African Swine Fever Outbreak Detection and Response in Wild Boars in Singapore

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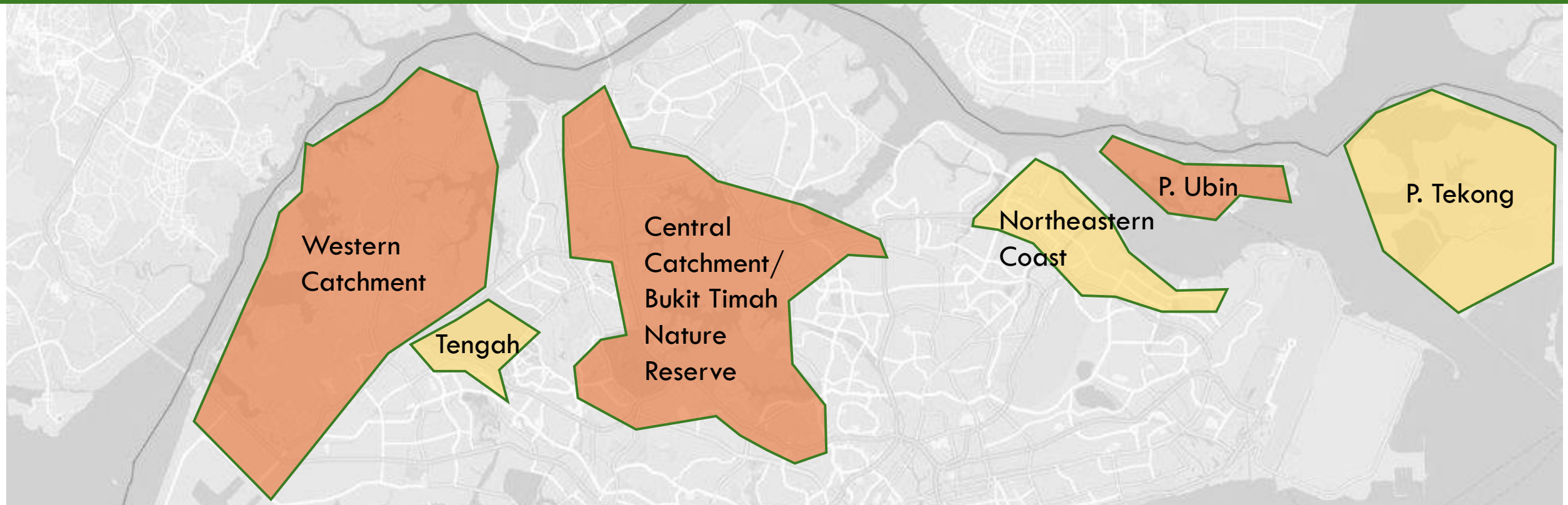
Wild boars in Singapore

- Found in most of Singapore's forested habitats
- Latest published population density of wild boar in the nature reserves of Singapore is 5.22 +/- 0.43 wild boars/ km^2, with the estimated abundance at 156+/- 13 (Khoo et al 2021)
- Wild boar population is well segregated from the captive pig population in Singapore
- Access of wild boars to urbanised areas where captive suids (e.g., zoological collections) are held are hindered by physical and geographical barriers (e.g., fencing, roads, expressways, canals)
- Wild boars that stray too far from the forests often end up as roadkill

Wild boar mortality monitoring



- Subpopulations of wild boars in Singapore are largely isolated, due to Singapore's fragmented forest and urban matrix
- Mortality is monitored by the respective land managers on site, who patrols their land daily. Any mortality observed will be consolidated for reporting
- Average report is 0 - 2 deaths recorded per month
- In late Jan/early Feb 2023, NParks recorded **9 wild boar deaths in one week** across three areas of Singapore as part of biosurveillance efforts

Laboratory investigation

Post-mortem performed on the 10th carcass (index case) in that week, found in northwestern part of Singapore

Gross examination of wild boar carcass (Sus scrofa)

- Wild boar carcass transported to the Centre for Animal & Veterinary Sciences (CAVS) for disease investigation
- Key findings include haemothorax, haemoperitoneum, and widespread subcutaneous and pulmonary hemorrhage
  - ASF and CSF were key differentials
- Organ samples (liver, lung, heart, spleen, lymph nodes, kidney, and tonsil) and two fluid samples (abdominal and thoracic fluids) were obtained for virological analysis
- Two adult ticks (1 male and 1 female) were found on the carcass and identified as *Dermacentor auratus* ticks by DNA barcoding
- ASFV was detected from all eleven (9 suid and 2 tick) samples by real-time PCR

