

# Global overview, evolution, and epidemiology of the highly pathogenic avian influenza viruses

Celia Abolnik

NRF-DSI SARCHI: Poultry Health and Production  
Faculty of Veterinary Science, University of Pretoria

SASVEPM webinar 14 March 2024

*Threat of Emerging H5 and H7 Avian Influenza Viruses to the Regional Poultry Market*



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

# What is HPAI, where does it come from?

**DEFAULT FORM:**

Low Pathogenicity Avian Influenza (LPAI) viruses occur naturally:

*N.B. H1 cannot mutate to H2 etc. Discrete lineages that diverged centuries ago*



- H1Nx
- H2Nx
- H3Nx
- H4Nx
- H5Nx
- H6Nx
- H7Nx
- H8Nx
- H9Nx
- H10Nx
- H11Nx
- H12Nx
- H13Nx
- H14Nx
- H15Nx
- H16Nx

where "x" is any neuraminidase (N) subtype- N1 to N9

and

"y" is N10 or N11

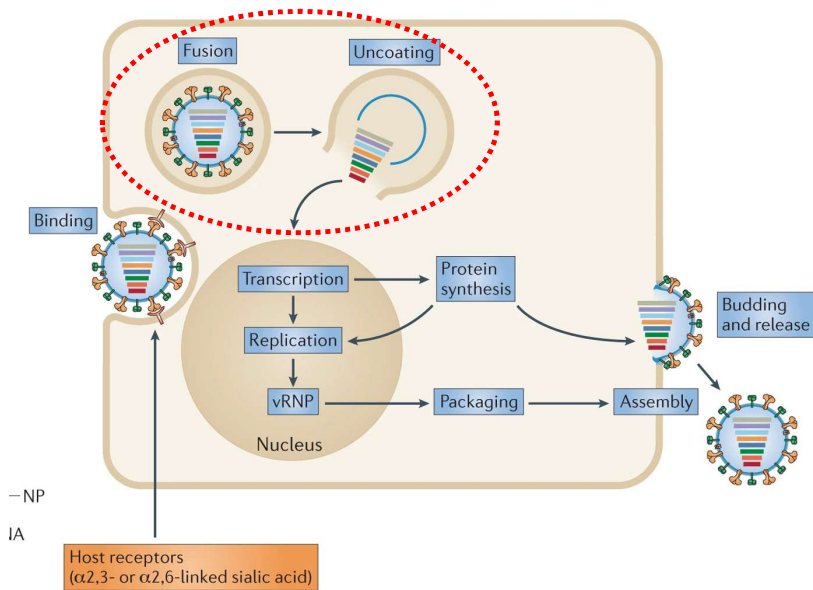
- H17Ny
- H18Ny

- All naturally occurring HPAI viruses are H5Nx or H7Nx subtype
- Not all H5Nx or H7Nx viruses are HPAI
- HPAI arises through mutation in terrestrial birds

WOAH criteria: a virus is HPAI if-  
 a) Lethal to chickens in *in vivo* tests (virus isolate kills 6-8 week-old susceptible chickens within 10- days or has an IVPI >1.2)  
 OR  
 b) The hemagglutinin protein cleavage site (HA0) sequence is typical of HPAIV

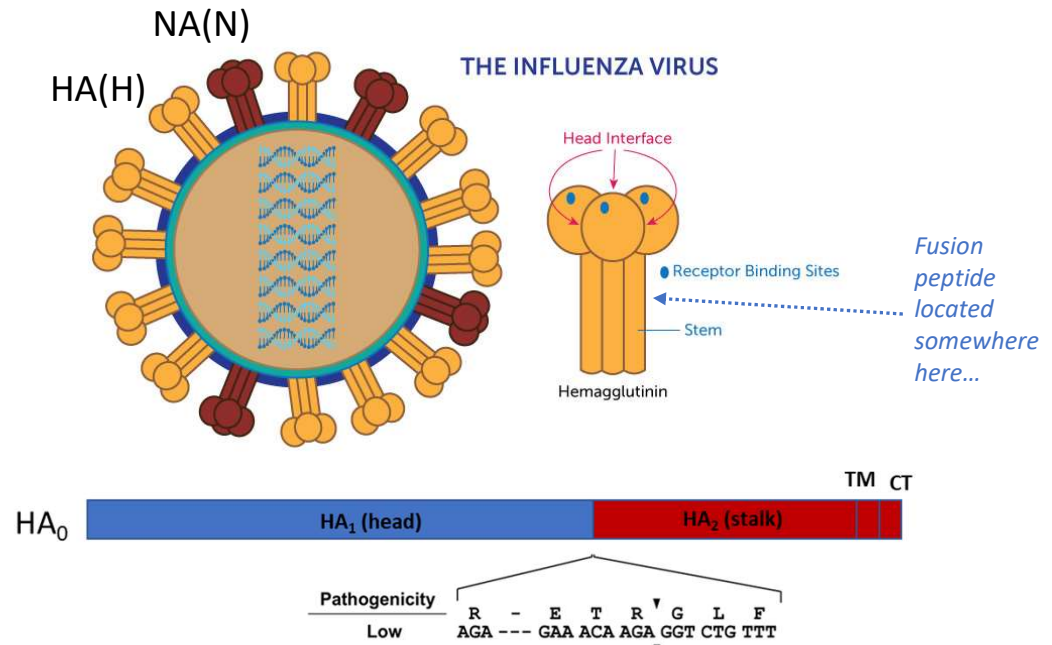


# Importance of the HA0 cleavage site sequence in viral pathogenicity (and HPAI classification)



Nature Reviews | Microbiology

Source: Shi et al., 2014; <https://www.nature.com/articles/nrmicro3362>



**Cleavage exposes fusion peptide: causes viral and cell membrane fusion**

**HA0 cleavage is facilitated by enzymes produced by the host cell:**

- **LPAIVs:** amino acid motif cleaved by **trypsin-like enzymes**, secreted **epithelial cells** of respiratory + gastrointestinal tracts = infection limited to these tissues
- **HPAIVs:** contains **additional basic amino acids (R/K)** or **extensions** derived through **non-homologous recombination** with host or viral RNA (rare). Motifs cleaved by a broad range of **furin/subtilisin-like enzymes**; ubiquitous in **many cell types** = systemic infection/ multiple organ infections = lethal

*A list of known HA0 HPAI sequences is maintained by OFFLU*

## Specie-specific ability to generate HPAI



Converts LPAI to HPAI

NO

NO

YES  
*H5 only??*

YES

YES

YES

### Sequential mutation/ incorporation of basic amino acids

**How long?** Weeks

*In ovo* experiments: H7N1: HPAI in 7 passages

H5N2: HPAI in 11 passages

*if passage = transmission events between individual hosts; typical infection dynamics*

