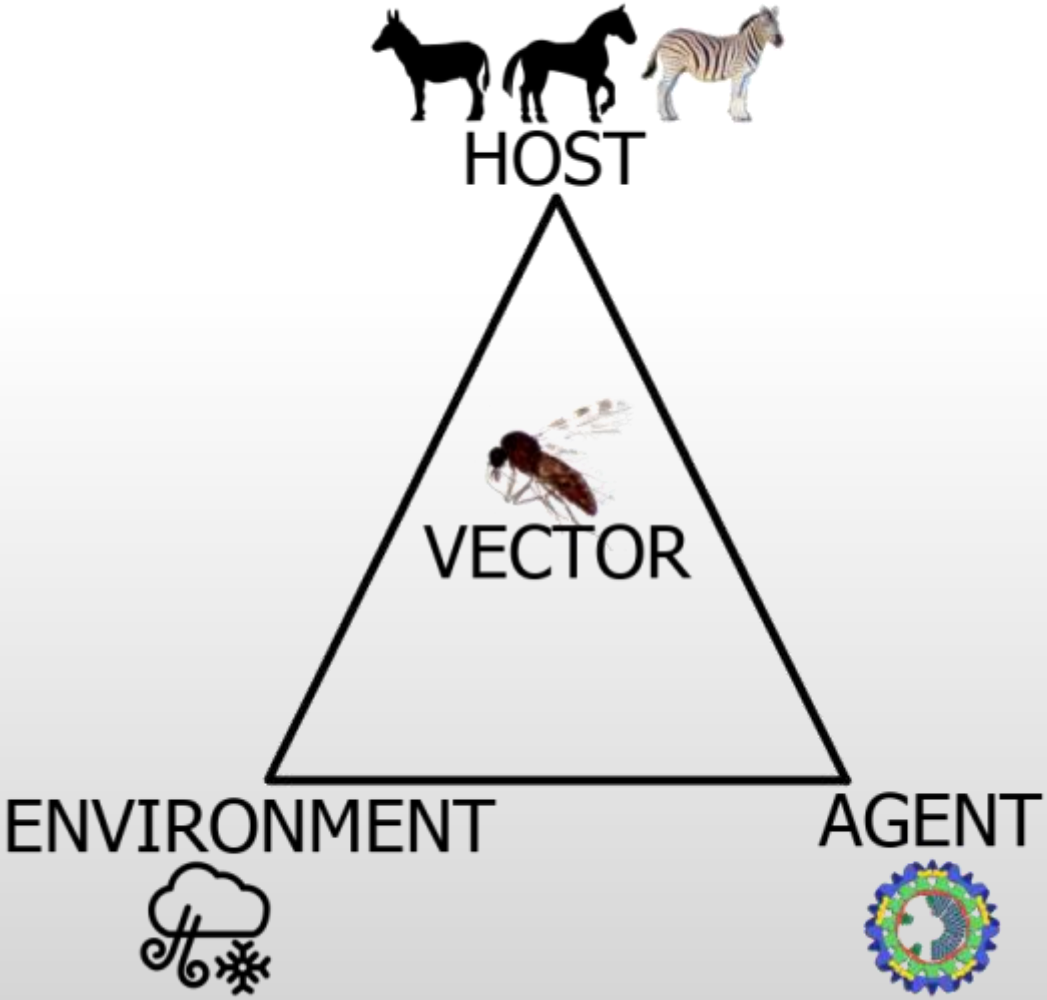
Drs John Grewar, Gary Buhrmann & Lesley van Helden
Government authorised veterinarians

African horse sickness
Outbreak investigation

Epidemiologic Triad



Investigation framework

1. Prepare and Contingency

- Existing materials on epidemiologic and clinical aspects of disease widely published
  - OIE repository
- Differential diagnoses of relevance NB to identify
  - Piroplasmosis and Equine Encepahlosis examples for South Africa
- Capacity
  - Field work
  - Laboratory personnel and testing
  - Technical capacity – GIS and surveillance
  - Public-Private Partnerships

1. Prepare and Contingency

- Communication channels
- Termination of outbreak and return to freedom
- Return to trade
- Legislative mandate regarding control and eradication

2. Confirm diagnosis

- Clinical presentation with standard RNA detection methodology for group and type specific determination
  - Type important for new/unexpected outbreak locations, particularly in face of use of LAV in region

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SOP
AHS Investigation - 1. Confirm Diagnosis

v1.1

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Potential Investigation Sources

- **Private vet passive survey**
  - Illness, death, +/? PCR
- **Active surveillance**
  - Sentinel
    - Illness/Death
    - Δ Sero-status
    - +/? PCR
- **Trace forward investigation**
- **Vaccination (legal & illegal)**

Primary Suspicion

Confirm Diagnosis

OIE Case Definition

The following defines an infection with AHSV:

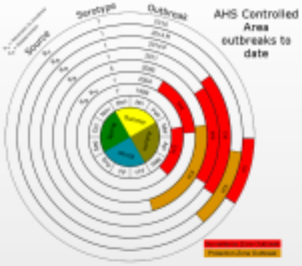
1) AHSV has been isolated and identified from an equid or a product derived from that equid; or
2) antigen or ribonucleic acid specific to AHSV has been identified in samples from an equid showing clinical signs consistent with AHS, or epidemiologically linked to a suspected or confirmed case; or
3) serological evidence of active infection with AHSV by detection of seroconversion with production of antibodies against structural or nonstructural proteins of AHSV that are not a consequence of vaccination have been identified
in an equid that either shows clinical signs consistent with AHS, or is epidemiologically linked to a suspected or confirmed case

LAV Vaccine

Establish likelihood of vaccine RNA association

Case vaccination history
Cohort vaccination history
Surrounding 3-5 km holding vaccination history

Vaccination only permitted June through October
Outside this period vaccination would be illegal
Vaccine RNA transmission associated with VECTOR
Likely transmission from Feb-April