Subcutaneous tissue showing multifocal haemorrhage

Multifocal ecchymoses in the lungs and thoracic effusion, note state of decomposition with bloating, and grey to green discoloration of organs

Laboratory investigation

EMERGING INFECTIOUS DISEASES®

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Volume 29, Number 12—December 2023

Research Letter

Detection of African Swine Fever Virus from Wild Boar, Singapore, 2023

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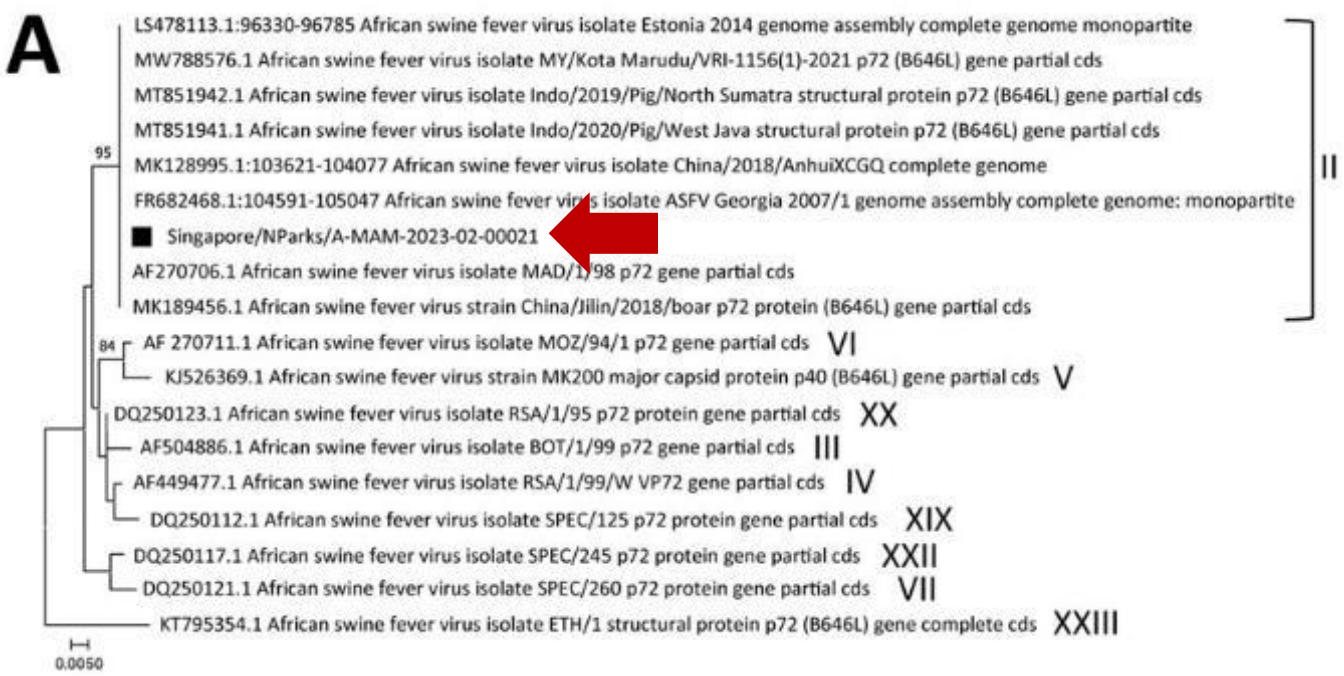
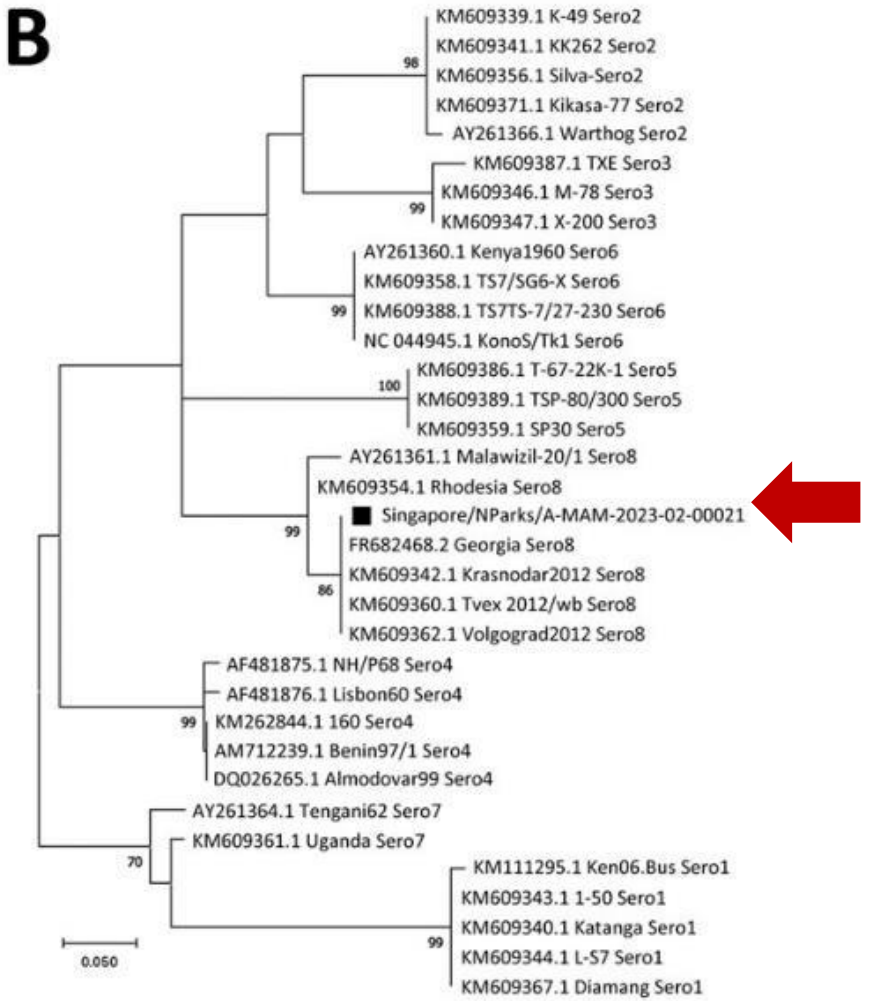