*Then theoretically: H7 HPAI could emerge within 3 to 7 weeks;*

*H5 HPAI could emerge within 5 to 11 weeks*

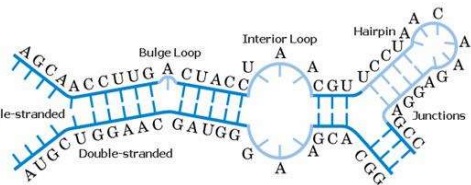
**How?**

Theory: Unstable secondary structure in the H5/H7 HA vRNA interacting with host transcription/replication machinery

RESEARCH ARTICLE

Emergence of highly pathogenic H5N2 and H7N1 influenza A viruses from low pathogenic precursors by serial passage *in ovo*

Agnes Tinuke Laleye<sup>1,2</sup>, Celia Abolnik<sup>2</sup>; [passage in ovo. PLoS ONE 15\(10\): e0240290. https://doi.org/10.1371/journal.pone.0240290](https://doi.org/10.1371/journal.pone.0240290)



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

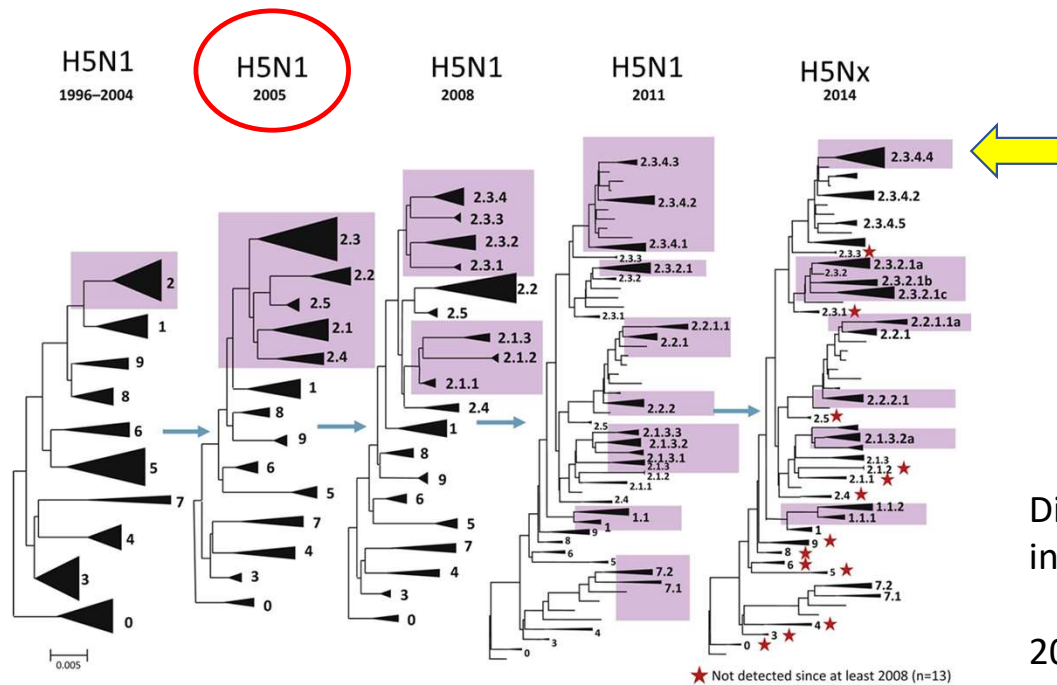
## Global HPAI events

- 45\* recognised global mutational events/ epidemics of H5Nx or H7Nx HPAI since 1959
  - \*SA's 2023 H7N6 HPAI outbreak is the 45<sup>th</sup> recorded global event
- 43/45 were **highly-localised** (single country) outbreaks or epidemics
  - most < 1 year, some < 2 years; eradicated by stamping out
  - Exceptions: Mexico H7N3, 2012-present; China H7N9, 2017-present
- 2/45 were **multi-country** outbreaks
  1. 2003's H7N7 HPAI chicken outbreaks in Netherlands, Belgium and Germany; eradicated by stamping out
  2. **Goose/Guangdong H5Nx HPAI pandemic**



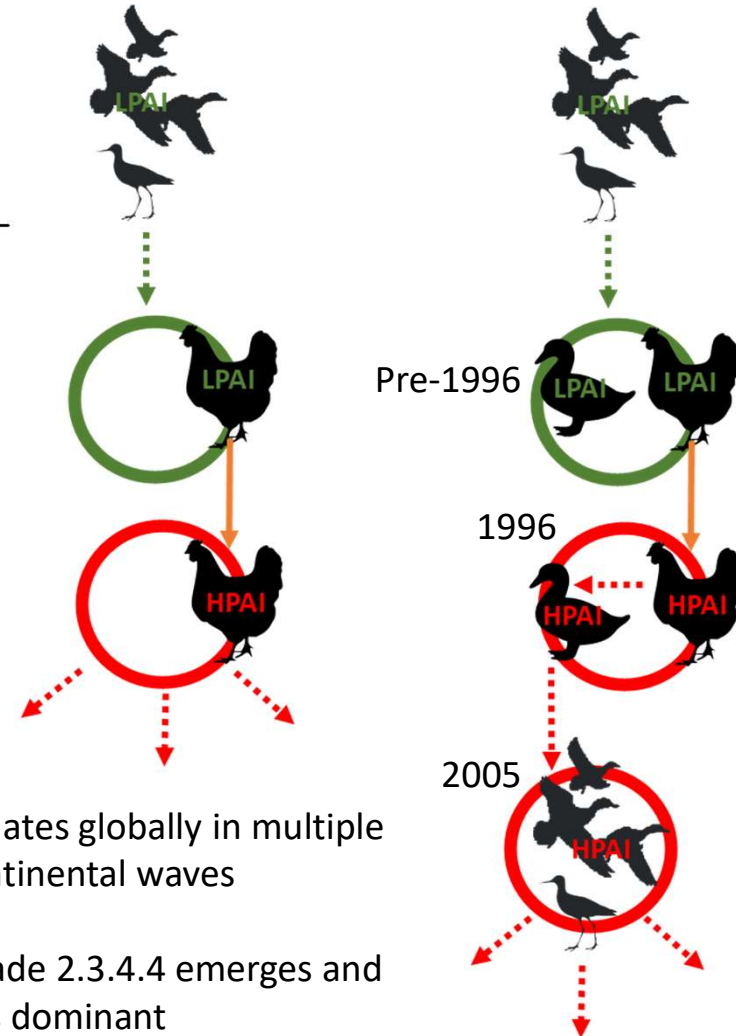
# Goose/Guangdong (Gs/GD) H5Nx HPAI pandemic

