal Discharge
- Anorexic
- Depressed

Symptoms

- Abscessation of head and neck lymph nodes

Symptoms

- Severity can vary greatly depending on level of immunity and stress
- Fully recover within 4-6 weeks
- Complications ~20 % of which 40 % may die or be euthanized
  - Metastatic spread of infection referred to as ‘bastard strangles’

Why can't antibiotics control an outbreak?

- Treatment with certain antibiotics is effective early before abscesses rupture in individual animals
- Must isolate any suspected animals as soon as possible
- Must be vigilant and follow infection control guidelines, eg always work from non-infected animals to infected animals, separate halters, brushes water troughs and dedicated overalls

Why is it a big deal?

- High transmission rate of 85-100 % in susceptible populations
- $ Costs to control an outbreak $100,000 plus
- Transmitted either directly by head to head contact or indirectly by fomites
- Difficult to control if chronic carriage, no symptoms and sporadic shedding over months to years
- Chronic Carriage
  - 10 % infected horses
  - Chondroids in the guttural pouch


Eardrum
Eustachian Tube
Guttural Pouch
Pharyngeal Opening of Eustachian Tube

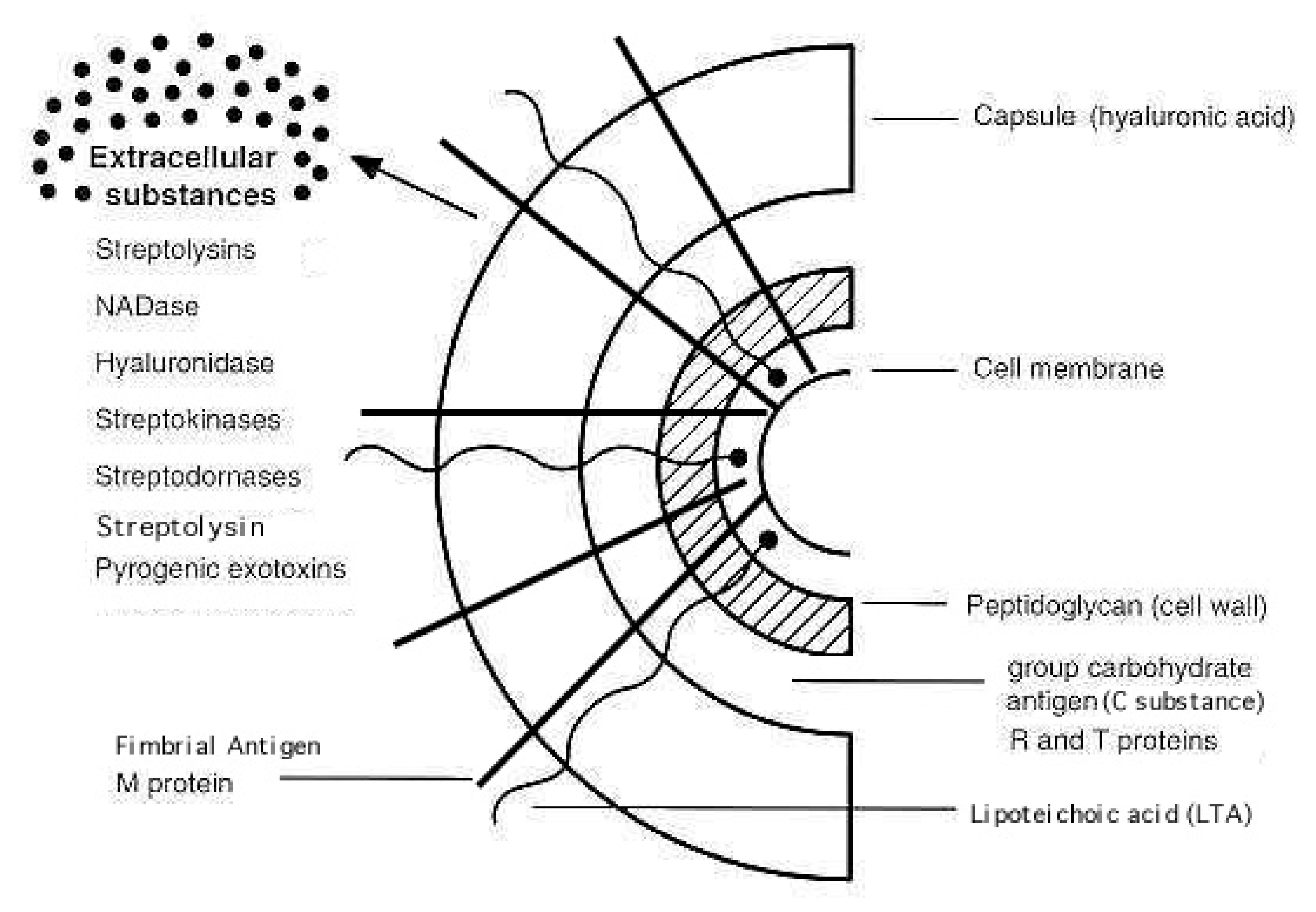
What causes strangles?

Streptococcus equi subsp. *equi*

- 128 different strains
- β-haemolytic
- Highly mucoid
-
- Lancefield group C
- Host-restricted

Courtesy Prof John Timoney

Strep Virulence Factors



How do you detect the bug?

- Culture
  - Was considered the gold standard
  - ≥ 2 days
  - Low initial numbers, overgrowth of contaminating bacteria
  - Complicated by other β-haemolytic streptococci *S. zooepidemicus* and *S. equisimilis.*

What is the best method of detection?

- Polymerase Chain reaction is now considered the best diagnostic test for *Streptococcus equi*
- Is highly sensitive "*Can find the needle in the hay-stack*"
- No interference from other equine Group C streptococci

Multiplex PCR **= Tetraplex**

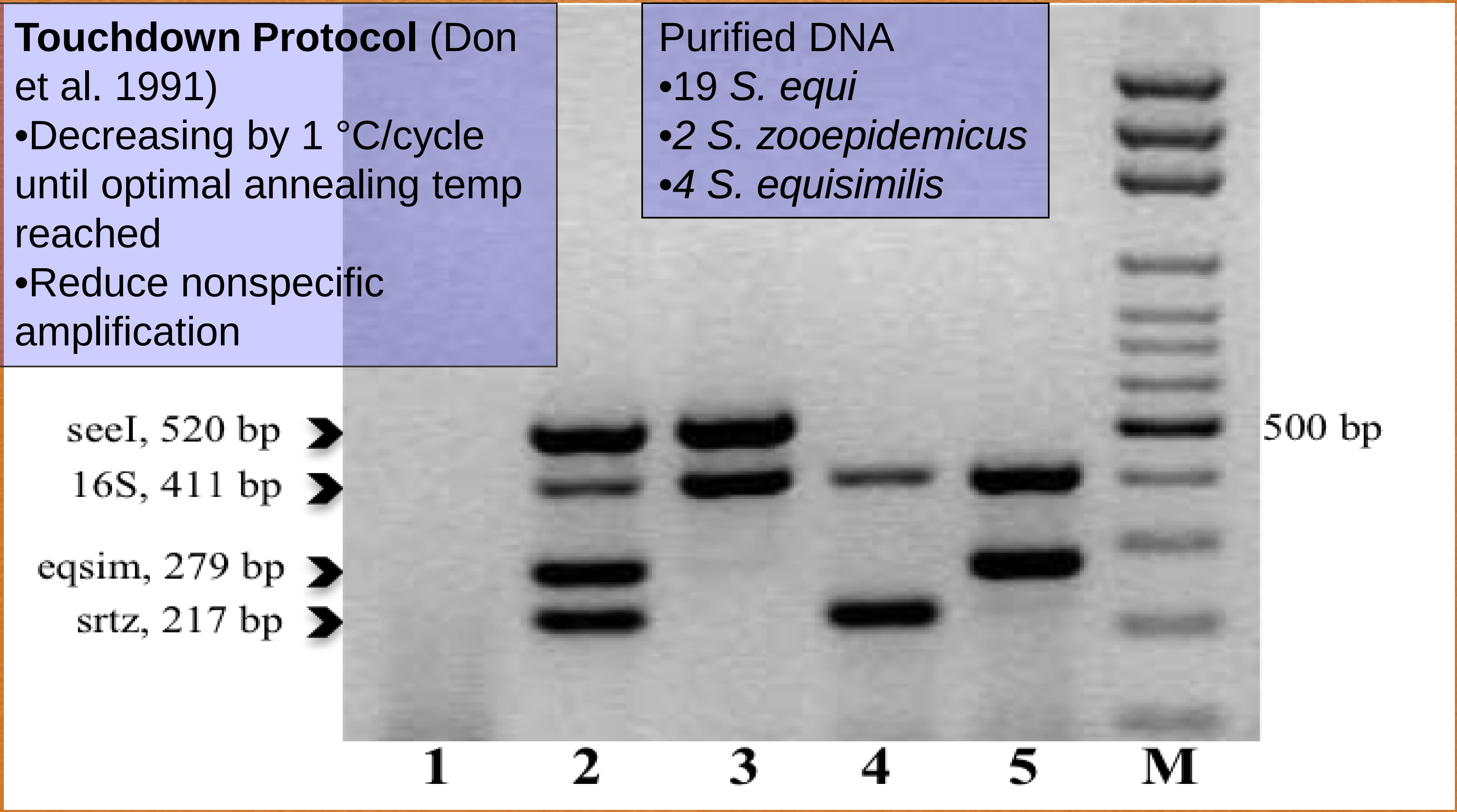
- Differentiate all three β-haemolytic streptococci
  - Species-specific primers

- *S. equi*
  - Super antigenic toxin SeeI gene (Alber et al. 2004) = ***seel***
- ***S. zooepidemicus***
  - Putative sortase gene *srtC2*, SZO 1827 = ***srtz***
- ***S. equisimilis***
  - Streptokinase precursor gene, SDEG 1020 (Preziuso et al. 2010) = ***equisim***
- ***16S rRNA***
  - Internal control to identify PCR inhibation
  - Universal to all bacteria (Boye et al. 1999) = ***16S***

Final Optimized Multiplex

Touchdown Protocol (Don et al. 1991)
- Decreasing by 1 °C/cycle until optimal annealing temp reached
- Reduce nonspecific amplification

Purified DNA
- 19 *S. equi*
- *2 S. zooepidemicus*
- *4 S. equisimilis*



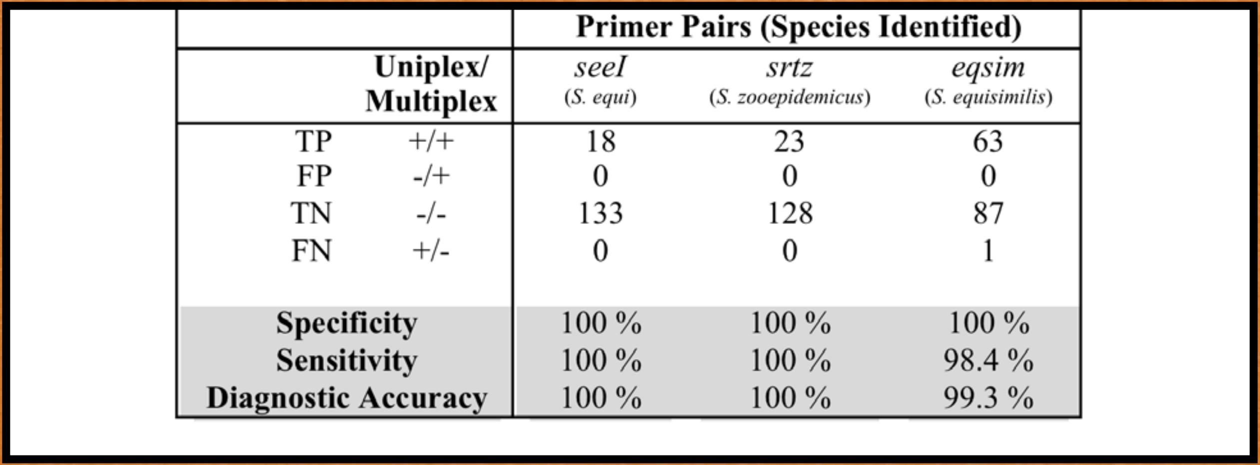
1= Neg, 2 = Mix, 3 = *S. equi*, 4 = *S. zooepidemicus, 5 = S. equisimilis*

What is the scientific-evidence that PCR is better?