Historically if the Guthrie PCR test has a Cq value of > 30 it may be suggestive of vaccine association (implies low viral load)

Type specific results of T5 or T9 are indicative of NON vaccine related RNA since these strains are not in the vaccine

Laboratory Testing

Establish AHS test profile

RNA Detection

Group Specific PCR
Real Time
- Negative results indicative of no active circulation
- Cq value indicative of viral load
  - Controlled area outbreaks generally <30
  - Lab test cut-off- 37 cycles

Hemi-nested PCR
- Highly sensitive but susceptible to contamination false +

Type Specific PCR
- Not as sensitive as GS PCR - use negative result with caution
- Types 5 & 9 - see LAV vaccine lab section

Virus Isolation and Sequencing

Virus isolation
Historically have taken time to return results
- ask lab for it as soon as GS PCR +
- poor sensitivity, particularly when Cq high

Sequencing
Been useful in proving LAV associated outbreaks
Thus far a research rather than primary investigation tool

Serology

ELISA (indirect)
Used in sentinel screening and export
NON-quantitative therefore paired sample evaluation of limited use

SNT
Excellent use in positive true-sentinel analysis of ELISA +
Across the board reactions (S1-S9) show
- Inadvertant vaccination or
- Incorrect sample taken/tested

Clinical Presentation

Establish likelihood of AHS given clinical presentation of case/s

Note: Clinical signs of AHS are not pathognomonic.
Piroplasmosis, EEV, EHV and any cause of acute death should be considered as differential Dx.
Forms of AHS include:

1. Pulmonary ('Dunkop')

The characteristic clinical signs in this form of AHS are fever severe dyspnoea, paroxysms of coughing, and sometimes, discharge of latrge quantities of frothy, serofibrinous fluid from the nostrils.

2. Cardiac ('Dikkop')

This form of AHS is characterized by subcutaneous oedema of the head and neck, and particularly the supraorbital fossae.

3. Mixed Form

This form is most common for AHS but most often confirmed on PM since clinically the pulmonary form or cardiac form predominates and is so classified

4. Horse sickness Fever

Horse sickness fever is a very mild form of the disease. The most characteristic finding is a rfever followed by a drop in temperature to normal, and recovery. Some horses may show partial loss of appetite, congestion of the conjunctivae, slightly laboured breathing, and increased heart rate, but these signs are transient.

Note Subclinical cases of AHS have been seen in outbreaks in the controlled area, and while they occur at relatively low prevalence the lack of clinical signs should not preclude AHS as a diagnosis. Thi said, animals with a low immunity (young animals with waning maternal antibody, unvaccinated imported horses or horses with compromised health status) are likely to show some clinical evidence of disease, so signalment is important in this evaluation

2016 Outbreak Case Def.

Positive
P1 Clinical and/or post-mortem signs synonymous with AHS with a positive RT-qPCR and/or virus isolation result
P2 Positive RT-qPCR and/or virus isolation result only (Subclinical cases)
P3 Clinical and/or post-mortem signs synonymous with AHS with no AHS positive laboratory confirmation but with epidemiological links to a confirmed case
Negative
N1 Clinical and/or post-mortem signs synonymous with AHS with confirmation of another cause of disease AND with a negative RT-qPCR
N2 Routine outbreak surveillance with negative RT-qPCR
N3 Clinical surveillance with no reported and/or detected clinical signs synonymous with AHS
Suspect
S1 Clinical and/or post-mortem signs synonymous with AHS with no lab positive confirmation of any associated disease
S2 NO clinical and/or post-mortem signs synonymous with AHS with AHS PCR result of CT value >=36

Current Role Players

SAEHP	**All enquiries**	Dr Camille Weyer Dr John Grewar	camille@saehp.com john@saehp.com
State Vet Boland Provincial Lab	**Epidemiology**	Dr Lesley van Heiden Dr Laura Roberts Dr Gary Bührmann Ms Renee Pieterse	lesleyvh@elsenburg.com laurar@elsenburg.com garyb@elsenburg.com reneep@elsenburg.com
ERC	**Equine Research Center**	Prof Alan Guthrie	alan.guthrie@up.ac.za
ARC + LNR	**Onderstepoort Veterinary Research** **All enquiries** **PCR** **Serology**	Dr Otto Koekemoer Dr Marco Romito Dr Alison Lubisi	KoekemoerO@arc.agric.za RomitoM@arc.agric.za lubisia@arc.agric.za
Deltamune	**Deltamune**	Oudtshoorn Lab	+27 (0)44 272 7159

LAV Outcome

- ☐ Case aro vaccine associated RNA
- ☐ No clarity as to likely LAV association
- ☐ LAV RNA unlikley to be involved

*** NOTE:**
If LAV is associated with a case,
clinical signs are not aro very recent vaccination,
transmission of LAV is shown and/or
reversion to virulence/re-assortment is suspected then an outbreak can be occurring

Lab Outcome

- ☐ AHS negative
- ☐ AHS suspect
- ☐ AHS positive

Lab results indicative of LAV influence?

- ☐ yes
- ☐ no
- ☐ maybe

Cx Outcome

- ☐ Clinically AHS associated
- ☐ Clinically AHS suspect
- ☐ Clinically AHS negative

Confirm Dx Outcome

- ☐ Likely AHS outbreak
  - LAV Influence?
    - ☐ yes
    - ☐ no
    - ☐ maybe
  - → **Implement Contingency Plan**
  - → **Perform further testing to confirm suspect**
- ☐ AHS unsure/suspect
- ☐ AHS vaccination event
- ☐ AHS negative

→ **Report findings to DAFF + WCDOA**

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3. Case definition

CHAPTER 12.1.

INFECTION WITH AFRICAN HORSE SICKNESS VIRUS

Article 12.1.1.

General provisions

For the purposes of the *Terrestrial Code*, African horse sickness (AHS) is defined as an *infection* of equids with African horse sickness virus (AHSV).