ASF genotype II, serogroup 8 based on p72 gene 90 nt from the EP402R gene respectively



Further field investigation and biosurveillance

1 *To determine extent of disease spread in Singapore through active sampling*

Reporting by ground officers
Enhanced by briefing to wildlife officers and land managers on ASF and biosecurity measures

Fresh carcasses directly transported to lab

On-site necropsy for off-shore islands

Bone harvesting for highly decomposed carcasses

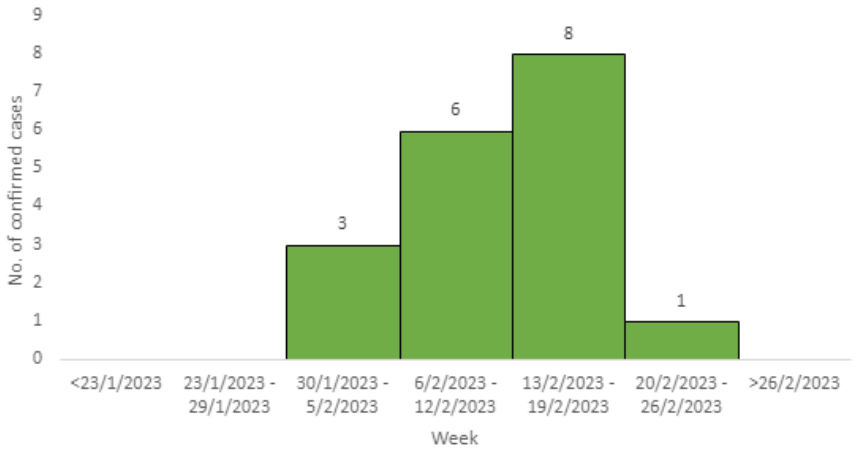
Laboratory analysis and epidemiological tracking