- Multiplex trial
- Specimens
    - Nasopharyngeal or abscess swabs
    - Guttural pouch washes
    - 151 from clinical and non-symptomatic

Specificity, Sensitivity and Diagnostic Accuracy

- Assumption that Uniplex superior = reference standard

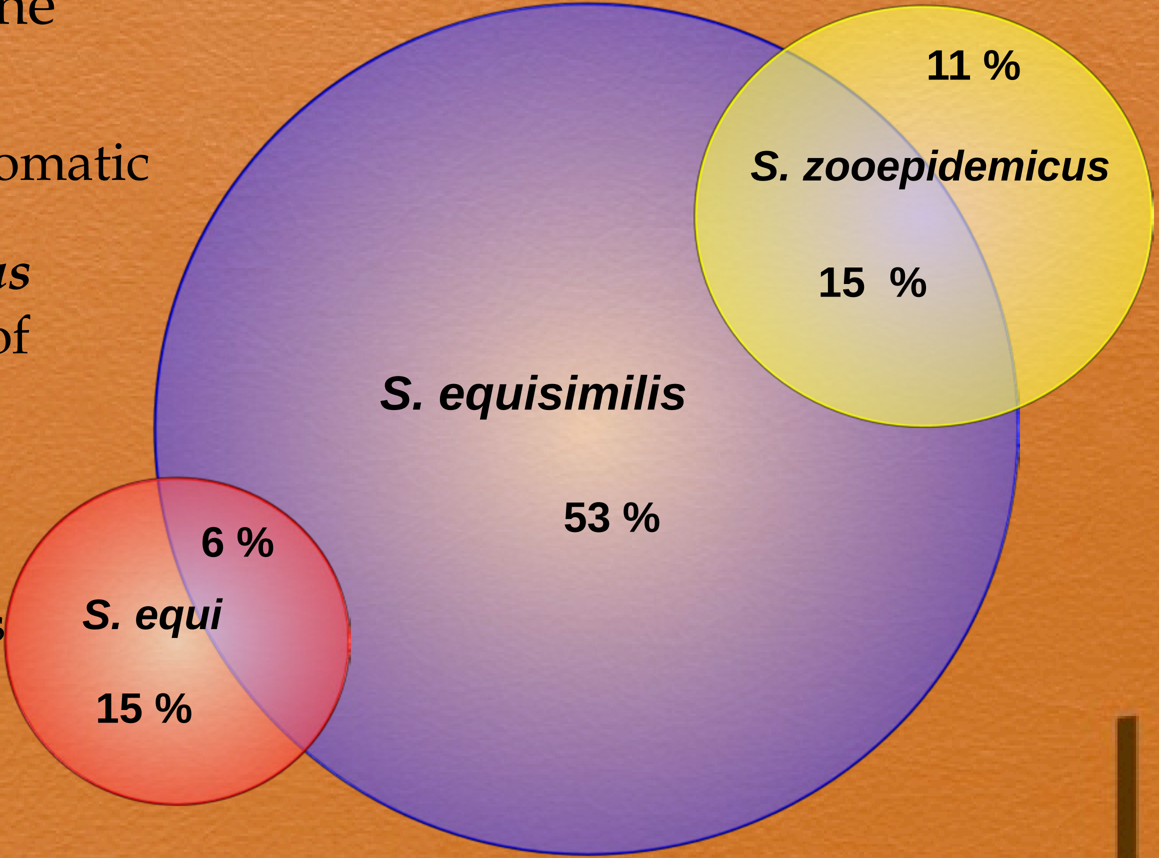


		Primer Pairs (Species Identified)		
	Uniplex/ Multiplex	*seeI* (*S. equi*)	*srtz* (*S. zooepidemicus*)	*eqsim* (*S. equisimilis*)
TP	+/+	18	23	63
FP	-/+	0	0	0
TN	-/-	133	128	87
FN	+/-	0	0	1
Specificity		100 %	100 %	100 %
Sensitivity		100 %	100 %	98.4 %
Diagnostic Accuracy		100 %	100 %	99.3 %

- TP = true positive; FP = false positive; TN = true negative; FN = false negative.
- Specificity = TN/(TN+FP); Sensitivity = TP/(TP+FN); Diagnostic Accuracy = (TP+TN)/(TP+FP+TN+FN).

Occurrence of Streptococci

- 151 Specimens
  - 58 % contained 1 of the 3 Strep of interest
  - All *S. equi* were sypmtomatic
  - 20% of ***S. zooepidemicus*** were displaying signs of respiratory disease
  - One ***S. zooepidemicus*** and ***S. equisimilis*** had submandibular abscess
  - 15% of ***S. equisimilis*** were displaying signs of respiratory disease



Diagnostic Conclusions

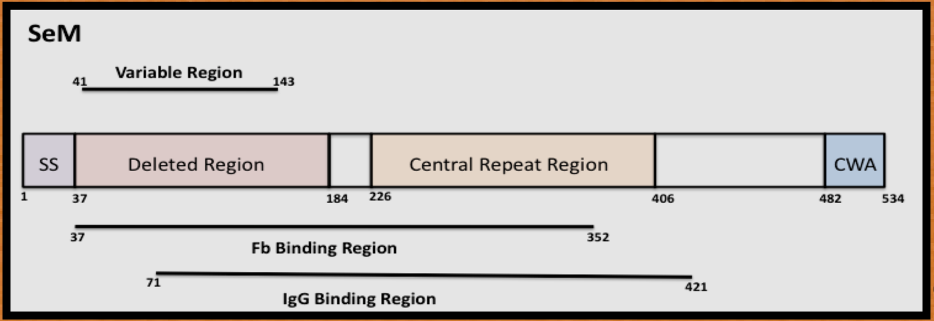
- Multiplex PCR
  - Rapidly, with equal accuracy of uniplex PCR, diagnose Strangles
  - Detect and differentiate *S. equi* from *S. zooepidemicus*, and *S. equisimilis*
  - Assist in determining the role and significance of *S. zooepidemicus* and *S. equisimilis* in respiratory diseases

Why is strain typing important?

- At least128 different strains of S equi worldwide
- Have strain-specific immunity for up to 5 years following recovery from infection
- NZ has only 2 known strains and are these strains covered by the commercial vaccine?
- Do both strains of S equi occur throughout NZ?

SeM Typing

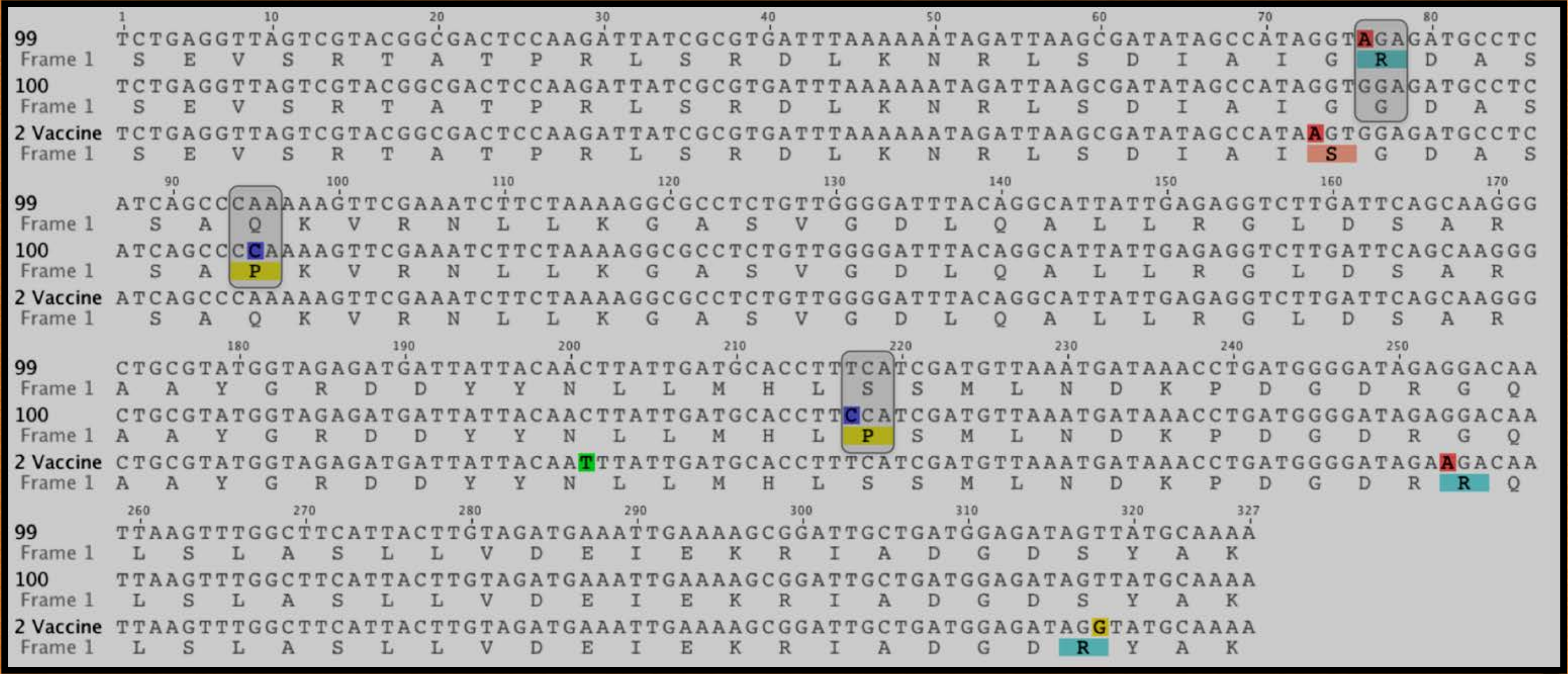
- Novel cell wall associated M-like protein – SeM
    - Proposed link for the increased virulence of S. equi over its archetype S. zooepidemicus (Timoney et al. 1997)
    - Antiphagocytic activity



(Adapted from Kelly et al. 2006)

- Sequencing the hypervariable region following the N-terminal signal sequence and Identifying SNP