- First emerged in China in 1996 →
- Reassortment and evolutionary adaptation; divergence into sub-lineages; 8/10 extinct, 2 persist...
- Reassortment with LPAI viruses and genetic drift: gained ability to sub-clinically infect some migratory bird species



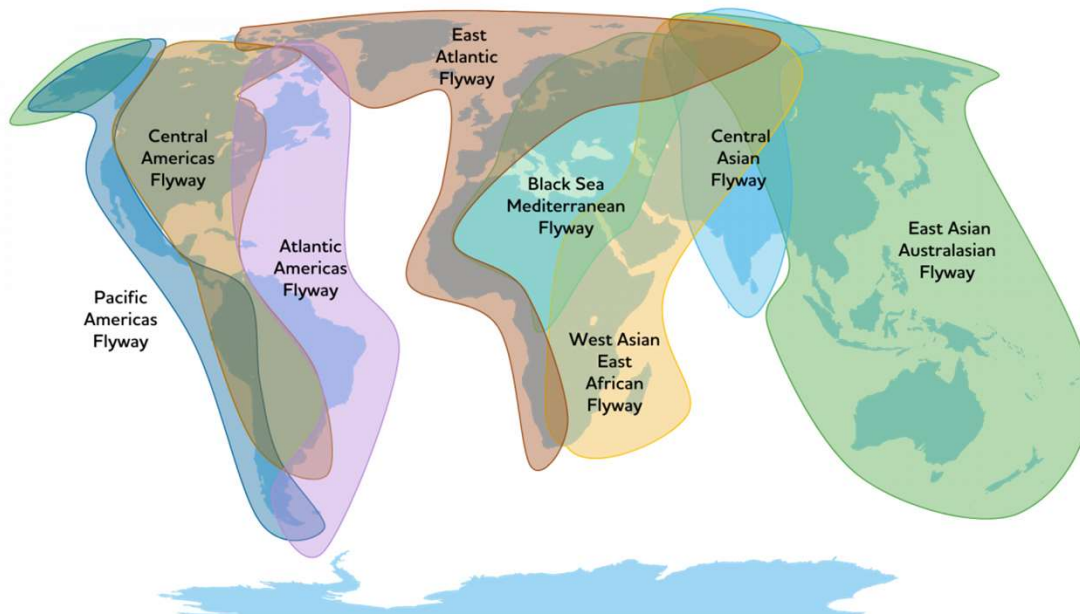
Source: [www.who.int/influenza/gisrs\\_laboratory/h5n1\\_nomenclature/en/](http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/en/).

“Classic” H5/H7 HPAI VS. Gs/GD H5 HPAI



# Role of wild birds in the introduction and spread of clade 2.3.4.4B in sub-Saharan Africa

Popular map of bird migration- depicts long-distance movements of waders, some storks and raptors to southern Africa (not ducks and geese)



<https://flyway.waddensea-worldheritage.org/flyway-conservation>



Little stint (*Calidris minuta*) Sanderling (*Calidris alba*) Ruff (*Philomachus pugnax*)

OPEN ACCESS Freely available online

PLOS ONE

## Investigating Avian Influenza Infection Hotspots in Old-World Shorebirds

Nicolas Gaidet<sup>1\*</sup>, Ahmed B. Ould El Mamy<sup>2</sup>, Julien Cappelle<sup>1</sup>, Alexandre Caron<sup>1</sup>, Graeme S. Cumming<sup>4</sup>, Vladimir Grosbois<sup>1</sup>, Patricia Gil<sup>3</sup>, Saliha Hammoumi<sup>3</sup>, Renata Servan de Almeida<sup>3</sup>, Sasan R. Fereidouni<sup>5</sup>, Giovanni Cattoli<sup>6</sup>, Celia Abolnik<sup>7</sup>, Josphine Mundava<sup>8</sup>, Boubou Fofana<sup>9</sup>, Mduduzi Ndlovu<sup>4</sup>, Yelli Diawara<sup>10</sup>, Renata Hurtado<sup>11,12</sup>, Scott H. Newman<sup>13</sup>, Tim Dodman<sup>14</sup>, Gilles Balança<sup>1</sup>

**Conclusions: No seasonal peaks in AIV prevalence  
Shorebirds do not play a significant role in AIV  
introduction to southern Africa**

If the implications of this map were true, southern Africa's clade 2.3.4.4 H5Nx outbreaks would start around **October/November-** but they don't; outbreaks start between **April and June.**



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

# What actually happens:

(courtesy of molecular epidemiological studies: surge in available H5 'flu genomes for analysis)

1. Palearctic-breeding ducks migrate south from Europe for the winter around October/November/December

e.g. Eurasian widgeon



2. Contaminated environment; inter-species mingling

3. Intra-African migrant ducks move south driven by rainfall patterns/ food availability

e.g. White-faced whistling duck



**No reverse gene movement:** environmental factors: higher temps, asynchronous duck breeding, unpredictable movements

## Understanding the ecological drivers of avian influenza virus infection in wildfowl: a continental-scale study across Africa

N. Gaidet<sup>1,\*</sup>, A. Caron<sup>1</sup>, J. Cappelle<sup>1</sup>, G. S. Cumming<sup>2</sup>, G. Balança<sup>1</sup>, S. Hammoumi<sup>3</sup>, G. Cattoli<sup>4</sup>, C. Abolnik<sup>5</sup>, R. Servan de Almeida<sup>3</sup>, P. Gil<sup>1</sup>, S. R. Fereidouni<sup>6</sup>, V. Grosbois<sup>1</sup>, A. Tran<sup>1</sup>, J. Mundava<sup>7</sup>, B. Fofana<sup>8</sup>, A. B. Ould El Mamy<sup>9</sup>, M. Ndlovu<sup>2</sup>, J. Y. Mondain-Monval<sup>10</sup>, P. Triplet<sup>11,12</sup>, W. Hagemeijer<sup>13</sup>, W. B. Karesh<sup>14</sup>, S. H. Newman<sup>15</sup> and T. Dodman<sup>13</sup>

NO. 12 | AUG 2012

## 2016–2018 Spread of H5N8 highly pathogenic avian influenza (HPAI) in sub-Saharan Africa: epidemiological and ecological observations