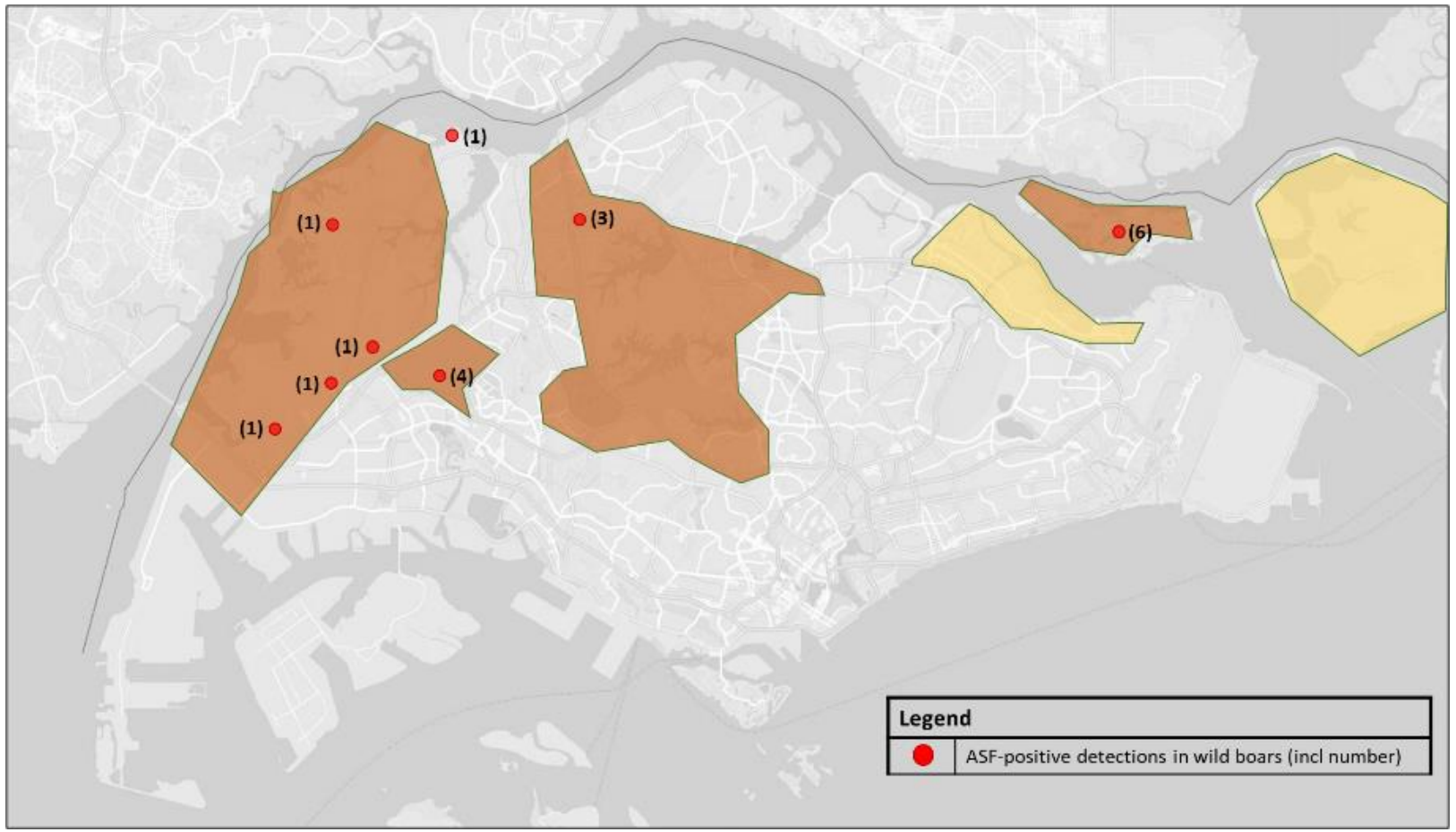
Swift removal and/or burial with decontamination of burial site

Further field investigation and biosurveillance

1 *To determine extent of disease spread in Singapore through active sampling*

Number of Confirmed ASF Detections in Wild Boars





Further field investigation and biosurveillance

1 *To determine extent of disease spread in Singapore through active sampling*

Soil Sampling for ASF?