- Vaccine types as *seM* 2
- 3 SNPs that differentiate SeM alleles 99 and 100
- *seM* gene is under strong diversifying selective pressure



```
           1        10        20        30        40        50        60        70        80
99         TCTGAGGTTAGTCGTACGGCGACTCCAAGATTATCGCGTGATTTAAAAAATAGATTAAGCGATATAGCCATAGGTAGAGATGCCTC
 Frame 1    S  E  V  S  R  T  A  T  P  R  L  S  R  D  L  K  N  R  L  S  D  I  A  I  G  R  D  A  S
100        TCTGAGGTTAGTCGTACGGCGACTCCAAGATTATCGCGTGATTTAAAAAATAGATTAAGCGATATAGCCATAGGTGGAGATGCCTC
 Frame 1    S  E  V  S  R  T  A  T  P  R  L  S  R  D  L  K  N  R  L  S  D  I  A  I  G  G  D  A  S
2 Vaccine  TCTGAGGTTAGTCGTACGGCGACTCCAAGATTATCGCGTGATTTAAAAAATAGATTAAGCGATATAGCCATAAGTGGAGATGCCTC
 Frame 1    S  E  V  S  R  T  A  T  P  R  L  S  R  D  L  K  N  R  L  S  D  I  A  I  S  G  D  A  S

              90        100       110       120       130       140       150       160       170
99         ATCAGCCCAAAAAGTTCGAAATCTTCTAAAAGGCGCCTCTGTTGGGGATTTACAGGCATTATTGAGAGGTCTTGATTCAGCAAGGG
 Frame 1     S  A  Q  K  V  R  N  L  L  K  G  A  S  V  G  D  L  Q  A  L  L  R  G  L  D  S  A  R
100        ATCAGCCCCAAAAGTTCGAAATCTTCTAAAAGGCGCCTCTGTTGGGGATTTACAGGCATTATTGAGAGGTCTTGATTCAGCAAGGG
 Frame 1     S  A  P  K  V  R  N  L  L  K  G  A  S  V  G  D  L  Q  A  L  L  R  G  L  D  S  A  R
2 Vaccine  ATCAGCCCAAAAAGTTCGAAATCTTCTAAAAGGCGCCTCTGTTGGGGATTTACAGGCATTATTGAGAGGTCTTGATTCAGCAAGGG
 Frame 1     S  A  Q  K  V  R  N  L  L  K  G  A  S  V  G  D  L  Q  A  L  L  R  G  L  D  S  A  R

                  180       190       200       210       220       230       240       250
99         CTGCGTATGGTAGAGATGATTATTACAACTTATTGATGCACCTTTCATCGATGTTAAATGATAAACCTGATGGGGATAGAGGACAA
 Frame 1   A  A  Y  G  R  D  D  Y  Y  N  L  L  M  H  L  S  S  M  L  N  D  K  P  D  G  D  R  G  Q
100        CTGCGTATGGTAGAGATGATTATTACAACTTATTGATGCACCTTCCATCGATGTTAAATGATAAACCTGATGGGGATAGAGGACAA
 Frame 1   A  A  Y  G  R  D  D  Y  Y  N  L  L  M  H  L  P  S  M  L  N  D  K  P  D  G  D  R  G  Q
2 Vaccine  CTGCGTATGGTAGAGATGATTATTACAATTTATTGATGCACCTTTCATCGATGTTAAATGATAAACCTGATGGGGATAGAAGACAA