Contributors:

Sergei Khomenko,<sup>1</sup> Celia Abolnik,<sup>1</sup> Laura Roberts,<sup>2</sup> Lauren Waller,<sup>2</sup> Kevin Shaw,<sup>3</sup> Isabella Monne,<sup>3</sup> Joanna Taylor,<sup>3</sup> Madhur Dhingra,<sup>3</sup> Claudia Piatigallo,<sup>3</sup> Mason Matyeom,<sup>3</sup> Xavier Roche,<sup>3</sup> Kwasia Fredrick,<sup>3</sup> Akiko Kamata,<sup>4</sup> Sam Okuthe,<sup>4</sup> Philippe Kone,<sup>4</sup> Lidewij Wiersma,<sup>4</sup> Sophie Von Dobschuetz,<sup>4</sup> Baba Soumare,<sup>4</sup> Yilma Makonnen,<sup>4</sup> Subhash Morzaria,<sup>4</sup> Juan Lubroth,<sup>4</sup> 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15



Article

### Wild Bird Surveillance in the Gauteng Province of South Africa during the High-Risk Period for Highly Pathogenic Avian Influenza Virus Introduction

Celia Abolnik<sup>1,\*</sup>, Thandeka P. Phiri<sup>1</sup>, Gerbrand van der Zel<sup>2</sup>, Jade Anthony<sup>1</sup>, Nadine Daniell<sup>1</sup> and Liesl de Boni<sup>2</sup>

### Why South Africa is more prone to AI outbreaks:

- bottom of migratory funnel
- Intensive agriculture- grazing ducks (index cases in the maize belt around the Vaal river)
  - higher poultry densities
- more temperate climate: environmental survival

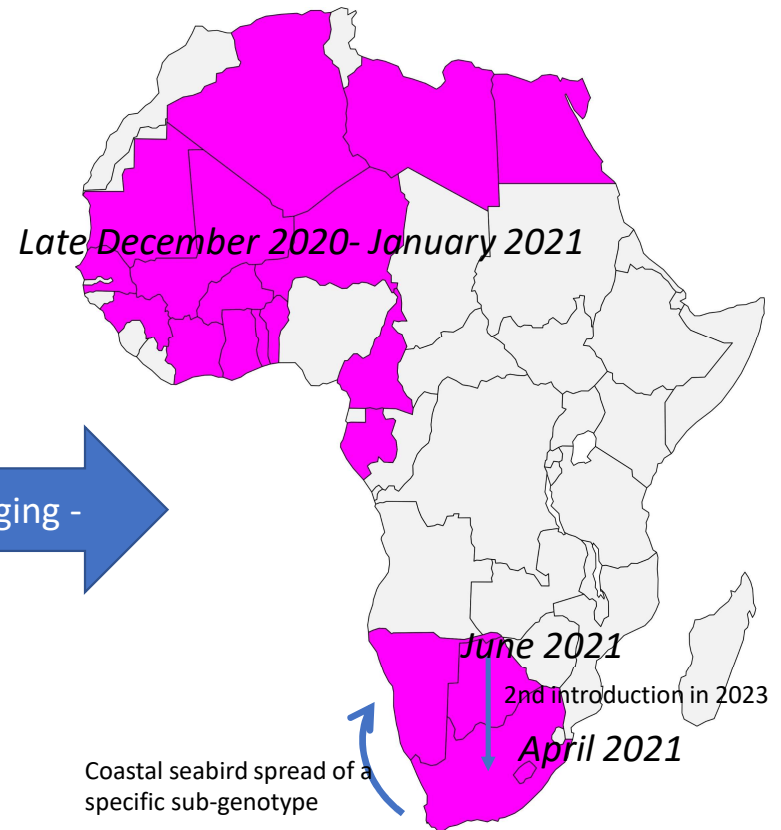
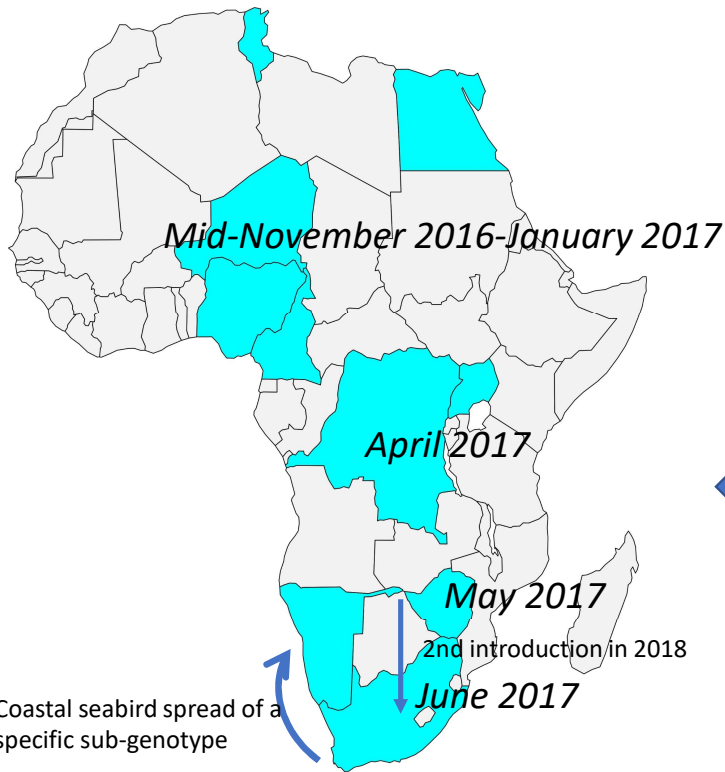


## Two waves of Clade 2.3.4.4B H5Nx HPAI spread to sub-Saharan Africa with wild migratory birds

index cases- reported cases in wild birds and/or poultry:

### 2016-2018: H5N8

### 2021-2023: H5N1



SA: 5.4 million broiler and layer chickens culled  
 Total economic losses: R1.66 billion  
 Mass coastal seabird mortalities- e.g. Swift terns; Cape cormorants

SA: 3.7 million layer chickens culled  
 Mass coastal seabird mortalities- e.g., African penguins