- Use of environmental sampling for ASF detection has been described (e.g., by Viltrop et al in Estonia)
  - Persistence of viral DNA appeared to be longer on sites where fresher carcasses were discovered
- Limited soil sampling was conducted in Singapore's outbreak –
  - Of the 18 confirmed cases, topsoil under 4 of the carcasses were sampled
  - Further study would need to be conducted on the utility of soil sampling for detecting ASFV in the tropical environment

S/N	Location of confirmed case	State of Carcass	Result of soil testing
1	Western Catchment	Fresh	+ve
2	Western Catchment	Wet decomposed	+ve
3	P. Ubin	Wet decomposed	+ve
4	P. Ubin	Dry and mainly bones	-ve

Further field investigation and biosurveillance

1 *To determine extent of disease spread in Singapore through active sampling*

- Advisory to establishments housing suids to reinforce clinical biosurveillance for suspect cases and biosecurity measures
- Sampling of captive suids was also conducted where feasible

Table 1: Results of surveillance in suids in Singapore from 2021 to 2024

	1 Jan- 31 Dec 2021	1 Jan- 31 Dec 2022	1 Jan- 19 April 2023	20 April – 31 Dec 2023	1 Jan- 1 May 2024
Wild boar Samples: blood, organs, long bones Test method: PCR, ELISA	0/23	0/6	18/82	0/11	0/2
Captive wild pigs Samples: blood Test method: PCR, ELISA			0/13	0/3	

Further field investigation and biosurveillance

2 *To investigate potential role of ticks in transmission*

Two adult ticks (1 male and 1 female) were obtained from the wild boar carcass and identified as ***Dermacentor auratus*** *ticks by DNA barcoding.*

Figure: Female (left) and male (right) ticks collected from the carcass

Digestion of whole tick after removal of host tissues from mouthparts

ASFV detected in tick samples by real-time PCR (Agűero et al. 2003)

ASFV not detected in tick samples by next-generation sequencing.

Significance of *D. auratus* tick in ASFV transmission in Singapore remains to be determined.

Further field investigation and biosurveillance

2

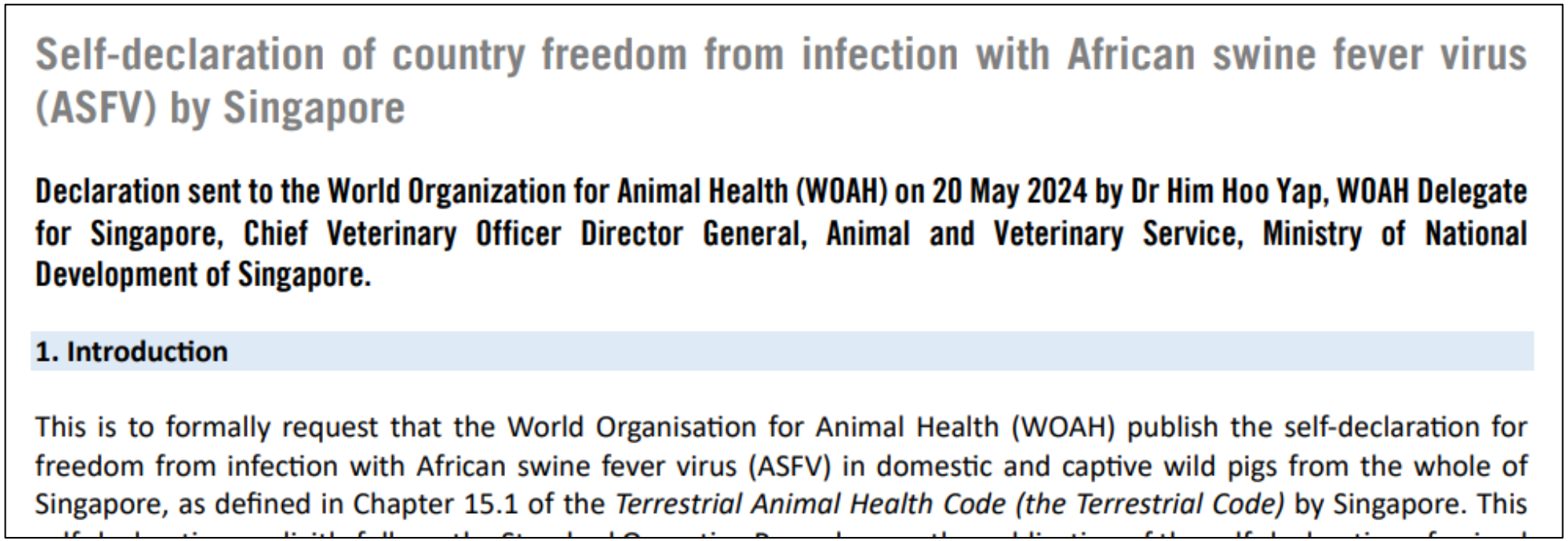
To investigate potential role of ticks in transmission