 Frame 1   A  A  Y  G  R  D  D  Y  Y  N  L  L  M  H  L  S  S  M  L  N  D  K  P  D  G  D  R  R  Q

            260       270       280       290       300       310       320    327
99         TTAAGTTTGGCTTCATTACTTGTAGATGAAATTGAAAAGCGGATTGCTGATGGAGATAGTTATGCAAAA
 Frame 1    L  S  L  A  S  L  L  V  D  E  I  E  K  R  I  A  D  G  D  S  Y  A  K
100        TTAAGTTTGGCTTCATTACTTGTAGATGAAATTGAAAAGCGGATTGCTGATGGAGATAGTTATGCAAAA
 Frame 1    L  S  L  A  S  L  L  V  D  E  I  E  K  R  I  A  D  G  D  S  Y  A  K
2 Vaccine  TTAAGTTTGGCTTCATTACTTGTAGATGAAATTGAAAAGCGGATTGCTGATGGAGATAGGTATGCAAAA
 Frame 1    L  S  L  A  S  L  L  V  D  E  I  E  K  R  I  A  D  G  D  R  Y  A  K
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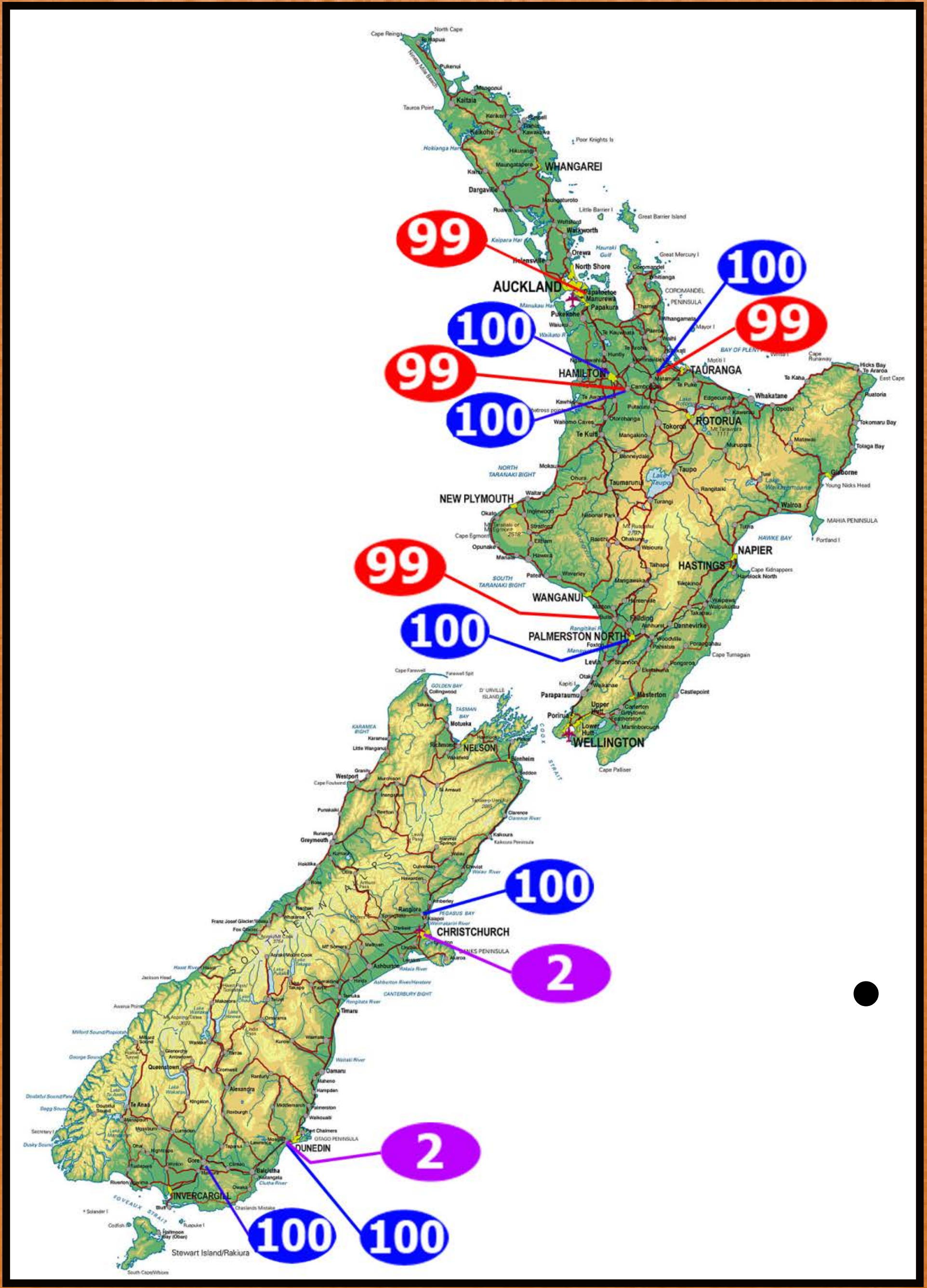
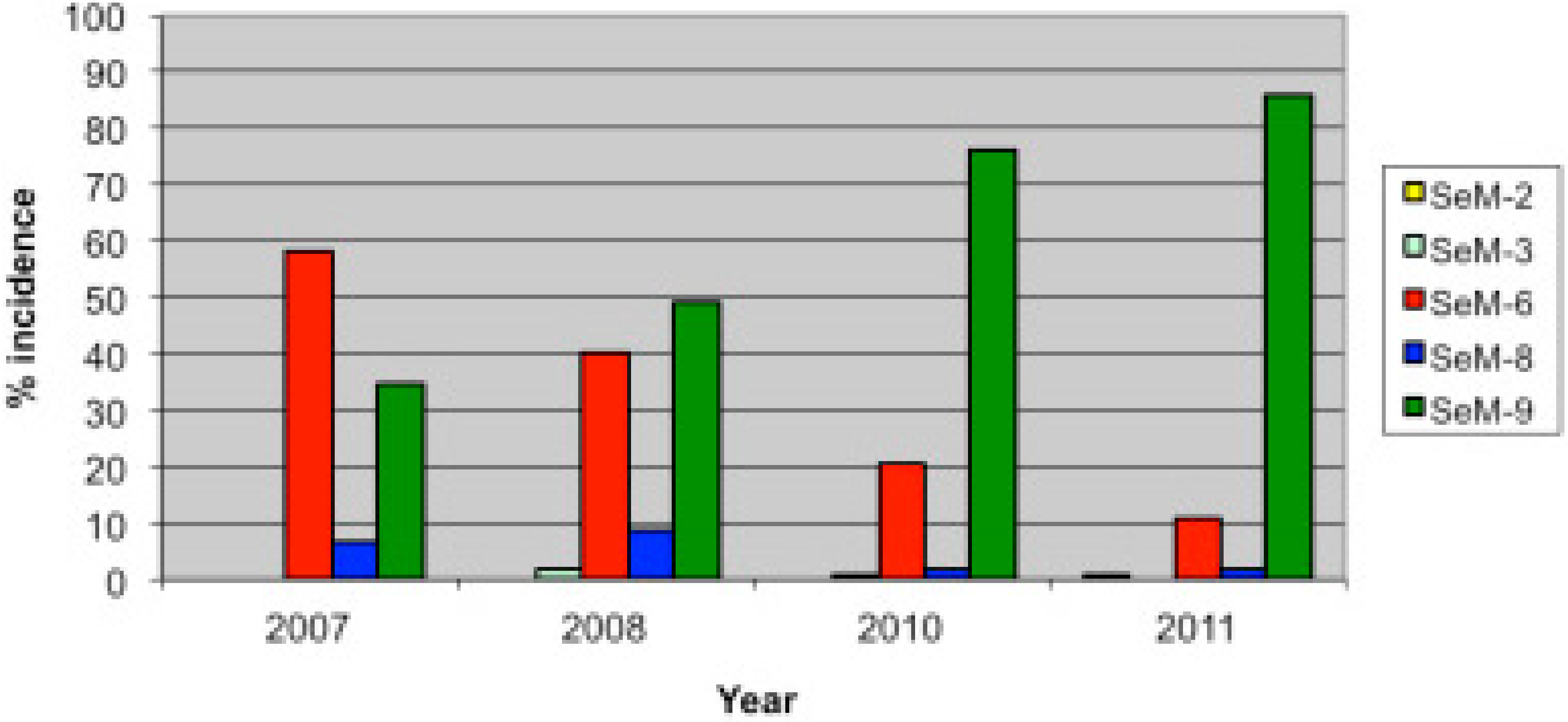
- SeM allele 99 only found on the North Island
- SeM allele 100 found on both Islands



- SeM allele 2, vaccine strain isolated from clinical foals


100
90
80
70
60
50
40
30
20
10
0
% incidence
2007
2008
2010
2011
Year
SeM-2
SeM-3
SeM-6
SeM-8
SeM-9

Are there vaccines to prevent strangles?

- Equivac S
- Pinnacle

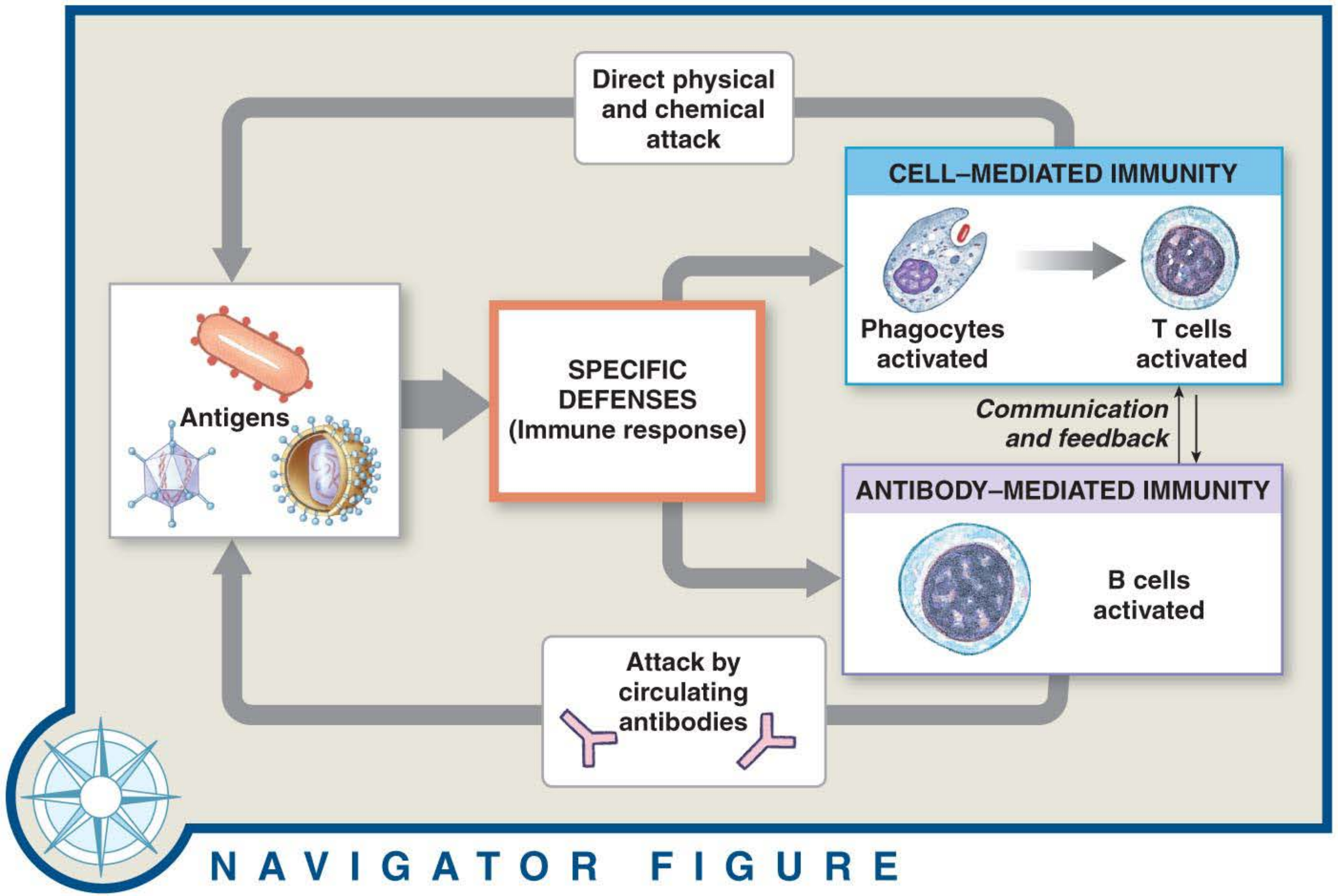
Equivac S Vaccine

- A sterile, cell-free extract rich in M-like and other proteins of *Streptococcus equi* (*S. equi*) adsorbed on an aluminium adjuvant.
- Produced by hot acid extraction of cells followed by acid precipitation, ammonium sulphate precipitation and column chromatography
- Administered by intramuscular injection X 3.

Pinnacle Vaccine

- Live, attenuated, non-encapsulated strain CF32 of *S equi*
- Produced by chemical mutagenesis of *S equi* strain CF32 with N-methyl-N′-nitro- nitrosoguanidine.
- Produces small dry colonies
- Differs from WT-*S equi* by 69 SNPs
- Induces local mucosal immunity via intra-nasal administration of the vaccine X 2.

Why must vaccinate animals more than once?



Body Responses to Bacterial Infection

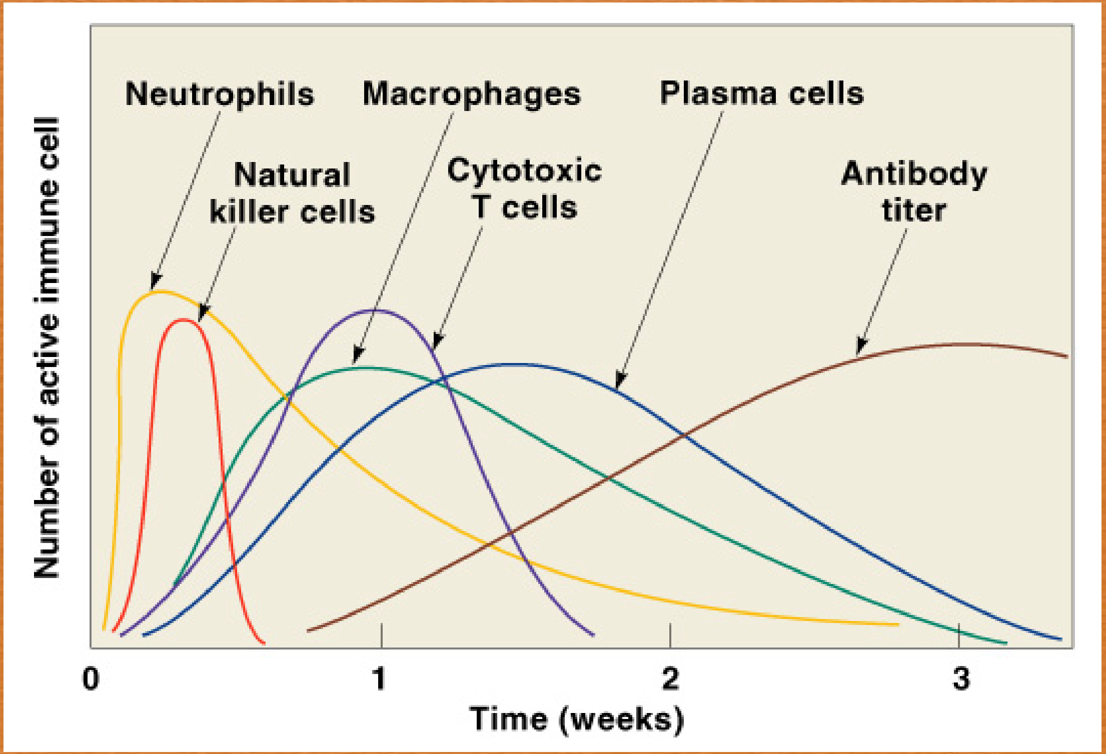


Figure 22–24

Primary and Secondary Responses

- Occur in both cell-mediated and antibody-mediated immunity

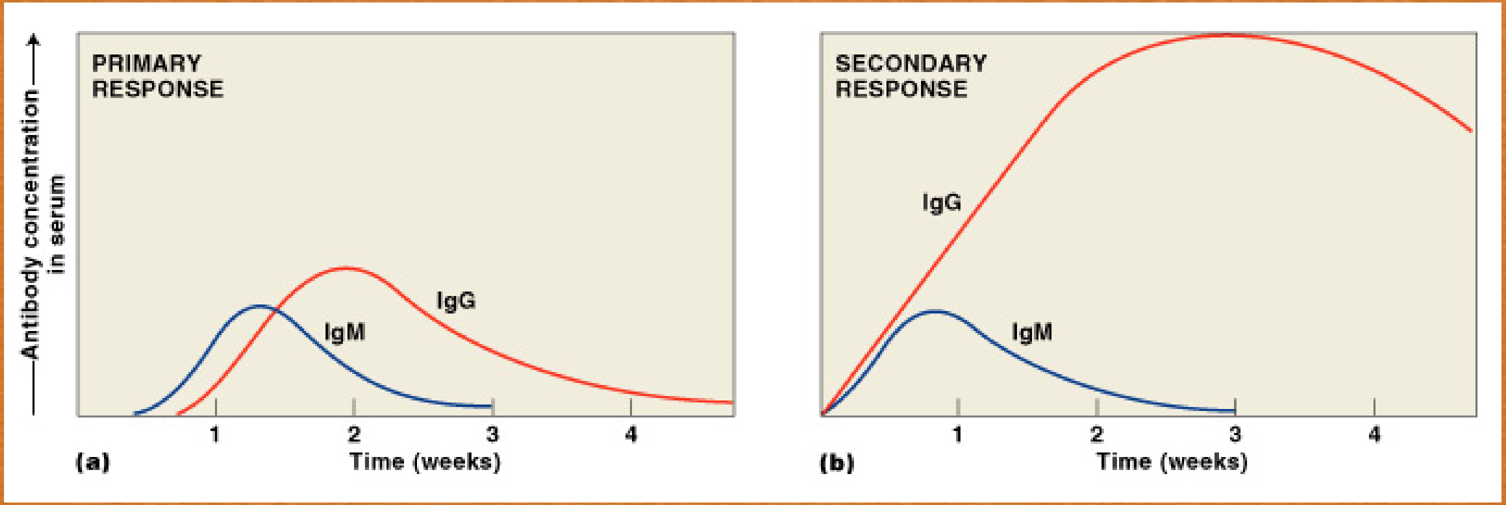
