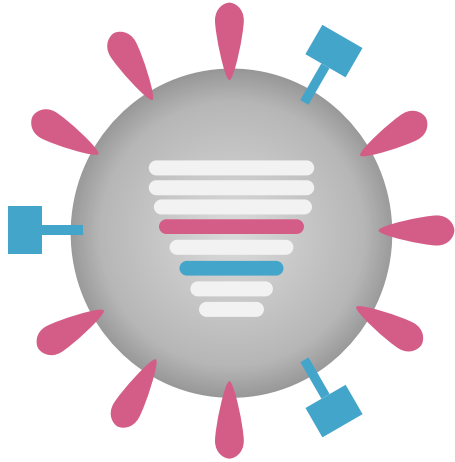


East Asia Wildlife health networking meeting
June 16, 2023

High-Pathogenicity Avian Influenza Outbreak among Vulnerable Crane Species in the Izumi Plain, Kagoshima, Japan

Kosuke Okuya, DVM, PhD
Transboundary Animal Disease Research Center,
Faculty of Joint Veterinary Medicine,
Kagoshima University

Avian influenza virus (AIV)



Genome: Eight segmented RNA
Negative-stranded

Hemagglutinin (HA)
Subtypes : H1-H16

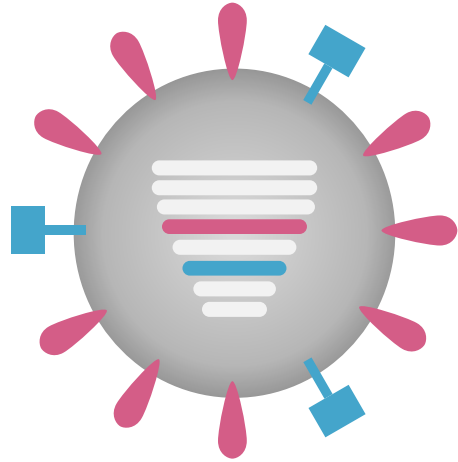
Neuraminidase (NA)
Subtypes: N1-N9

Natural reservoirs

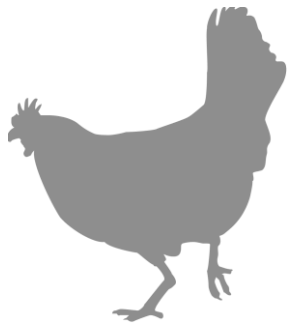


Wild ducks

High Pathogenicity Avian Influenza Viruses (HPAIVs)



Part of H5 and H7 subtypes



High mortality in chickens ($\geq 75\%$)