# South African H5N1 HPAI outbreaks- 2023



## The Molecular Epidemiology of Clade 2.3.4.4B H5N1 High Pathogenicity Avian Influenza in Southern Africa, 2021–2022

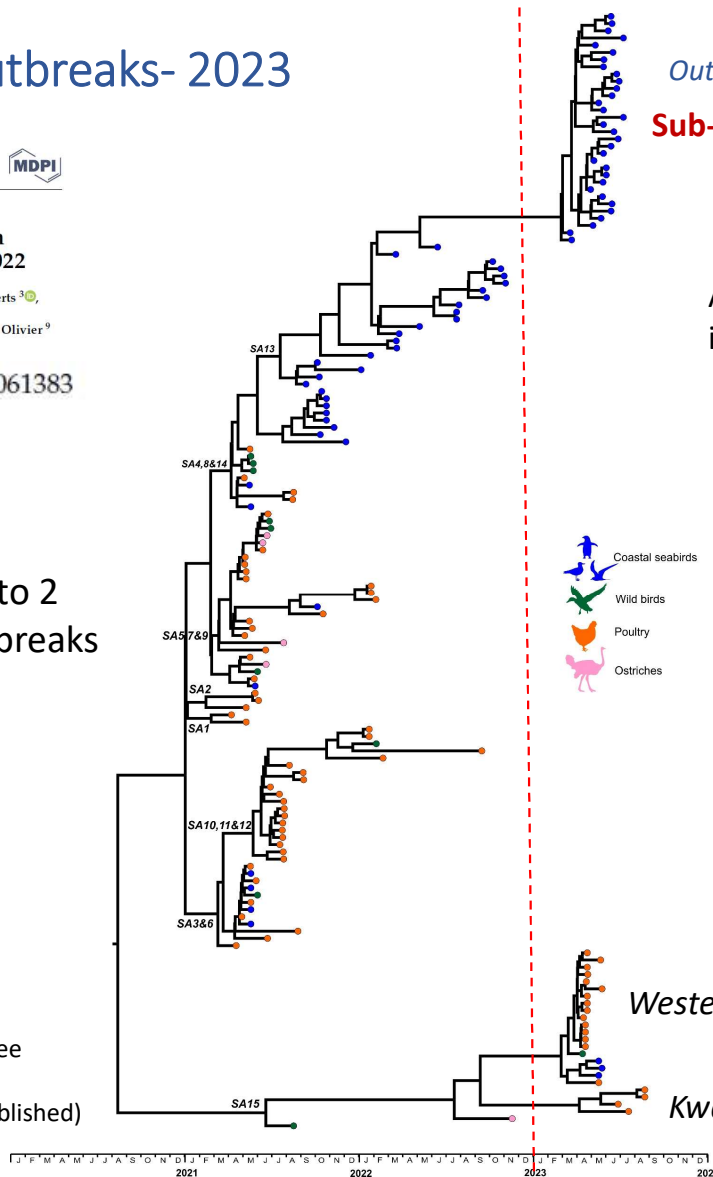
Celia Abolnik <sup>1,\*</sup>, Thandeka Phiri <sup>1</sup>, Belinda Peyrot <sup>2</sup>, Renee de Beer <sup>2</sup>, Albert Snyman <sup>3</sup>, David Roberts <sup>3</sup>, Katrin Ludynia <sup>3,4</sup>, Frances Jordaan <sup>5</sup>, Michele Maartens <sup>5</sup>, Zehaad Ismail <sup>6</sup>, Christine Strydom <sup>1,6</sup>, Gerbrand van der Zel <sup>7</sup>, Jade Anthony <sup>1</sup>, Nadine Daniell <sup>1</sup>, Liesl De Boni <sup>7</sup>, John Grewar <sup>1,8</sup>, Adriaan Olivier <sup>9</sup> and Laura Roberts <sup>1,10</sup>

*Viruses* 2023, 15, 1383. <https://doi.org/10.3390/v15061383>

- >100 complete genomes
- High diversity introduced; reduced to 2
- Relationships between country outbreaks
- >80 % primary introductions



Time-scaled Maximum Clade Credibility tree  
Concatenated H5N1 genomes  
Southern African strains 2021-2023 (unpublished)



*Outbreaks in coastal seabirds, March to June*

**Sub-genotype SA13**

Antigenic differences- overcame “herd” immunity in wild bird reservoir

**H5N1 persisted in wild bird reservoir in 2023:**

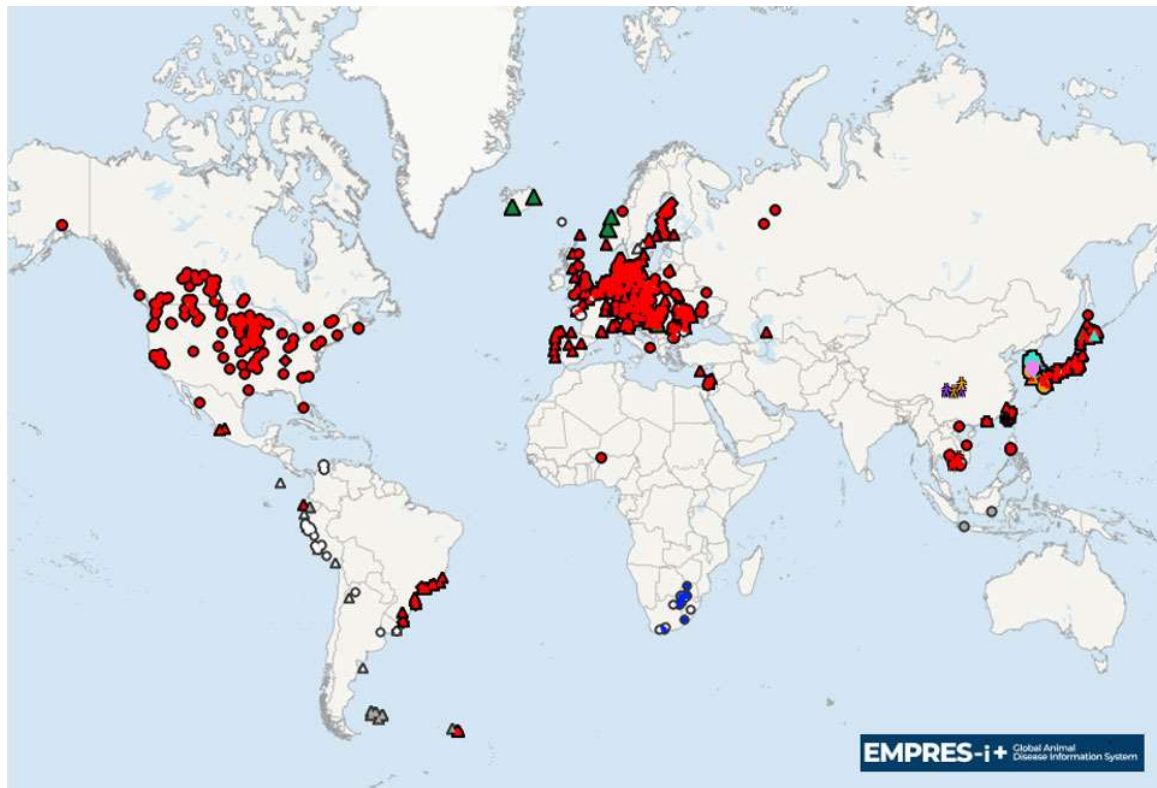
Wild bird surveillance  
42.2 % of all AIV positive duck fecal swab pools = H5 positive

*Outbreaks in poultry, April to August*

*Western Cape outbreaks* **Sub-genotype SA15**  
tMRCA: entered SA ~July 2022

*KwaZulu-Natal outbreaks*

# Current global HPAI situation



FAO map: 1 October 2023- 22 February 2024

Mammals...