The following defines an *infection* with AHSV:

1. AHSV has been isolated and identified from an equid or a product derived from that equid; or
2. antigen or ribonucleic acid specific to AHSV has been identified in samples from an equid showing clinical signs consistent with AHS, or epidemiologically linked to a suspected or confirmed *case*; or
3. serological evidence of active *infection* with AHSV by detection of seroconversion with production of antibodies against structural or nonstructural proteins of AHSV that are not a consequence of *vaccination* have been identified in an equid that either shows clinical signs consistent with AHS, or is epidemiologically linked to a suspected or confirmed *case*.

2016 Outbreak Case Def.

Positive

P1 Clinical and/or post-mortem signs synonymous with AHS with a positive RT-qPCR and/or virus isolation result

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P3 Clinical and/or post-mortem signs synonymous with AHS with no AHS positive laboratory confirmation but with epidemiological links to a confirmed case

Negative

N1 Clinical and/or post-mortem signs synonymous with AHS with confirmation of another cause of disease AND with a negative RT-qPCR

N2 Routine outbreak surveillance with negative RT-qPCR

N3 Clinical surveillance with no reported and/or detected clinical signs synonymous with AHS

Suspect

S1 Clinical and/or post-mortem signs synonymous with AHS with no lab positive confirmation of any associated disease

S2 NO clinical and/or post-mortem signs synonymous with AHS with AHS PCR result of CT value >=36

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4. Data and Information

- Demographic
- Risk factor
- Population and population at risk
- Case definition
  - Clinical information
  - History of vaccination
- Sampling and results
- Spatial
- Future use of information to inform surveillance

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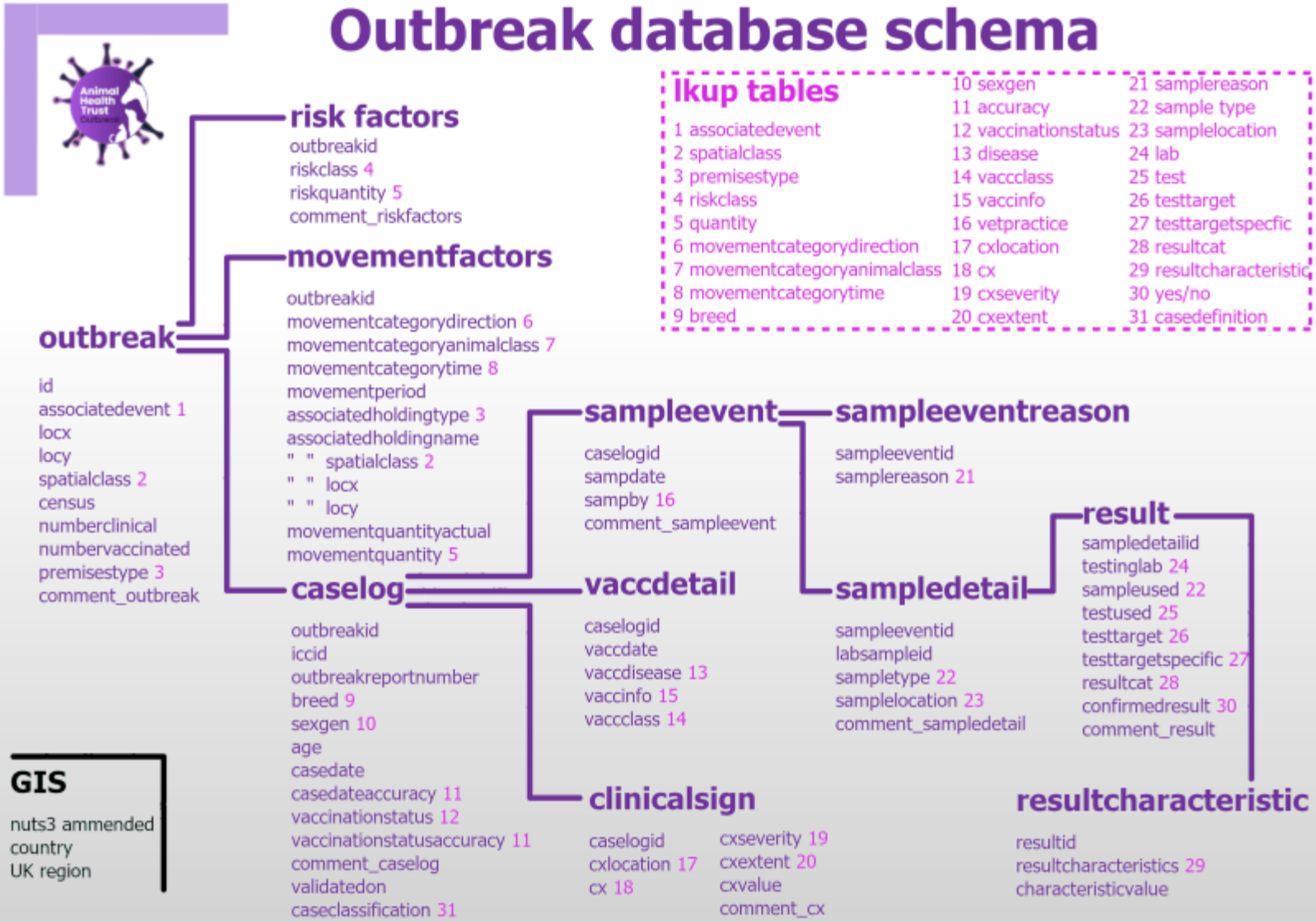
4. Data and Information