Table 2: Tick surveillance results conducted in 2023

Number of surveys traps	3
Number of ticks collected via flagging	51
Number of ticks obtained from carcasses of ASF-positive wild boar	27
Results (presented as number of ticks tested as *Ornithodoros* spp/ total number of ticks tested)	0/78
Results (presented as number of ticks testing positive for ASFV/ total number of ticks tested)	2/78

Conclusion and Further Research

- No case of infection with ASF virus in wild boars and captive suids since April 2023
- Biosurveillance of wild boars has demonstrated no evidence of involvement of *Ornithodoros* ticks in the ASF transmission



Self-declaration of country freedom from infection with African swine fever virus (ASFV) by Singapore

Declaration sent to the World Organization for Animal Health (WOAH) on 20 May 2024 by Dr Him Hoo Yap, WOAH Delegate for Singapore, Chief Veterinary Officer Director General, Animal and Veterinary Service, Ministry of National Development of Singapore.

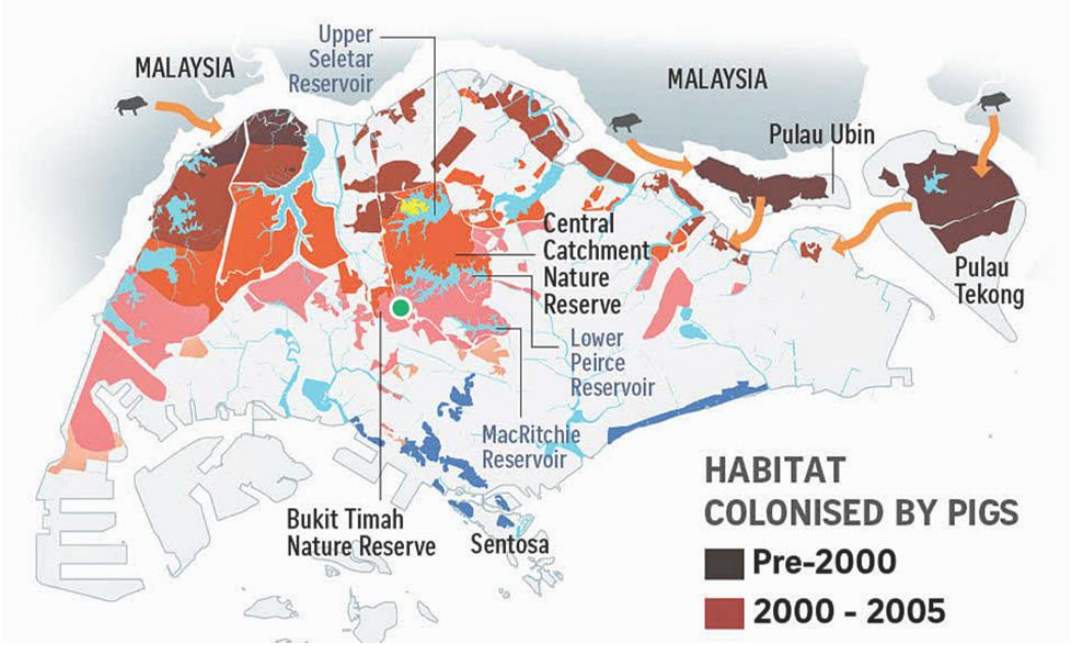
1. Introduction

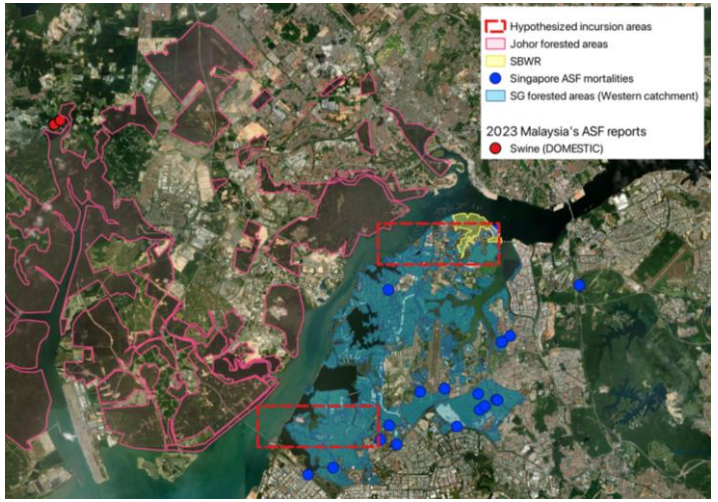