## Countries reporting HPAI H5Nx outbreaks since

**22 Feb:**

**USA:** wild birds + poultry

**Canada:** wild birds + poultry

**Ecuador:** poultry

**Brazil:** wild birds

**Panama:** wild birds

**Mexico:** wild birds + poultry

**South Georgia & Sandwich Islands:** wild birds

**Sweden:** wild birds

**Norway:** wild birds + poultry

**UK:** wild birds + poultry

**Poland:** wild birds + poultry

**Italy:** wild birds + poultry

**Finland:** wild birds

**Slovenia:** wild birds

**Switzerland:** wild birds

**Romania:** wild birds

**Hungary:** wild birds + poultry

**Czech Republic:** wild birds + poultry

**Germany:** wild birds + poultry

**Denmark:** wild birds

**Austria:** wild birds

**Ukraine:** wild birds

**Bulgaria:** poultry

**Slovakia:** poultry

**Moldova:** poultry

**Israel:** wild birds

**Cambodia:** poultry

**Nigeria:** poultry

**Senegal:** wild birds + poultry

## Natural infections of H5 HPAI reported in mammals

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 30, No. 3, March 2024 Plaza et al

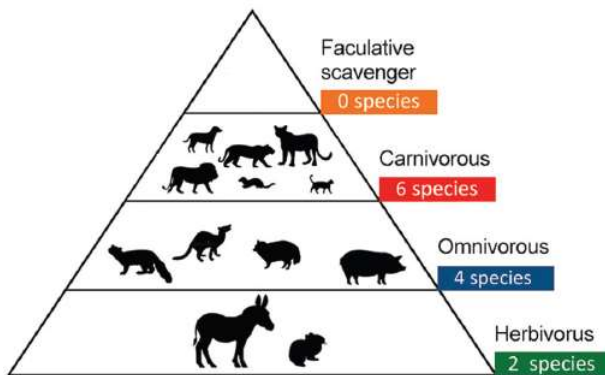
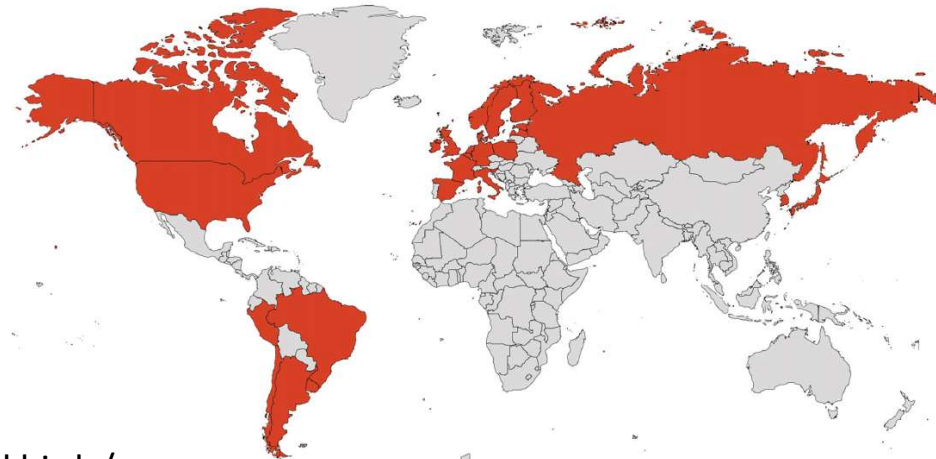
(16 years)

Gs/GD H5Nx, 2003-2019

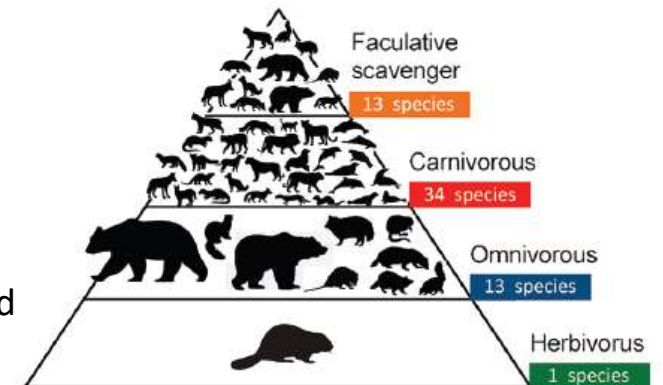


(3 years)

Gs/GD clade 2.3.4.4B H5N1, 2020-2023



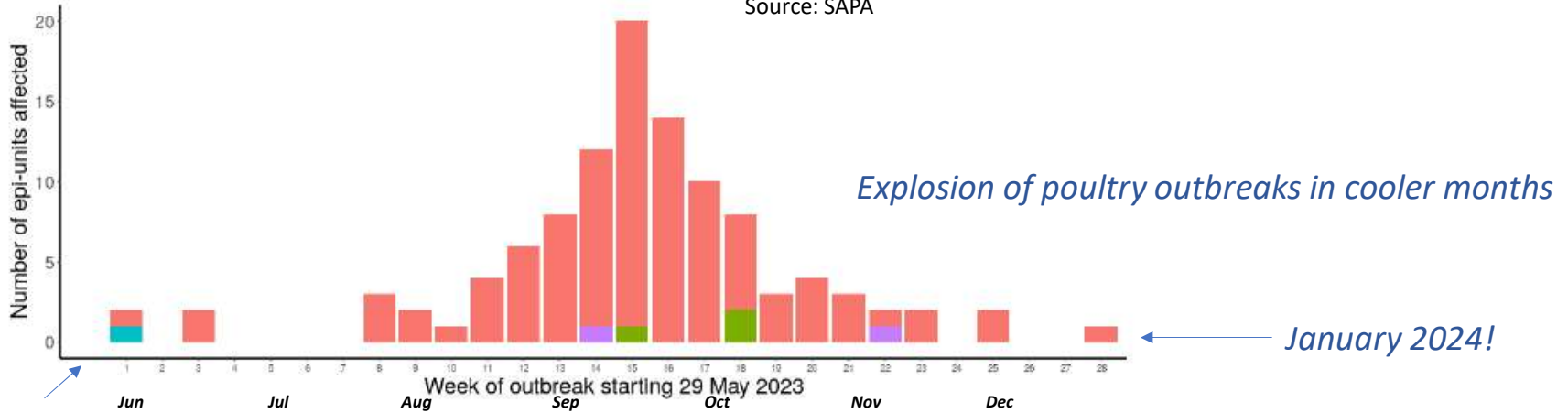
- Contact with infected birds/contaminated environment; water
- Scavenging on dead or dying birds
- Loss of appetite, respiratory and neurologic signs; death
- So far no sustained mammal-mammal transmission
- Molecular markers being monitored
- **Increasing zoonotic potential**