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5. Descriptive epidemiology

		Epicentre				Overall containment zone			
Factor	Category	Total	Positive	Proportion (95% CI)	*p*-value[b]	Total	Positive	Proportion (95% CI)	*p*-value[b]
Vaccination status	Vaccinated	320	9	0.03 (0.01–0.05)	0.04	1,184	10	0.01 (0–0.02)	0.10
	Unvaccinated	73	6	0.08 (0.03–0.17)		408	8	0.02 (0.01–0.04)	
	Unknown status	155	3			225	3		
Breed[a]	American Saddlebred	161	1	0.01 (0, 0.03)	0.02	167	1	0.01 (0, 0.03)	0.04
	Arab	7	1	0.14 (0, 0.58)		64	1	0.02 (0, 0.08)	
	Boerperd	19	2	0.11 (0.01,0.33)		37	2	0.05 (0.01, 0.18)	
	Friesian	20	0	0 (0, 0.17)		32	0	0 (0, 0.11)	
	SA Warmblood	32	1	0.03 (0, 0.16)		70	1	0.01 (0, 0.08)	
	Thoroughbred	123	2	0.02 (0, 0.06)		1,070	5	0 (0, 0.01)	
	Cross/Other/Unknown	186	11			377	11		
Sex	Male	254	12	0.05 (0.02, 0.08)	0.15	695	13	0.02 (0.01, 0.03)	0.12
	Female	262	6	0.02 (0.01, 0.05)		917	8	0.01 (0, 0.02)	
	Unknown/Not Captured	32	0			205	0		
Colour[a]	Bay	201	8	0.04 (0.02, 0.07)	0.16	774	11	0.01 (0.01, 0.03)	0.25
	Black	39	0	0 (0, 0.09)		56	0	0 (0, 0.06)	
	Chestnut	170	4	0.02 (0.01, 0.06)		385	4	0.01 (0, 0.03)	
	Grey	64	5	0.08 (0.03, 0.17)		153	5	0.03 (0.01, 0.07)	
	Unknown/Other	74	1			449	1		
Total horses		548	18	0.03 (0.02, 0.05)		1,817	21	0.01 (0.01–0.02)	
Properties visited		48	6	0.13 (0.05,0.25)		118	8	0.07 (0.03,0.13)	

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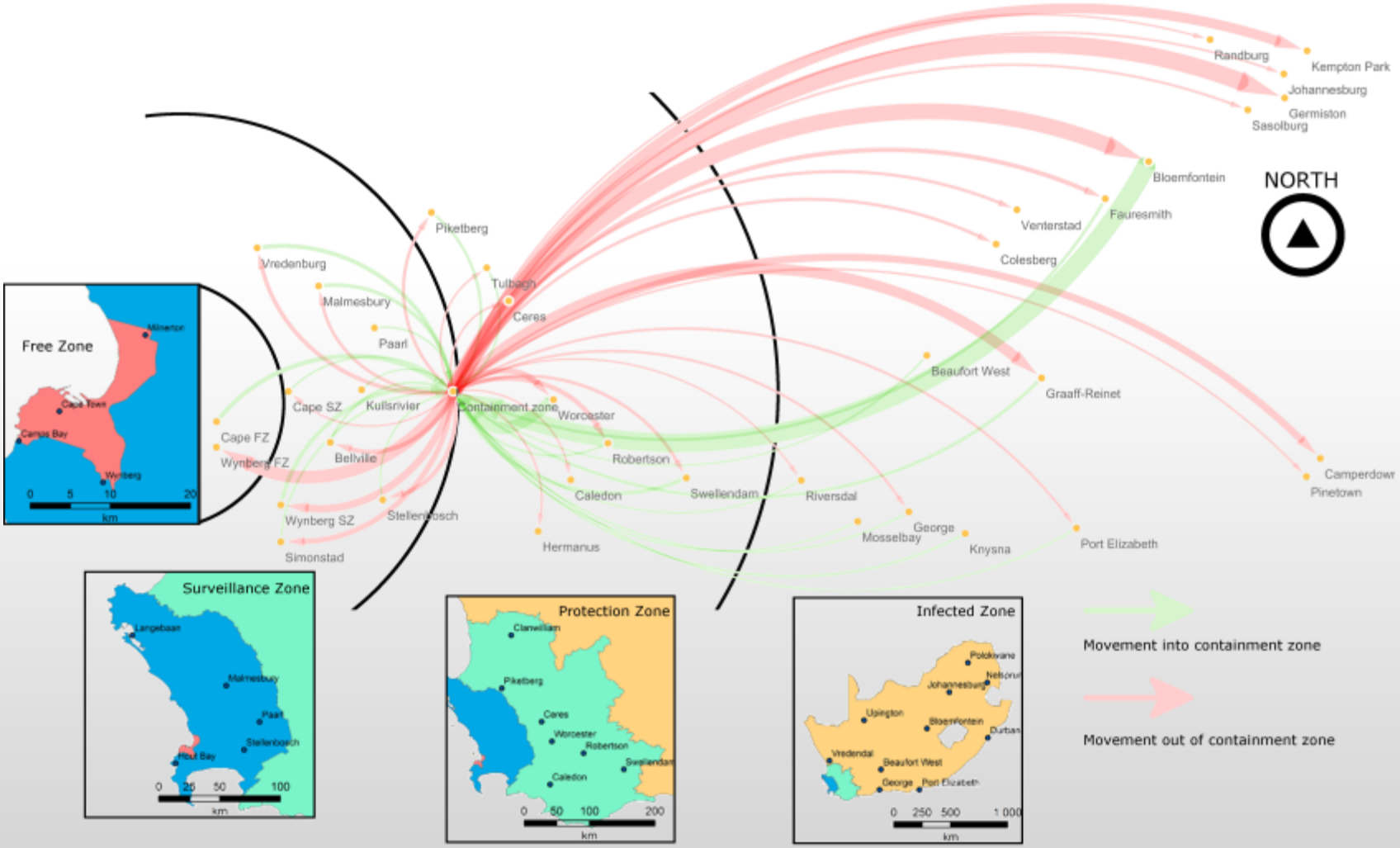
5. Descriptive epidemiology