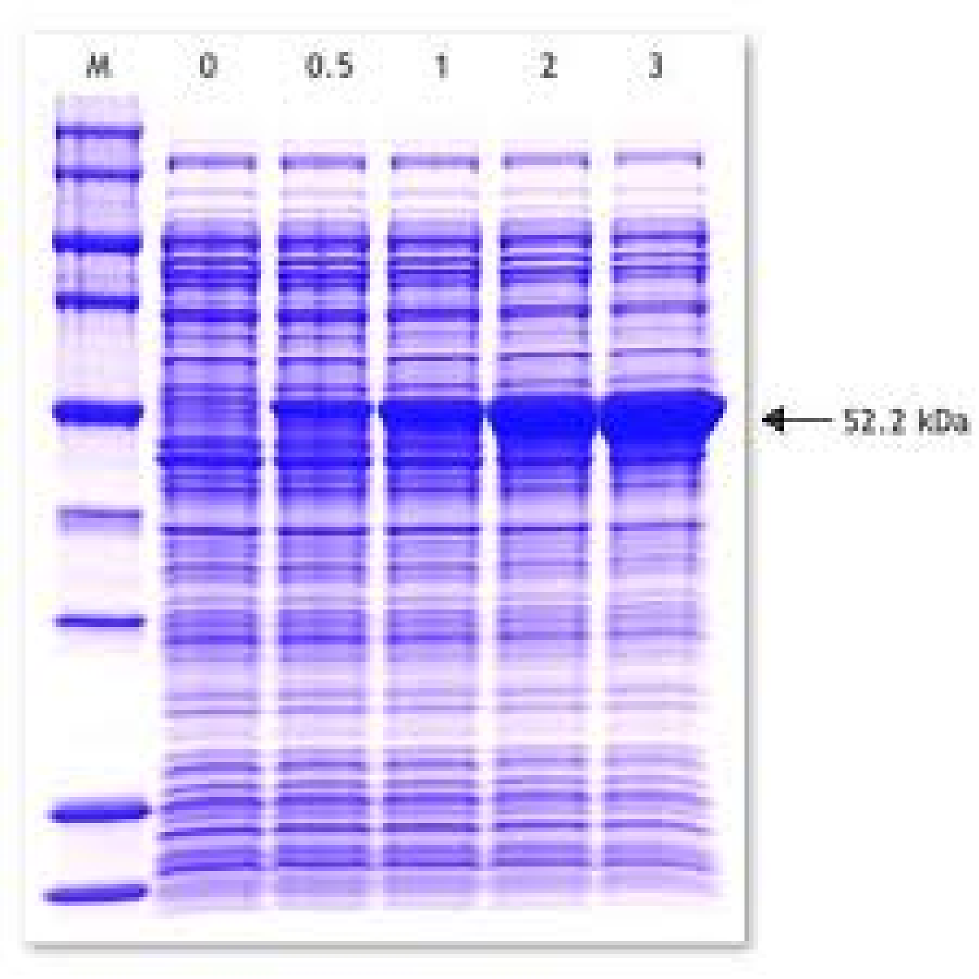


Figure 22–22

Do the vaccines work against NZ strains of *S equi* ?

- Serology of S equi
- Group 1. Pre-vaccinated weanling horses
- Group 2. Pinnacle post-vaccinated horses
- Group 3. Equivac S post-vaccinated horses
- Group 4. Horses from a stud undergoing a severe *S. equi* epidemic
- Group 5. Horses that had recovered from a natural infection of *S. equi*.

Western blot



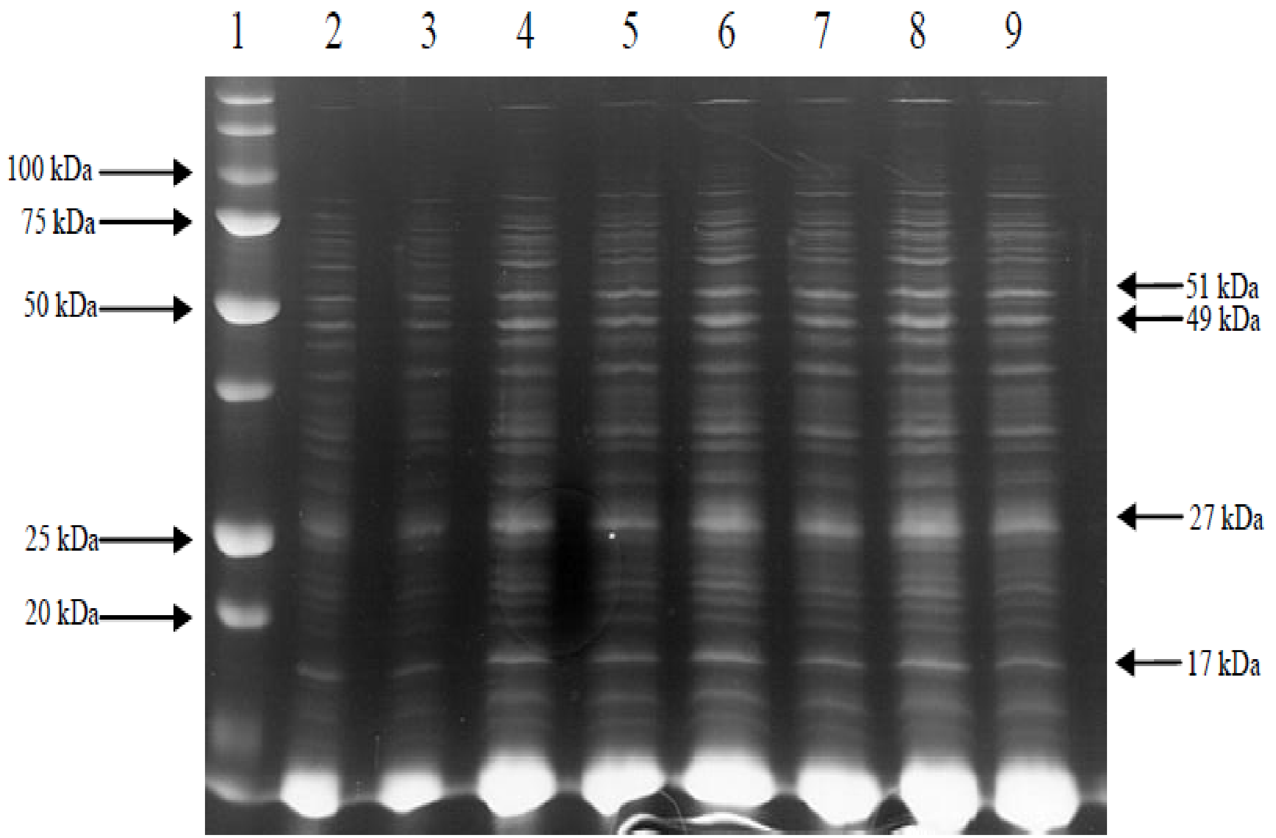
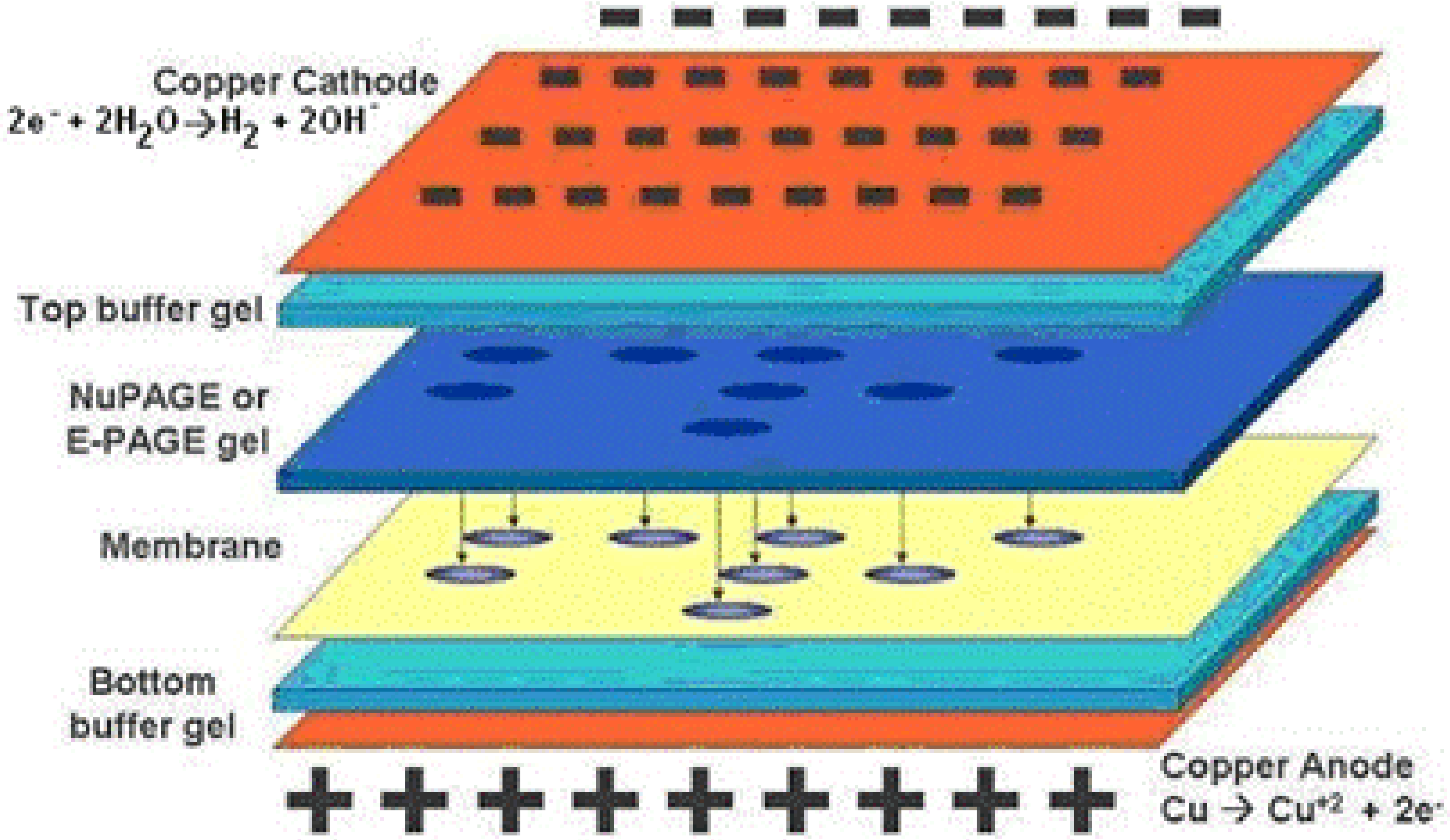


Figure 3.1 SDS-PAGE gel results of proteins extracted from *S. equi* strain 99, 100 and vaccine strain. Lane 1, protein ladder; lane 2 to 4, 5 uL of protein samples from *S. equi* strain 99, 100 and vaccine strain, respectively; lane 5 to 7, 10 uL of protein samples from *S. equi* strain 99, 100 and vaccine strain, respectively; lane 8 and 9, 15 uL of protein samples from *S. equi* strain 99 and 100 separately.


Copper Cathode
$2e^- + 2H_2O \rightarrow H_2 + 2OH^-$
Top buffer gel
NuPAGE or E-PAGE gel
Membrane
Bottom buffer gel
Copper Anode
$Cu \rightarrow Cu^{+2} + 2e^-$

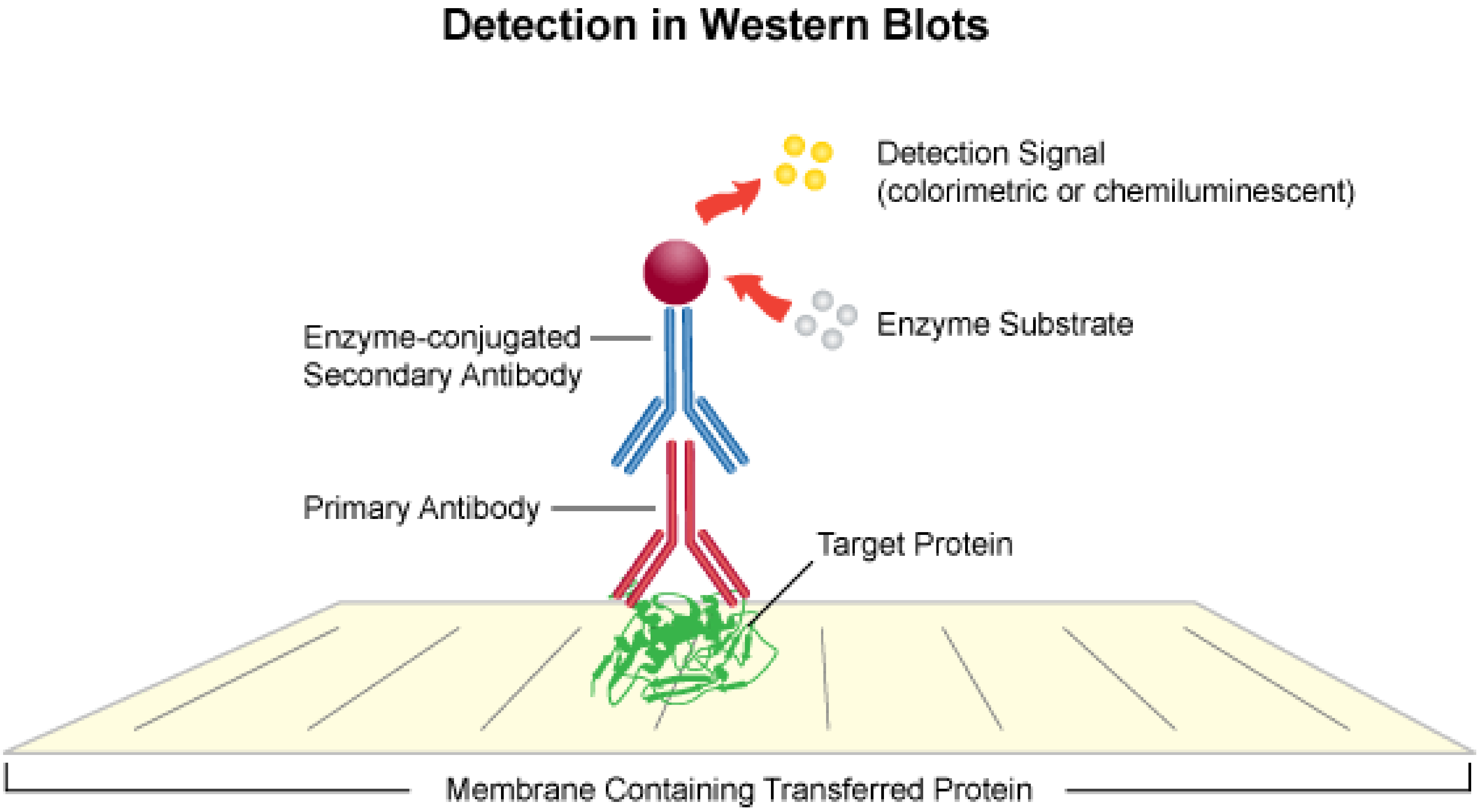
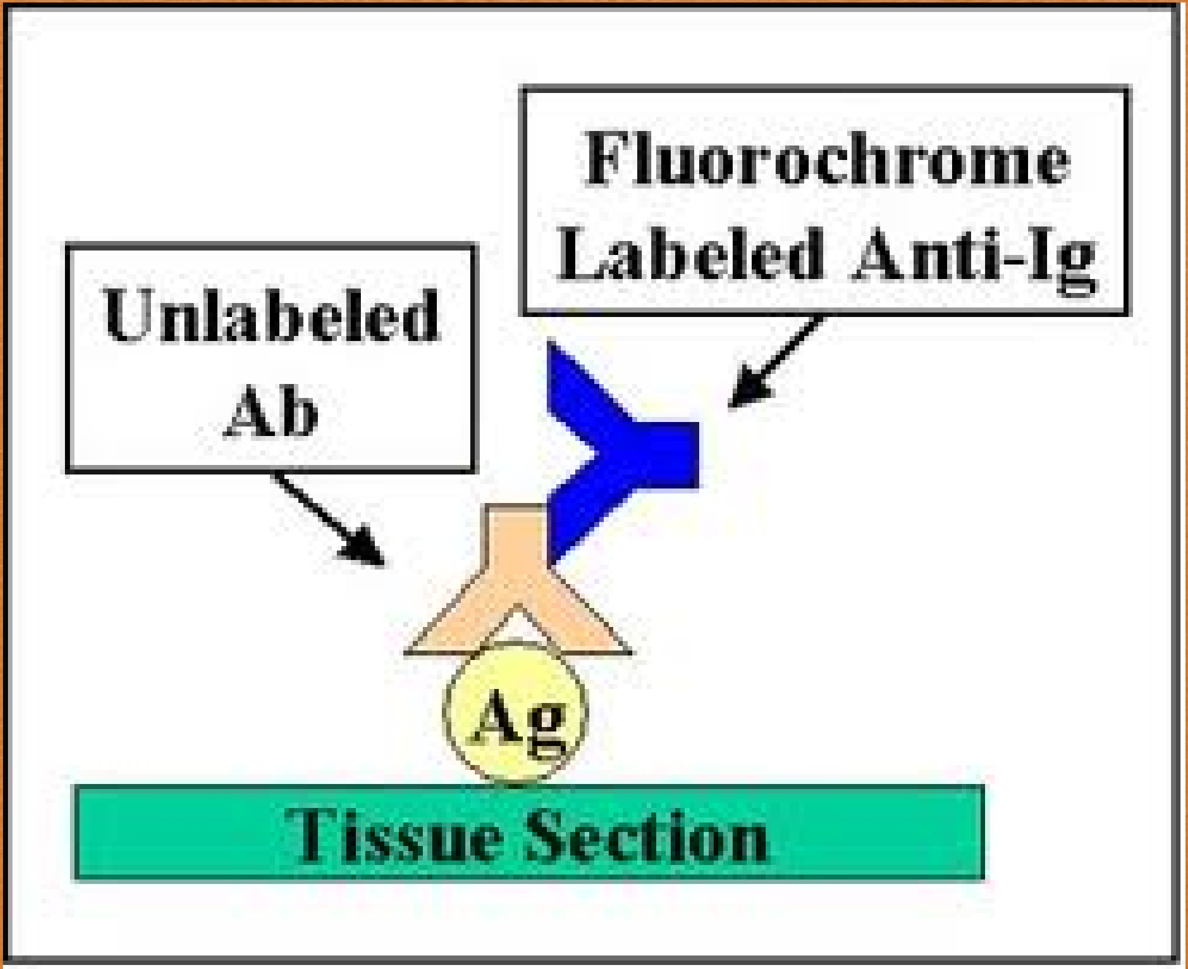


Diagram 2: *Illustration of detection in Western Blots.*

Indirect fluorescent antibody test



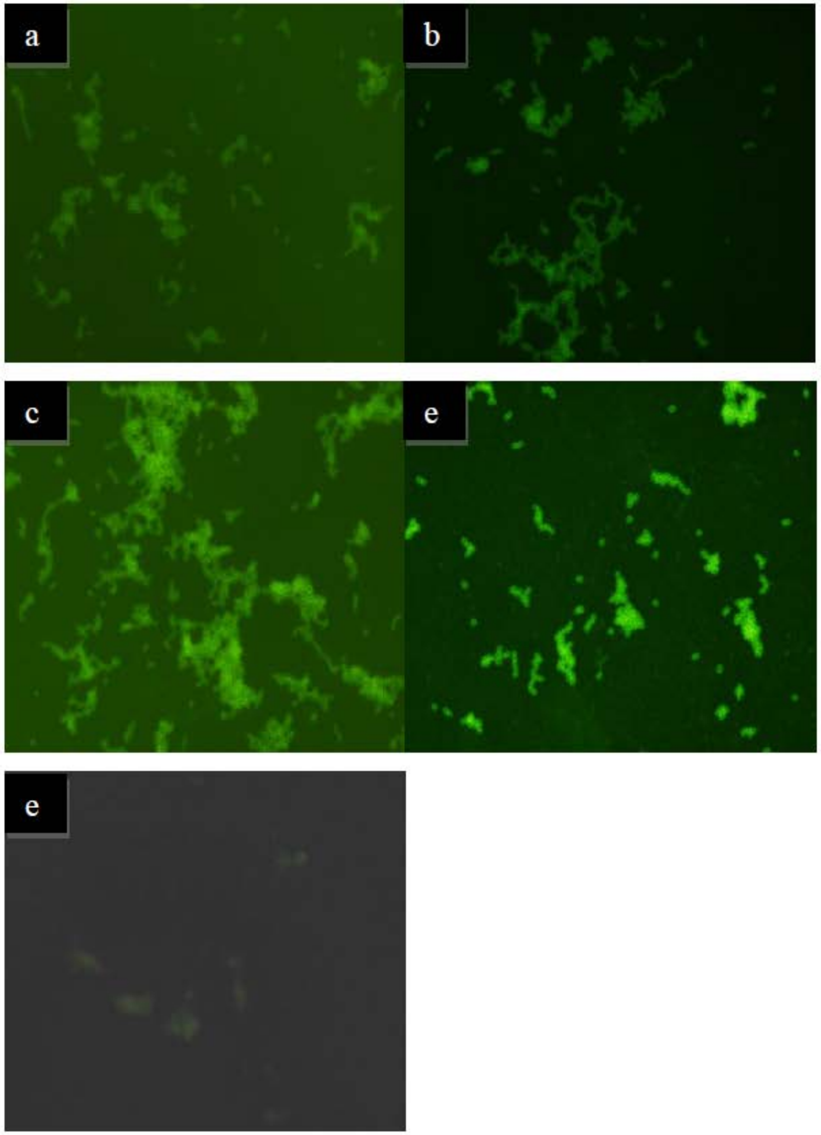
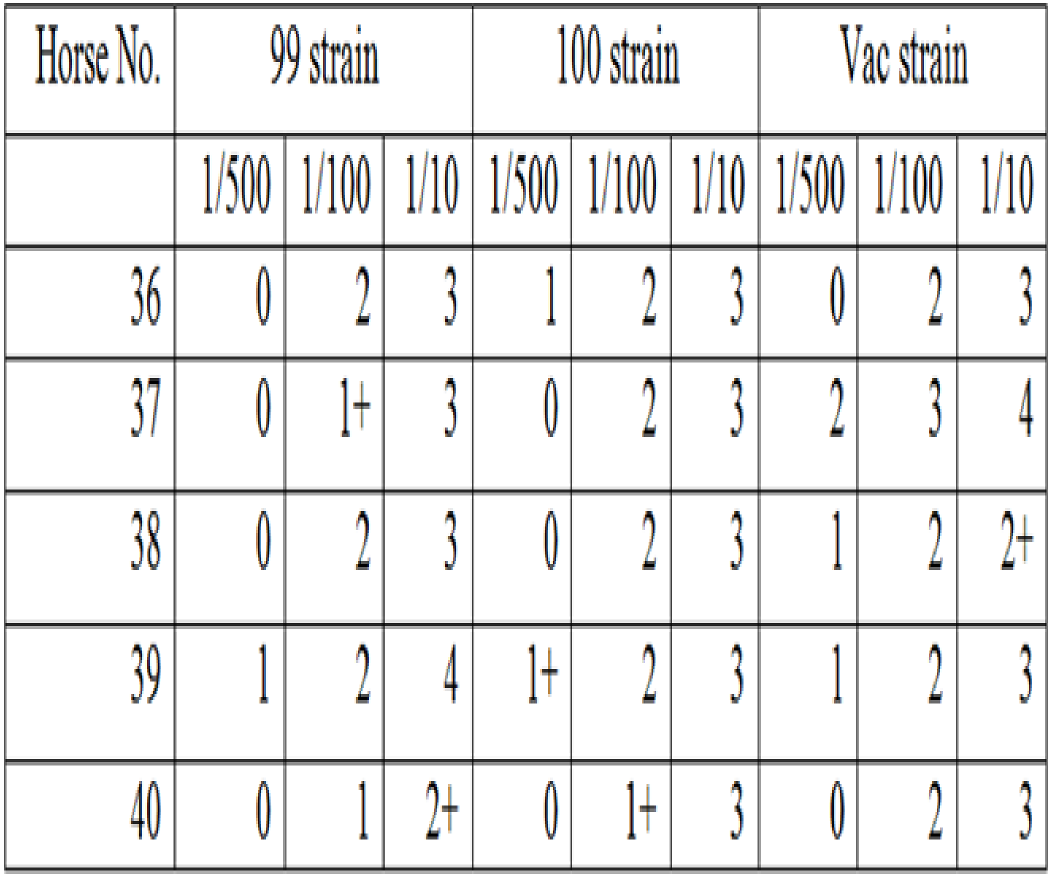


Figure 3.8 Different scores of IFA results. a.) score 1. b.) score 2. c.) score 3. d.) score 4. e.) score 0 (negative control).

Table 3.1 IFA result of pre-vaccinated sera from stud O



Horse No.	99 strain			100 strain			Vac strain		
	1/500	1/100	1/10	1/500	1/100	1/10	1/500	1/100	1/10
36	0	2	3	1	2	3	0	2	3
37	0	1+	3	0	2	3	2	3	4
38	0	2	3	0	2	3	1	2	2+
39	1	2	4	1+	2	3	1	2	3
40	0	1	2+	0	1+	3	0	2	3

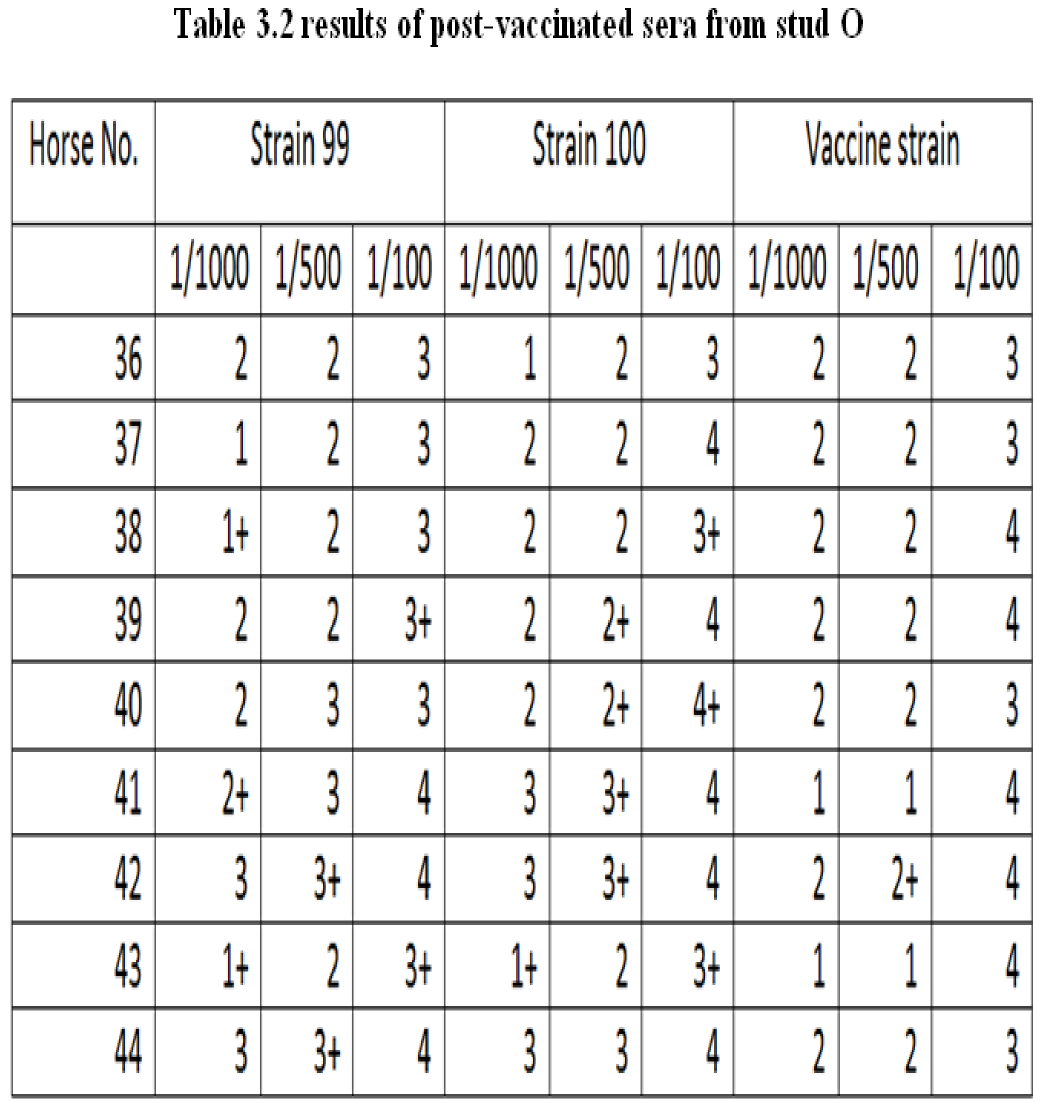


Table 3.2 results of post-vaccinated sera from stud O

Horse No.	Strain 99			Strain 100			Vaccine strain		
	1/1000	1/500	1/100	1/1000	1/500	1/100	1/1000	1/500	1/100
36	2	2	3	1	2	3	2	2	3
37	1	2	3	2	2	4	2	2	3
38	1+	2	3	2	2	3+	2	2	4
39	2	2	3+	2	2+	4	2	2	4
40	2	3	3	2	2+	4+	2	2	3
41	2+	3	4	3	3+	4	1	1	4
42	3	3+	4	3	3+	4	2	2+	4