## Epidemiologic curve for H7N6 HPAI outbreaks

Source: SAPA



HPAI emergence:  
tMRCA = mid-late  
May

Phase of unreported/  
undetected cases

Enterprise type

- commercial
- free range
- small holder
- wildbirds

### H7N6 HPAI not maintained in or spread by wild birds:

Wild bird surveillance: duck environmental fecal swabs  
477 fecal swab pools representing ~2385 individual fecal swabs  
received for testing from 6/9 provinces in 2023; Mostly  
Gauteng, KZN, WC



Subtyping of positive cases (60.1%):

42.2% = H5+

0.3% = H9+

0.3% = H11+

**0.7% = H7 positive;**

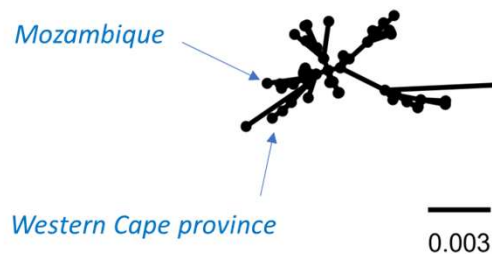
**(Not N6+)**

**KZN, WC.** No H7 detected in outbreak areas

# H7N6 HPAI was disseminated by movements of infected chickens and fomites

- Adapted to replication chickens; lower bird infectious dose (?):
- Highly contagious and more so than clade 2.3.4.4 Gs/GD H5N1; biosecurity measures that kept H5N1 out failed with H7N6
- Routes of spread:
  - Poultry movements
    - Incubation phase- birds moved before the flock showed clinical signs; owners selling/dumping sick birds
  - Fomites
    - Vehicles (e.g., feed trucks), mud, feathers, possibly wind (dust, feathers) over short distances

Phylogenetic analysis: single source outbreaks; genetic and epidemiologic data links suppliers and clients (e.g., sale of point-of-lay pullets)



EPI\_ISL\_12852376\_A\_ostrich\_South\_Africa\_070595\_2020\_H7N1

Emerging Microbes & Infections  
2024, VOL. 13, 2321993 (5 pages)  
<https://doi.org/10.1080/22221751.2024.2321993>



LETTER TO THE EDITOR

OPEN ACCESS Check for updates

## H7N6 highly pathogenic avian influenza in Mozambique, 2023

Iolanda Vieira Anahory Monjane<sup>a</sup>, Hernâni Djedje<sup>a</sup>, Esmeralda Tamele<sup>a</sup>, Virginia Nhabomba<sup>a</sup>, Almiro Rogério Tivane<sup>b</sup>, Zacarias Elias Massicame<sup>c</sup>, Dercília Mudanisse Arone<sup>c</sup>, Ambra Pastori<sup>d</sup>, Alessio Bortolami<sup>d</sup>, Isabella Monne<sup>e,d</sup>, Timothy Woma<sup>e</sup>, Charles E. Lamien<sup>e</sup> and William G. Dondon<sup>e,f</sup>

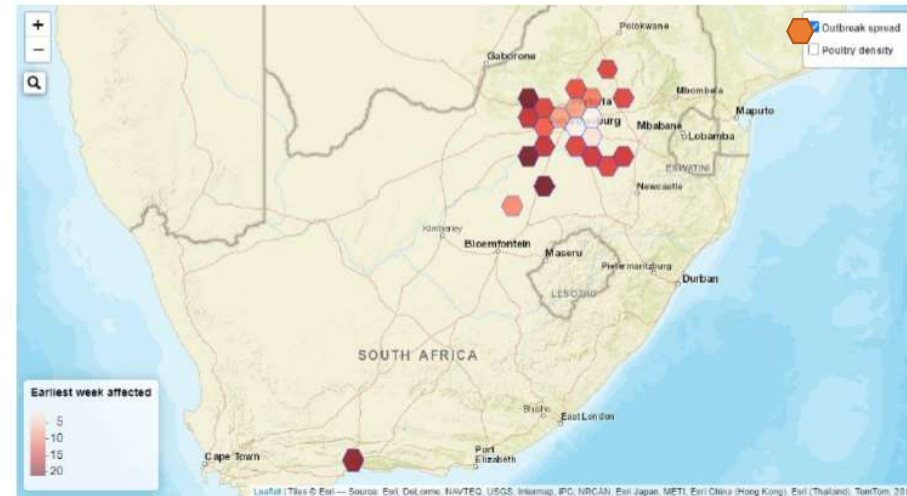


Figure 13: H7N6 outbreak spread (29 May to 8 December 2023)



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

# Summary and Conclusions

## Two contrasting HPAI epidemiological situations

### CLASSICAL

#### H7N6 HPAI

- LPAI progenitor present in southern African wild birds
- HPAI emergence in local poultry
- Highly localized outbreaks
- Spread by poultry movements and fomites
- Chicken-adapted: efficient chicken-chicken spread
- No wild bird involvement in HPAIV spread
- Low zoonotic potential
- Poor control facilitated spread

Is it gone? Winter will tell.

vs.