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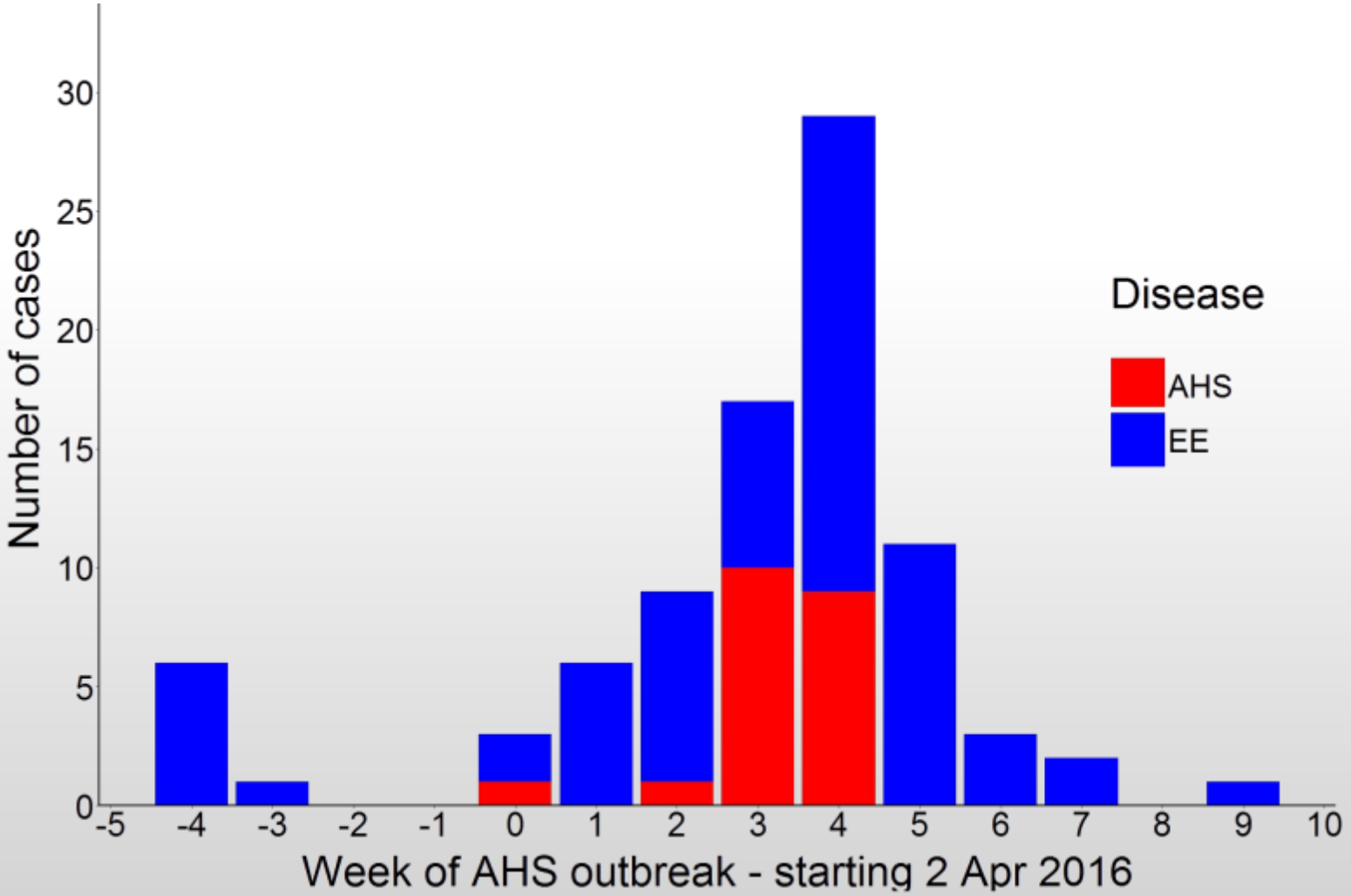
5. Descriptive epidemiology