43	1+	2	3+	1+	2	3+	1	1	4
44	3	3+	4	3	3	4	2	2	3

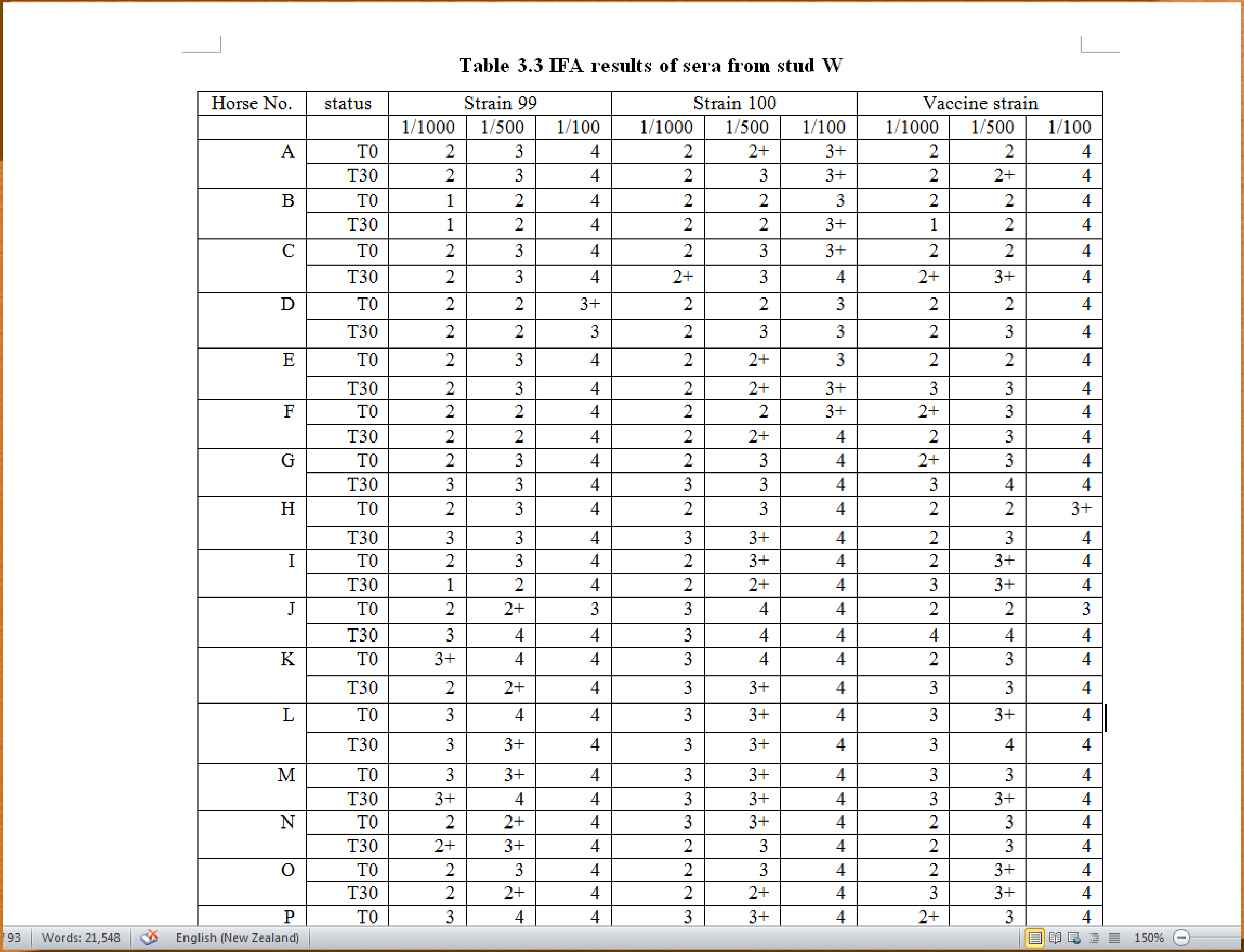


Table 3.3 IFA results of sera from stud W

Horse No.	status	Strain 99			Strain 100			Vaccine strain		
		1/1000	1/500	1/100	1/1000	1/500	1/100	1/1000	1/500	1/100
A	T0	2	3	4	2	2+	3+	2	2	4
	T30	2	3	4	2	3	3+	2	2+	4
B	T0	1	2	4	2	2	3	2	2	4
	T30	1	2	4	2	2	3+	1	2	4
C	T0	2	3	4	2	3	3+	2	2	4
	T30	2	3	4	2+	3	4	2+	3+	4
D	T0	2	2	3+	2	2	3	2	2	4
	T30	2	2	3	2	3	3	2	3	4
E	T0	2	3	4	2	2+	3	2	2	4
	T30	2	3	4	2	2+	3+	3	3	4
F	T0	2	2	4	2	2	3+	2+	3	4
	T30	2	2	4	2	2+	4	2	3	4
G	T0	2	3	4	2	3	4	2+	3	4
	T30	3	3	4	3	3	4	3	4	4
H	T0	2	3	4	2	3	4	2	2	3+
	T30	3	3	4	3	3+	4	2	3	4
I	T0	2	3	4	2	3+	4	2	3+	4
	T30	1	2	4	2	2+	4	3	3+	4
J	T0	2	2+	3	3	4	4	2	2	3
	T30	3	4	4	3	4	4	4	4	4
K	T0	3+	4	4	3	4	4	2	3	4
	T30	2	2+	4	3	3+	4	3	3	4
L	T0	3	4	4	3	3+	4	3	3+	4
	T30	3	3+	4	3	3+	4	3	4	4
M	T0	3	3+	4	3	3+	4	3	3	4
	T30	3+	4	4	3	3+	4	3	3+	4
N	T0	2	2+	4	3	3+	4	2	3	4
	T30	2+	3+	4	2	3	4	2	3	4
O	T0	2	3	4	2	3	4	2	3+	4
	T30	2	2+	4	2	2+	4	3	3+	4
P	T0	3	4	4	3	3+	4	2+	3	4

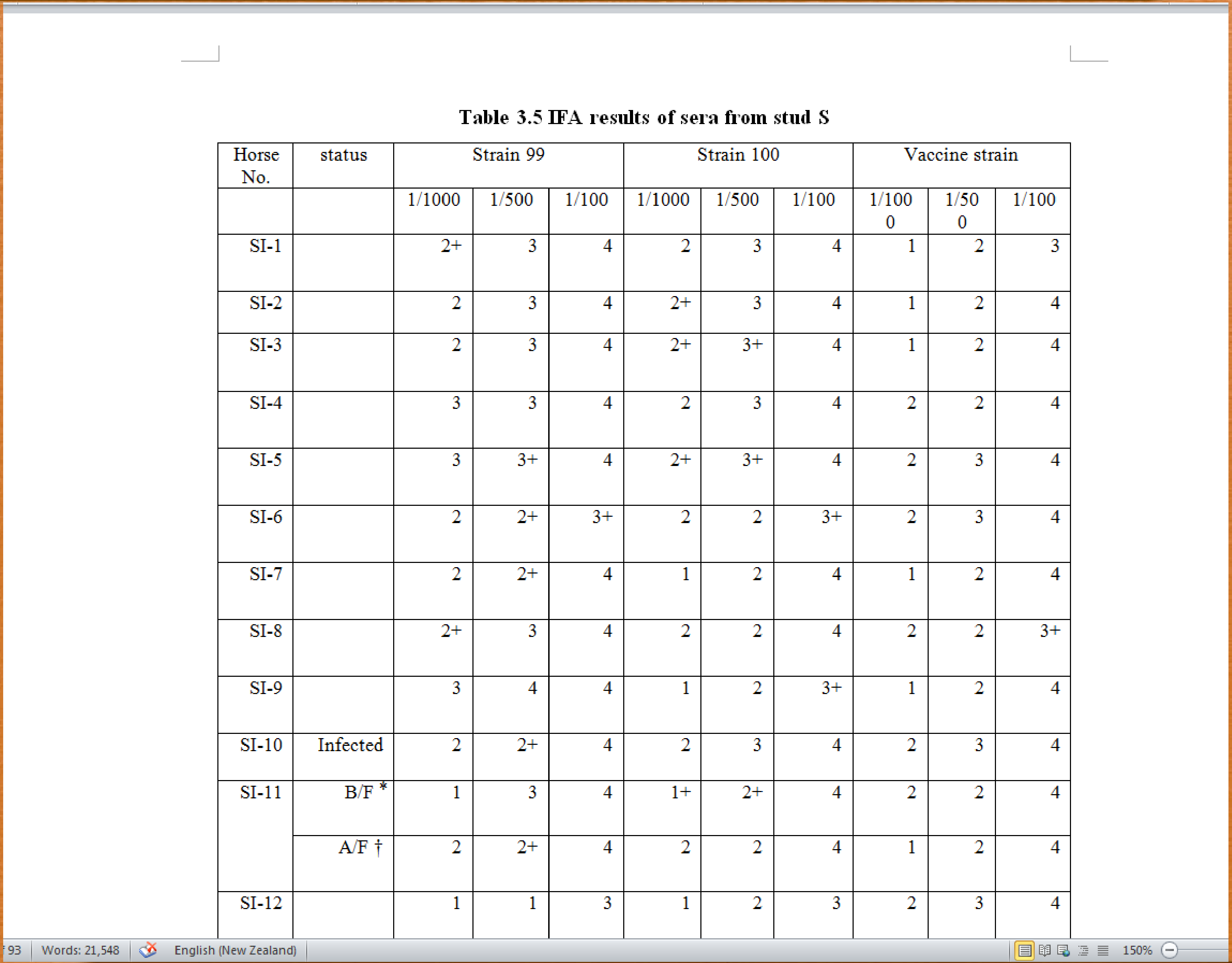


Table 3.5 IFA results of sera from stud S

Horse No.	status	Strain 99			Strain 100			Vaccine strain		
		1/1000	1/500	1/100	1/1000	1/500	1/100	1/1000	1/500	1/100
SI-1		2+	3	4	2	3	4	1	2	3
SI-2		2	3	4	2+	3	4	1	2	4
SI-3		2	3	4	2+	3+	4	1	2	4
SI-4		3	3	4	2	3	4	2	2	4
SI-5		3	3+	4	2+	3+	4	2	3	4
SI-6		2	2+	3+	2	2	3+	2	3	4
SI-7		2	2+	4	1	2	4	1	2	4
SI-8		2+	3	4	2	2	4	2	2	3+
SI-9		3	4	4	1	2	3+	1	2	4
SI-10	Infected	2	2+	4	2	3	4	2	3	4
SI-11	B/F *	1	3	4	1+	2+	4	2	2	4
	A/F †	2	2+	4	2	2	4	1	2	4
SI-12		1	1	3	1	2	3	2	3	4

Conclusions

- The results of western blotting, IFA show that the post-vaccinated horses had high levels of pre-existing antibodies within their sera to all three *S. equi* strains examined, even one year after vaccination.
- The existing high titres of pre-existing antibodies suggest that the serological response to the vaccines have plateaued and that vaccine boosters could be delayed for 2 years.
- IFA results showed that antibodies induced after vaccination or infection had similar immunological reactivity to those three *S. equi* strains tested.