### EXCEPTIONAL

#### Gs/GD clade 2.3.4.4B H5Nx HPAI

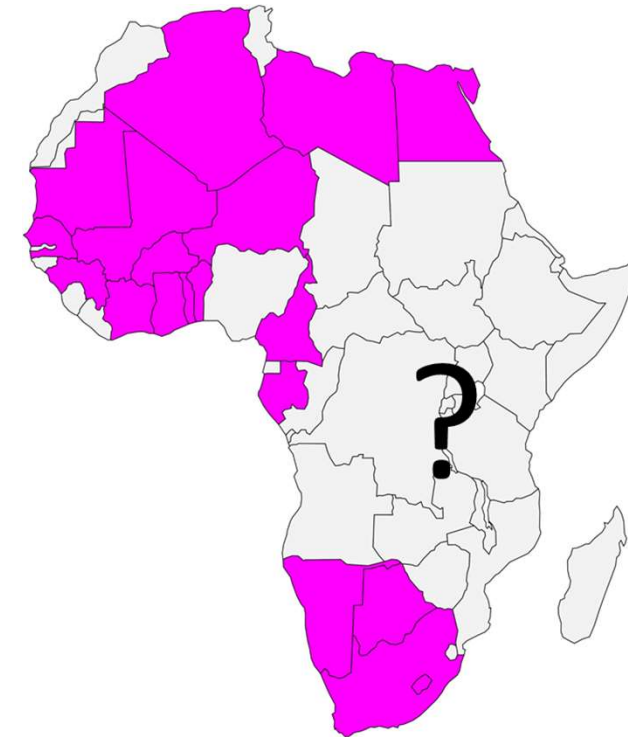
- LPAI progenitor in Chinese wild birds in early 1990's
- Emergence in Chinese poultry
- Circulation in domestic chickens and ducks (China)
- Reassortment events with LPAIVs
- Decades of evolution
- Adaptation to wild birds and poultry
- High environmental fecal contamination by ducks
- Lower ability for chicken secondary spread
- Northern hemisphere reservoir
- Disseminated by migratory wild birds
- Increasing zoonotic potential
- Annual, unavoidable risk





## Conclusions: clade 2.3.4.4B H5N1 HPAI

- Highly unlikely that other southern African countries had no H5N1 cases in 2021-2022; either not diagnosed (e.g., assumed NDV; botulism), or not reported
- Wild ducks don't respect borders- all at risk
- Encouraging to see more AI surveillance, virus genome data from SADC; regional institutions in collaboration with northern hemisphere partners
- Gaps in sequence database: need more AIV sequencing from SADC countries:
  - Essential for deeper understanding of HPAI epidemiology; risk assessment and mitigation; updating of diagnostic tests



### Highly pathogenic avian influenza (A/H5N1) virus outbreaks in Lesotho, May 2021

Mabusetsa R.J. Makalo <sup>a</sup>, William G. Dundon <sup>b</sup>, Tirumala B.K. Settypalli <sup>b</sup>, Sneha Datta <sup>b</sup>, Charles E. Lamien <sup>b</sup>, Giovanni Cattoli <sup>b</sup>, Moeketsi S. Phalatsi <sup>c</sup>, Relebohile J. Lepheana<sup>a</sup>, Mpaliseng Matlali<sup>a</sup>, Relebohile G. Mahloane<sup>a</sup>, Marosi Molomo<sup>a</sup> and Palesa C. Mphaka<sup>a</sup>

#### Research Article







### Surveillance and Phylogenetic Characterisation of Avian Influenza Viruses Isolated from Wild Waterfowl in Zambia in 2015, 2020, and 2021

Annic Kalonda <sup>1,2,3</sup>, Ngonda Saasa,<sup>2</sup> Masahiro Kajihara,<sup>4,5</sup> Naganori Nao,<sup>4,5,6</sup> Ladislav Moonga,<sup>7</sup> Joseph Ndebe,<sup>2</sup> Akina Mori-Kajihara,<sup>8</sup> Andrew Nalishuwa Mukubesa,<sup>2</sup> Mulemba Samutela <sup>1,3,7</sup>, Samuel Munjita,<sup>1,2,3</sup> Yoshihiro Sakoda,<sup>5,9,10,11</sup> Hirofumi Sawa,<sup>2,3,4,5,6,9,11,12,13</sup> Ayato Takada <sup>2,3,6,8,9</sup> and Edgar Simulundu <sup>2,14</sup>



#### Communication

### Emergence of High Pathogenicity Avian Influenza Virus H5N1 Clade 2.3.4.4b in Wild Birds and Poultry in Botswana

Samantha L. Letsholo <sup>1,\*</sup>, Joe James <sup>2</sup>, Stephanie M. Meyer <sup>2</sup>, Alexander M. P. Byrne <sup>2</sup>, Scott M. Reid <sup>2</sup>, Tirumala B. K. Settypalli <sup>3</sup>, Sneha Datta <sup>3</sup>, Lethogile Oarabile <sup>4</sup>, Obakeng Kemolathe <sup>4</sup>, Kgagamatso T. Pebe <sup>4</sup>, Bruce R. Mafonko <sup>4</sup>, Tebogo J. Kgotlele <sup>1</sup>, Kago Kumile <sup>1</sup>, Boitumelo Modise <sup>1</sup>, Carter Thanda <sup>1</sup>, John F. C. Nyange <sup>1</sup>, Chandapiwa Marobela-Raborokgwe <sup>1</sup>, Giovanni Cattoli <sup>3</sup>, Charles E. Lamien <sup>3</sup>, Ian H. Brown <sup>2</sup>, William G. Dundon <sup>3</sup> and Ashley C. Banyard <sup>2,\*</sup>



### Highly pathogenic avian influenza H5N1 virus outbreak among Cape cormorants (*Phalacrocorax capensis*) in Namibia, 2022

Umberto Molini, John Yabe, Irene K. Meki, Hatem Ouled Ahmed Ben Ali, Tirumala B.K. Settypalli, Sneha Datta, Lauren Michelle Coetzee, Ellini Hamunyela, Siegfried Khaiseb, Giovanni Cattoli, Charles E. Lamien & William G. Dundon

## Conclusions

- Reintroduction of H5N1 in 2024? Senegal and Nigeria reported cases in poultry and/or wild birds since February; new antigenic variants from Europe may overcome herd (flock) immunity
- Mitigation of risk:
  - Keep wild birds, rodents and insects out of poultry houses; do not use untreated surface water
  - Poultry farms should be located as far away from natural water sources as possible; high environmental load- wind blowing across wet feces can aerosolize virus
  - Do not mix species.
    - Duck farms and chicken farms should be separated
    - Domestic ducks must not have access to a natural water source frequented by wild ducks
  - Remove and bury domestic and wild bird carcasses – risk of mammalian infections through predation
  - Vigilance; diagnose suspicious deaths in mammals in vicinity of avian outbreaks



Thank you for your attention

Email: [celia.Abolnik@up.ac.za](mailto:celia.Abolnik@up.ac.za)

Questions?