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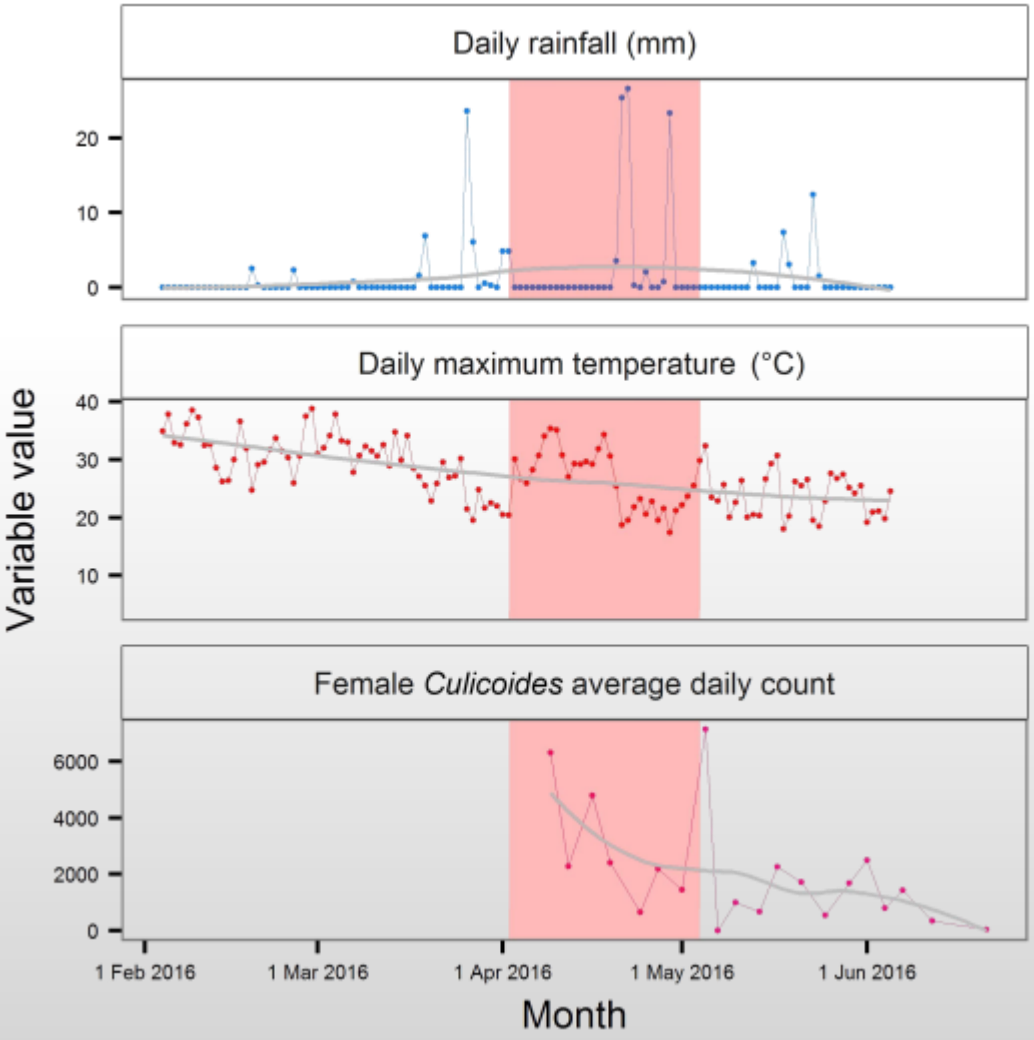
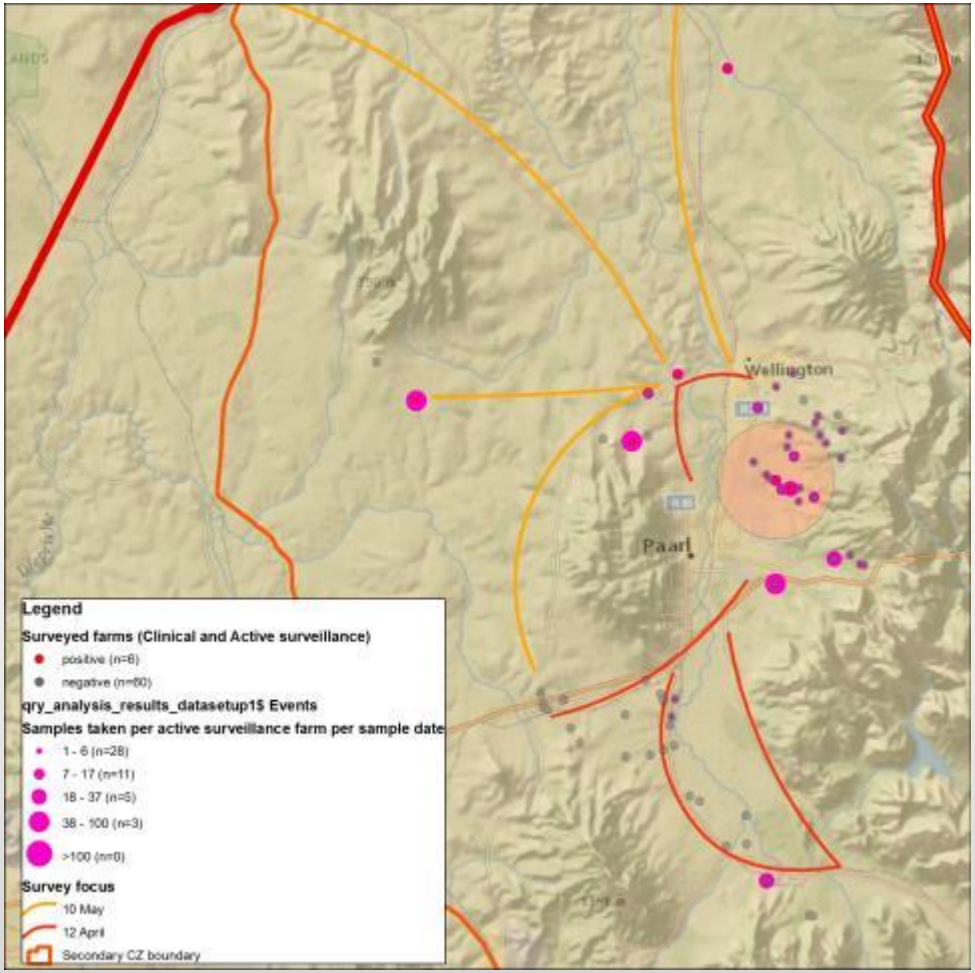
5. Descriptive epidemiology



Culicoides species	Total collected	Proportion of total collected (%)
C. imicola	244,881	95.5
C. subschultzei	4,159	1.6
C. bolitinos	1,659	0.65
C. zuluensis	1,580	0.62
C. nivosus	1,456	0.57
Other species (*n* = 12)	2,585	1.06
Total	256,320	100

6. Surveillance

- Passive surveillance
- Active surveillance
  - In outbreak
    - Capacity
  - Post-outbreak