Future Work WHY?

- Investigate the role of *Streptococcus zooepidemicus* in respiratory disease and genitourinary disease
- Incidence of respiratory disease is 5/100 per month.
- Clinical signs for each episode is about 8 weeks
- Large diversity between S zooepidemicus
- Traditional typing schemes cannot distinguish between outbreak strains and colonizing strains

Table 1. The distribution of beta-hemolytic streptococci isolates among fetuses, foals, and adult horses between 2000 and 2010.*.

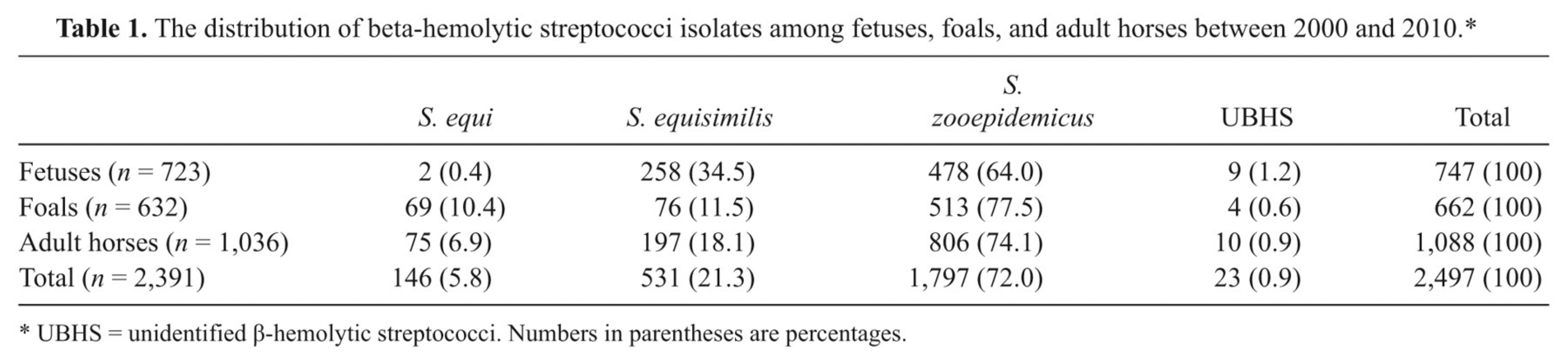


Table 1. The distribution of beta-hemolytic streptococci isolates among fetuses, foals, and adult horses between 2000 and 2010.*

	S. equi	*S. equisimilis*	*S. zooepidemicus*	UBHS	Total
Fetuses (*n* = 723)	2 (0.4)	258 (34.5)	478 (64.0)	9 (1.2)	747 (100)
Foals (*n* = 632)	69 (10.4)	76 (11.5)	513 (77.5)	4 (0.6)	662 (100)
Adult horses (*n* = 1,036)	75 (6.9)	197 (18.1)	806 (74.1)	10 (0.9)	1,088 (100)
Total (*n* = 2,391)	146 (5.8)	531 (21.3)	1,797 (72.0)	23 (0.9)	2,497 (100)

* UBHS = unidentified β-hemolytic streptococci. Numbers in parentheses are percentages.

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Table 2. Beta-hemolytic streptococci historical data between 2000 and 2010: organism and organ and/or tissue distributions.*.

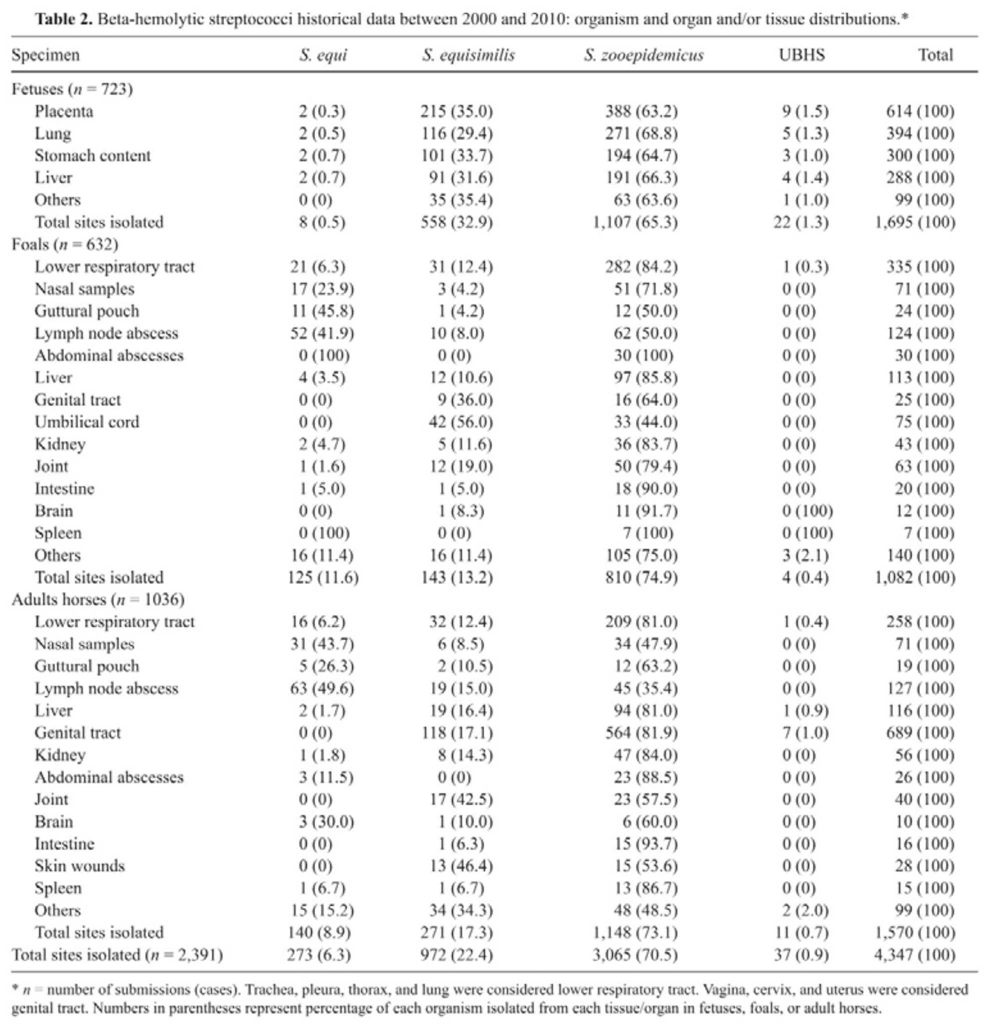

Table 2. Beta-hemolytic streptococci historical data between 2000 and 2010: organism and organ and/or tissue distributions.*

Specimen	*S. equi*	*S. equisimilis*	*S. zooepidemicus*	UBHS	Total
Fetuses (*n* = 723)					
Placenta	2 (0.3)	215 (35.0)	388 (63.2)	9 (1.5)	614 (100)
Lung	2 (0.5)	116 (29.4)	271 (68.8)	5 (1.3)	394 (100)
Stomach content	2 (0.7)	101 (33.7)	194 (64.7)	3 (1.0)	300 (100)
Liver	2 (0.7)	91 (31.6)	191 (66.3)	4 (1.4)	288 (100)
Others	0 (0)	35 (35.4)	63 (63.6)	1 (1.0)	99 (100)
Total sites isolated	8 (0.5)	558 (32.9)	1,107 (65.3)	22 (1.3)	1,695 (100)
Foals (*n* = 632)					
Lower respiratory tract	21 (6.3)	31 (12.4)	282 (84.2)	1 (0.3)	335 (100)
Nasal samples	17 (23.9)	3 (4.2)	51 (71.8)	0 (0)	71 (100)
Guttural pouch	11 (45.8)	1 (4.2)	12 (50.0)	0 (0)	24 (100)