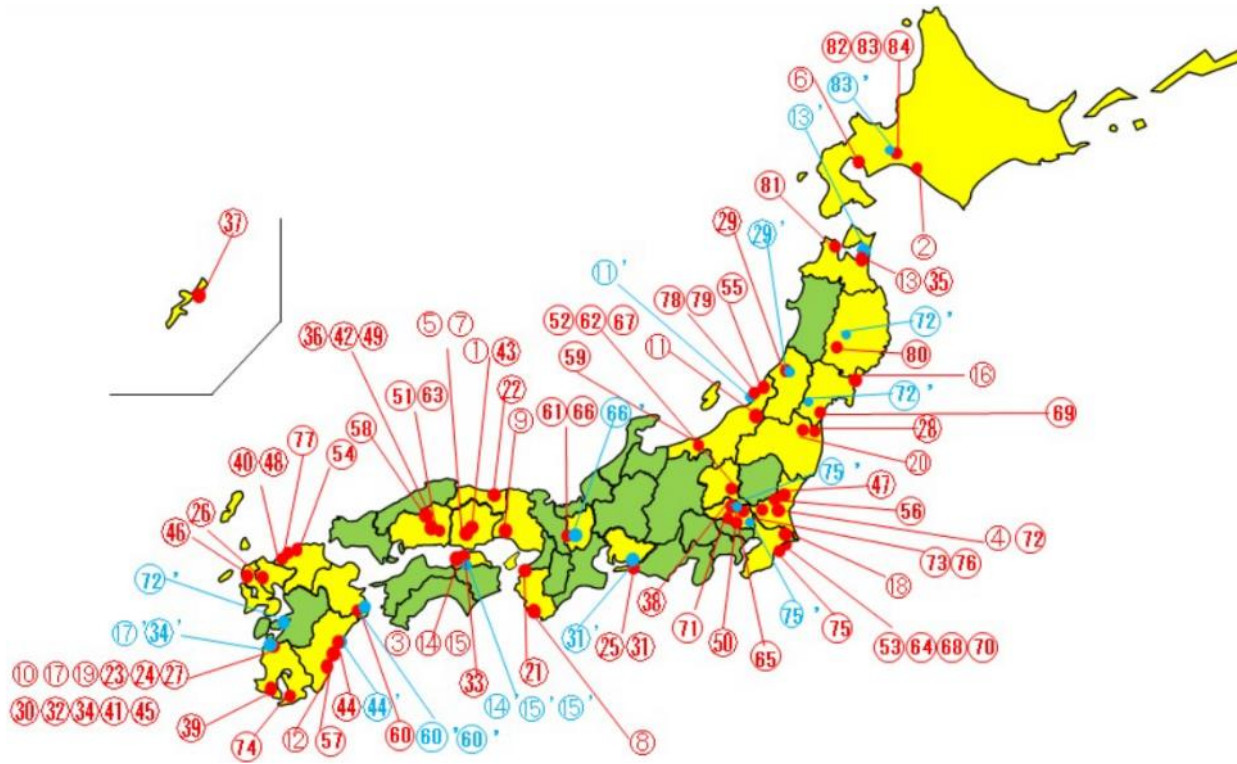


Wild ducks

Relatively low mortality in wild ducks

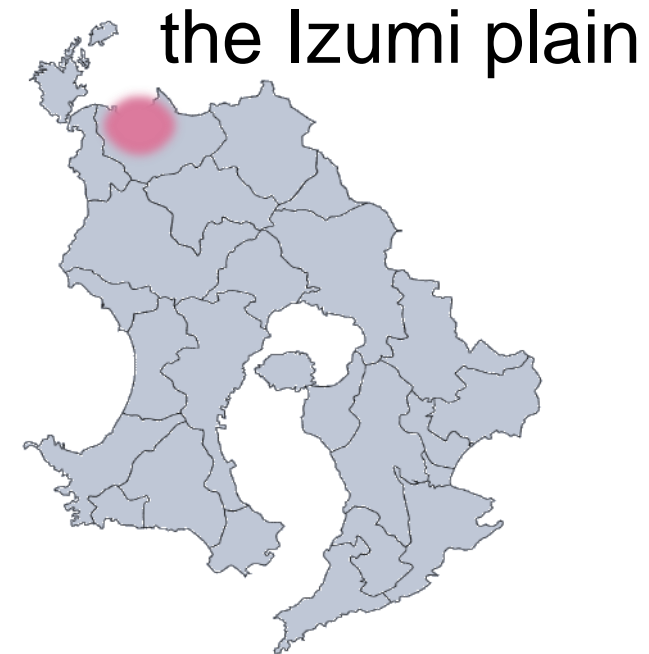
Global spread by migration of wild ducks

HPAI in Japan during the 2022/23 winter season



Poultry farms: 84 cases
Wild birds: 242 cases

Ministry of Agriculture, Forestry and Fisheries



The Izumi plain, Kagoshima

Known as a wintering ground for more than 12,000 endangered cranes



The Izumi plain, Kagoshima

Known as a wintering ground for more than 12,000 endangered cranes

Registered as a Ramsar Convention site in 2021



Hooded crane
(*Grus monacha*)



White-naped crane
(*Grus vipio*)

Crane conservation activities

Preparing roost



Feeding



Monitoring



The Izumi plain, Kagoshima



Wild ducks also visit the Izumi plain.