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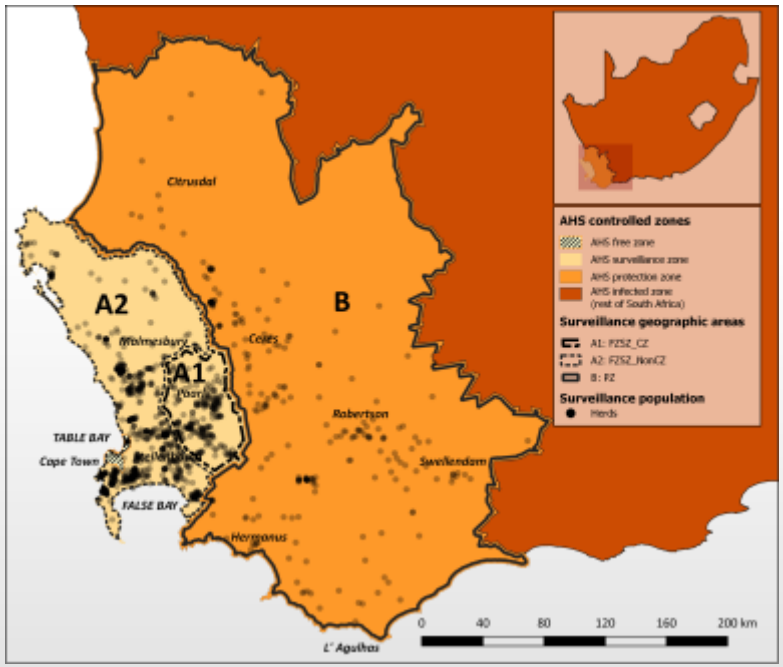
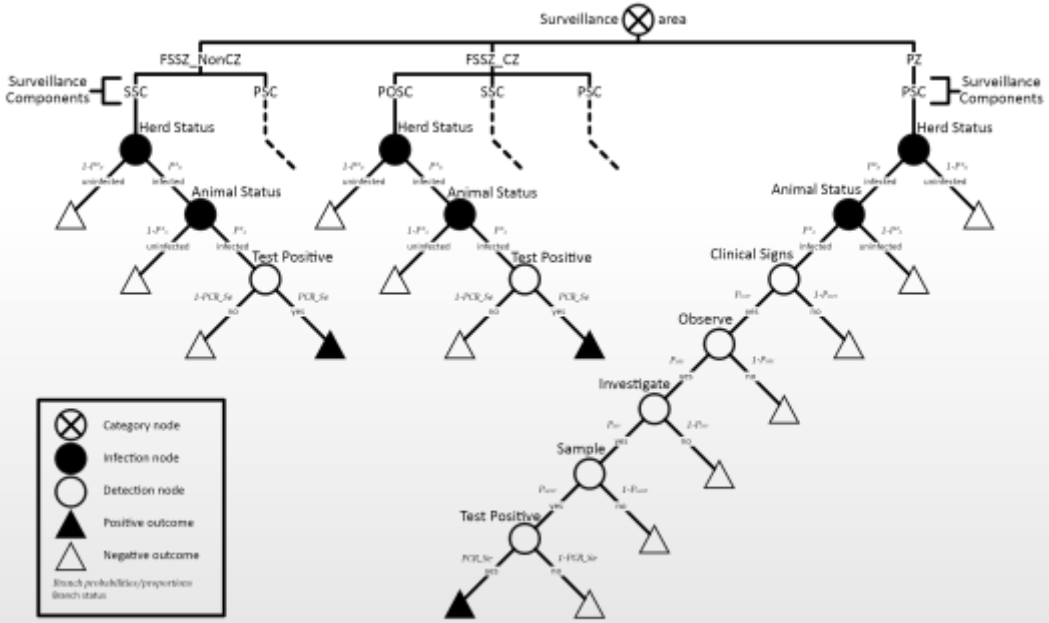
6. Surveillance





7. Outbreak hypotheses

- Source and ongoing threat of introduction (backward and forward tracing)
  - Movement of an infected horse
  - If vaccinating then reversion to virulence/re-assortment
  - Midge dispersal

7. Outbreak hypotheses

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AHS Contingency - Tracing

Goal is to perform a **trace back** for all horses moving from the AHS infected zone that might be a potential source of the outbreak under investigation. Secondly **trace forward** from restricted area holdings will assist in establishing potential spread of outbreak

1. Variable Input
Input Destination (trace back) or Origin (trace forward) space and time variable

Rule of thumb is use 2 months for investigation purposes

Find permits issued within Area - Click on map OR type in GPS address

Either enter GPS coordinates or click on the Map to generate the buffer window

2. Spatial Visualisation
The buffer zone selected will show on the map and show all locations where permits were associated with.

If holdings are close to each other then the points will automatically cluster - zoom in or click on a cluster to get to the individual farm level

Green dots indicate where the location is a destination of a permit associated movement

Red dots indicate where the location is an orgin of a permit associated movement

3. Identifying locations
Hovering over a point will give the holding ID's of the associated movement - in this example horses moved from 8106 TO 6470, and since the dot is green the location shown here represents holding 6470

4. List of permits
The list can be extensive - a table is shown of the basic details of all permit associated movements within the buffer - click on the DOWNLOAD RESULTS button to export that to a CSV file which can be used in further investigation

5. Identifying individual horses
Using the permit ID's either the actual permits can be obtained from State Vet Boland (the SAEHP current role players will assist given they issue the permits) or contact the myhorse database administrator to run a query extracting all horses moved from the permits downloaded in the CSV