This is to formally request that the World Organisation for Animal Health (WOAH) publish the self-declaration for freedom from infection with African swine fever virus (ASFV) in domestic and captive wild pigs from the whole of Singapore, as defined in Chapter 15.1 of the *Terrestrial Animal Health Code (the Terrestrial Code)* by Singapore. This

Conclusion and Further Research

- Disease could have been introduced through cross-border movement of infected wild boars/ carcasses from the region

- Investigation of ASF transmission dynamics in Singapore through simulation modelling
  - To identify high impact areas/populations (e.g., P. Ubin)
  - To predict and validate geographic extent of outbreak (W. Catchment)





WOAH guidelines

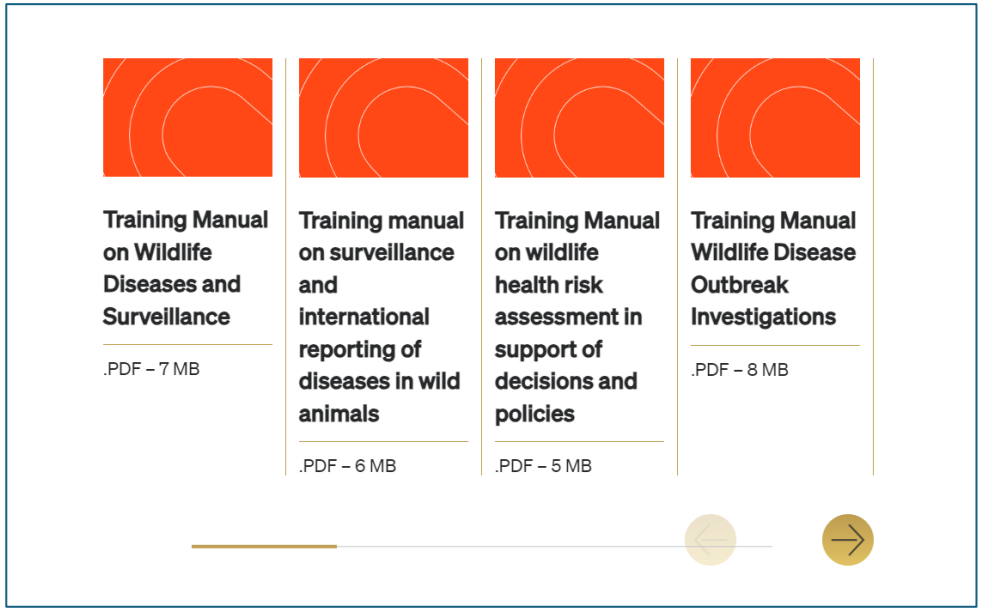
- A well-designed animal disease surveillance system allows for the early detection of health threats. Preventive action and early reaction to outbreaks could contain dangerous diseases before they cause damage and serve to preserve the health of animals and humans alike.
- Varied stakeholders at multiple levels can be involved in disease surveillance
- WAHIS, the World Animal Health Information System makes available to the international community, the information on animal diseases, including those in wildlife, reported by Member Countries and territories.