Lymph node abscess	52 (41.9)	10 (8.0)	62 (50.0)	0 (0)	124 (100)
Abdominal abscesses	0 (100)	0 (0)	30 (100)	0 (0)	30 (100)
Liver	4 (3.5)	12 (10.6)	97 (85.8)	0 (0)	113 (100)
Genital tract	0 (0)	9 (36.0)	16 (64.0)	0 (0)	25 (100)
Umbilical cord	0 (0)	42 (56.0)	33 (44.0)	0 (0)	75 (100)
Kidney	2 (4.7)	5 (11.6)	36 (83.7)	0 (0)	43 (100)
Joint	1 (1.6)	12 (19.0)	50 (79.4)	0 (0)	63 (100)
Intestine	1 (5.0)	1 (5.0)	18 (90.0)	0 (0)	20 (100)
Brain	0 (0)	1 (8.3)	11 (91.7)	0 (100)	12 (100)
Spleen	0 (100)	0 (0)	7 (100)	0 (100)	7 (100)
Others	16 (11.4)	16 (11.4)	105 (75.0)	3 (2.1)	140 (100)
Total sites isolated	125 (11.6)	143 (13.2)	810 (74.9)	4 (0.4)	1,082 (100)
Adults horses (*n* = 1036)					
Lower respiratory tract	16 (6.2)	32 (12.4)	209 (81.0)	1 (0.4)	258 (100)
Nasal samples	31 (43.7)	6 (8.5)	34 (47.9)	0 (0)	71 (100)
Guttural pouch	5 (26.3)	2 (10.5)	12 (63.2)	0 (0)	19 (100)
Lymph node abscess	63 (49.6)	19 (15.0)	45 (35.4)	0 (0)	127 (100)
Liver	2 (1.7)	19 (16.4)	94 (81.0)	1 (0.9)	116 (100)
Genital tract	0 (0)	118 (17.1)	564 (81.9)	7 (1.0)	689 (100)
Kidney	1 (1.8)	8 (14.3)	47 (84.0)	0 (0)	56 (100)
Abdominal abscesses	3 (11.5)	0 (0)	23 (88.5)	0 (0)	26 (100)
Joint	0 (0)	17 (42.5)	23 (57.5)	0 (0)	40 (100)
Brain	3 (30.0)	1 (10.0)	6 (60.0)	0 (0)	10 (100)
Intestine	0 (0)	1 (6.3)	15 (93.7)	0 (0)	16 (100)
Skin wounds	0 (0)	13 (46.4)	15 (53.6)	0 (0)	28 (100)
Spleen	1 (6.7)	1 (6.7)	13 (86.7)	0 (0)	15 (100)
Others	15 (15.2)	34 (34.3)	48 (48.5)	2 (2.0)	99 (100)
Total sites isolated	140 (8.9)	271 (17.3)	1,148 (73.1)	11 (0.7)	1,570 (100)
Total sites isolated (*n* = 2,391)	273 (6.3)	972 (22.4)	3,065 (70.5)	37 (0.9)	4,347 (100)

* *n* = number of submissions (cases). Trachea, pleura, thorax, and lung were considered lower respiratory tract. Vagina, cervix, and uterus were considered genital tract. Numbers in parentheses represent percentage of each organism isolated from each tissue/organ in fetuses, foals, or adult horses.

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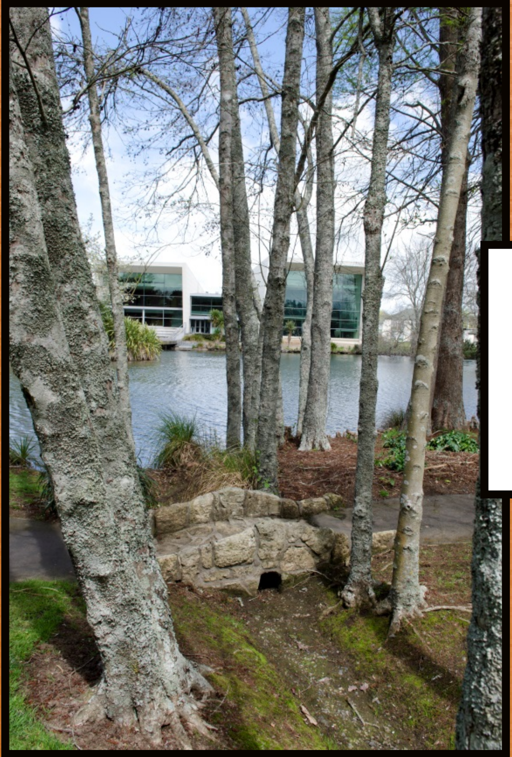

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