Final Notes

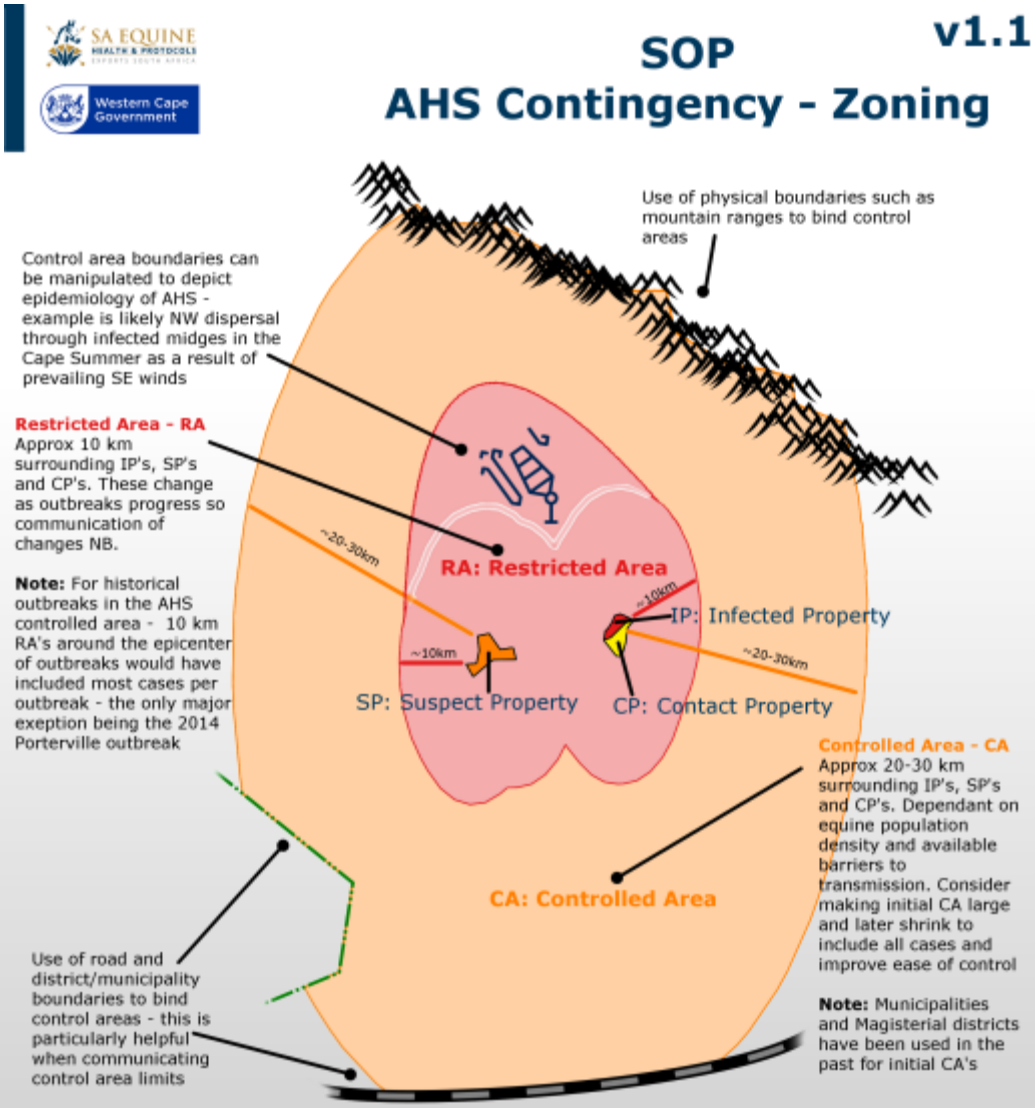
Tracing investigations should follow up on any traced horse to establish recent clinical history, recent location history (particularly in the infected zone) and vaccination history.

The online system is limited to permit associated movenments. Tracing interview questions should be included in primary investigations in the **Restricted Area**; this is particularly relevant for trace forward investiogations since local movements won't be recorded if the movement is within or to a AHS zone of less control

8. Control and Prevent

- Risk reduction
  - Movement control
    - Permits
    - Health checks
    - Vaccination status
    - Use of vector protection pre- and/or post movement
  - Vaccination
  - Original source
  - Zoning

8. Control and Prevent



9. Communication

- Clear, concise and timely
- Single, trusted source
- Include role-players to buy into communication strategy
- Avoid and mitigate for fake-news

References

CDC Centers for Disease Control and Prevention

DOI: 10.1111/tbed.13077

ORIGINAL ARTICLE

WILEY

A field investigation of an African horse sickness outbreak in the controlled area of South Africa in 2016

John Duncan Grewar[1,2] | Camilla Theresa Weyer[3] | Gert Johannes Venter[4] | Lesley Susan van Helden[5] | Phillippa Burger[2] | Alan John Guthrie[3] | Peter Coetzee[3] | Karien Labuschagne[4] | Gary Bührmann[5] | Beverley Joan Parker[2] | Peter Neil Thompson[1]

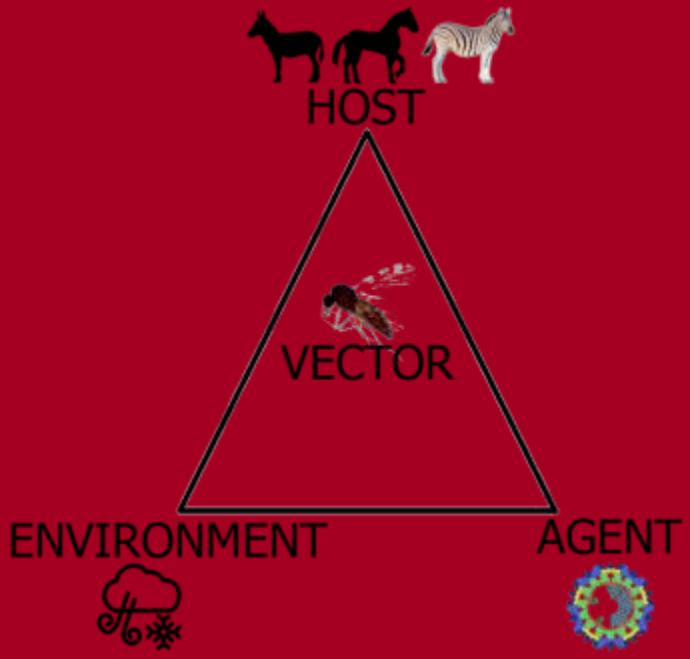
DOI: 10.1111/tbed.13566

ORIGINAL ARTICLE

WILEY

Post-outbreak African horse sickness surveillance: A scenario tree evaluation in South Africa's controlled area

John Duncan Grewar[1,2] | Thibaud Porphyre[3] | Evan S. Sergeant[4] | Camilla Theresa Weyer[2,5] | Peter Neil Thompson[1]



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