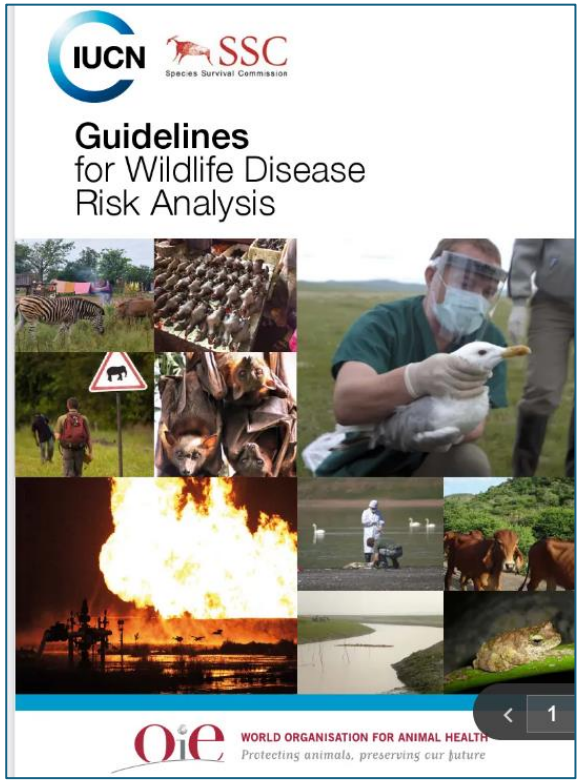
WOAH reporting

- As a key activity of the Wildlife Health Framework, WOAH helps support Members in the mandatory reporting of listed diseases affecting wildlife, and helps encourage Members to voluntarily report the non-listed diseases affecting wildlife.
- *WAHIS-WILD Beta* gathers and presents information on wildlife diseases that are not included in the WOAH List but are considered to require surveillance.

WOAH training activities and guides for wildlife health

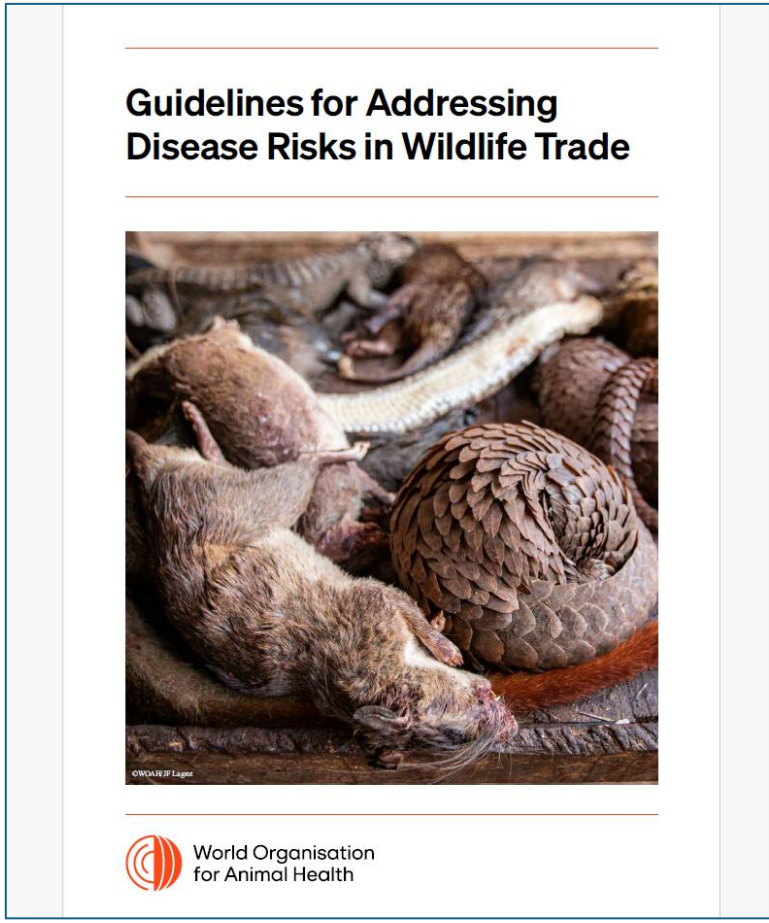
- In the context of better identifying, assessing and managing disease risks to and from wildlife, WOAH in collaboration with IUCN produced the Guidelines for Wildlife Disease Risk Analysis and a Manual of Procedures for Wildlife Disease Risk Analysis.





New publication

- The **Guidelines for Addressing Disease Risks in Wildlife Trade** present a high-level framework to assess risk and identify risk-management strategies for wildlife trade.
  - to help users to determine appropriate measures to reduce risk.
  - provide support in applying risk analysis to the wildlife trade system.
  - highlight some of the challenges that result from the complexity of the landscape and significant knowledge gaps.
- In summary, these Guidelines enable key actors in wildlife trade to identify and select pragmatic, flexible, practical, adaptable and relevant risk-management strategies based on identified disease risks and available capacity, and to ensure their effective and sustainable implementation.



Thank you