The Izumi city, Kagoshima

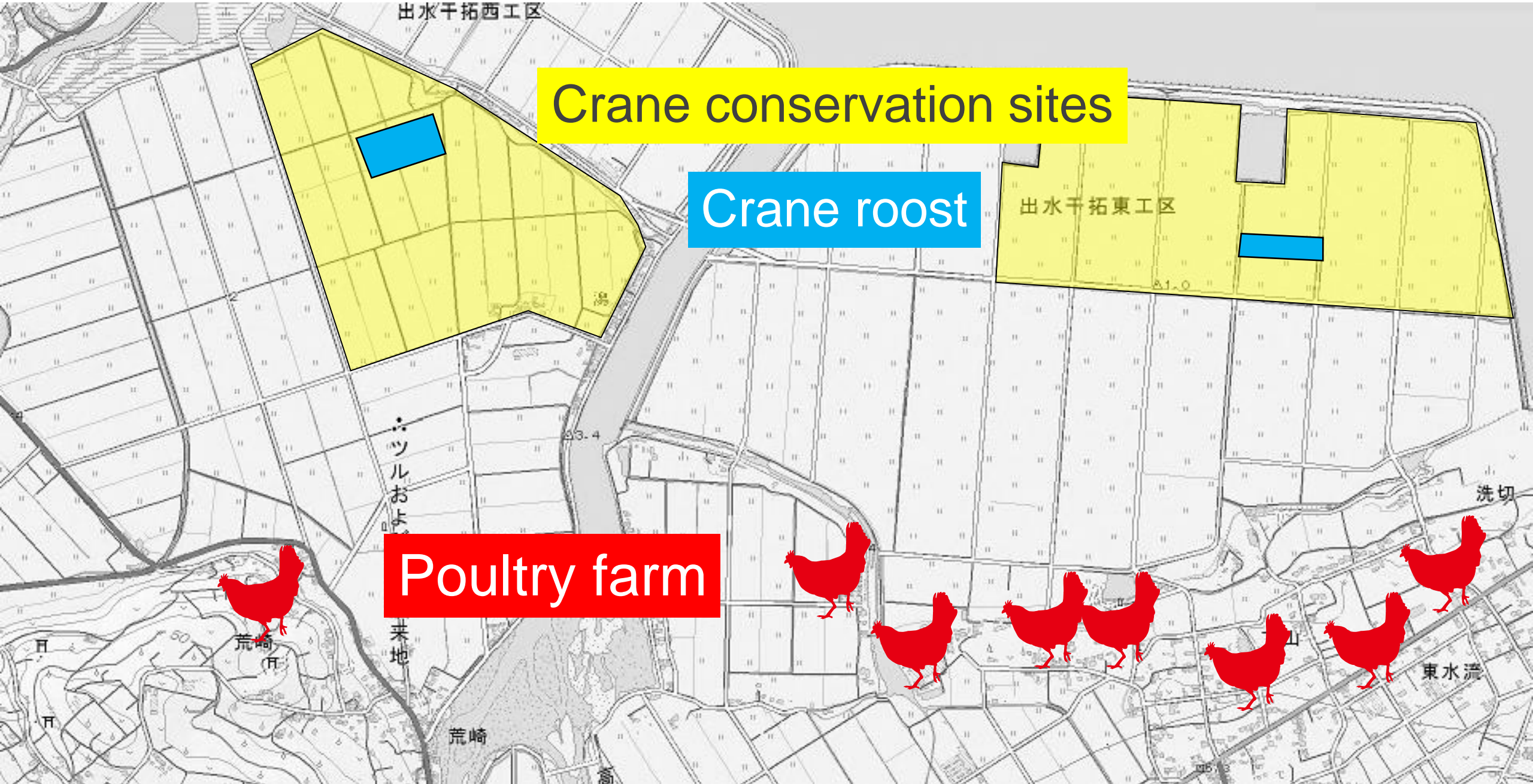
Poultry farming is thriving in Izumi city.



Izumi city HP

Izumi city had the highest egg production in Japan in 2021.

The Izumi plain, Kagoshima



Crane conservation sites

Crane roost

Poultry farm

The Izumi plain, Kagoshima



出水干拓西工区

Crane conservation sites

出水干拓東工区

This map shows the Izumi plain in Kagoshima, Japan. It features a grid of agricultural fields and waterways. Two specific areas are highlighted with grey rectangles: one in the upper left and one in the upper right. The text '出水干拓西工区' (Mizumi Kankaku Seibu Kouku) is located at the top left, and '出水干拓東工区' (Mizumi Kankaku Toubu Kouku) is at the top right. A large black text box is overlaid in the center.

Crane roost

To protect “**endangered crane species**” and “**poultry farming**”, AIV surveillance is essential in this area.



荒崎

菜地

荒崎

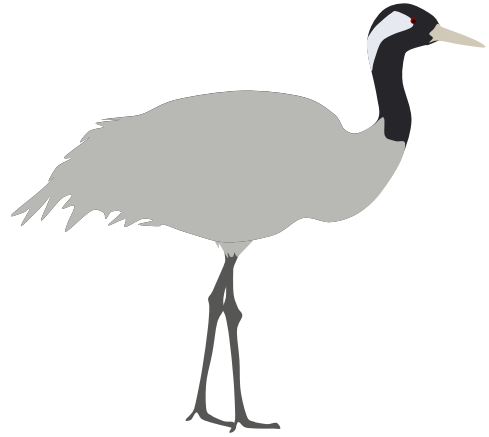
東水流

洗切

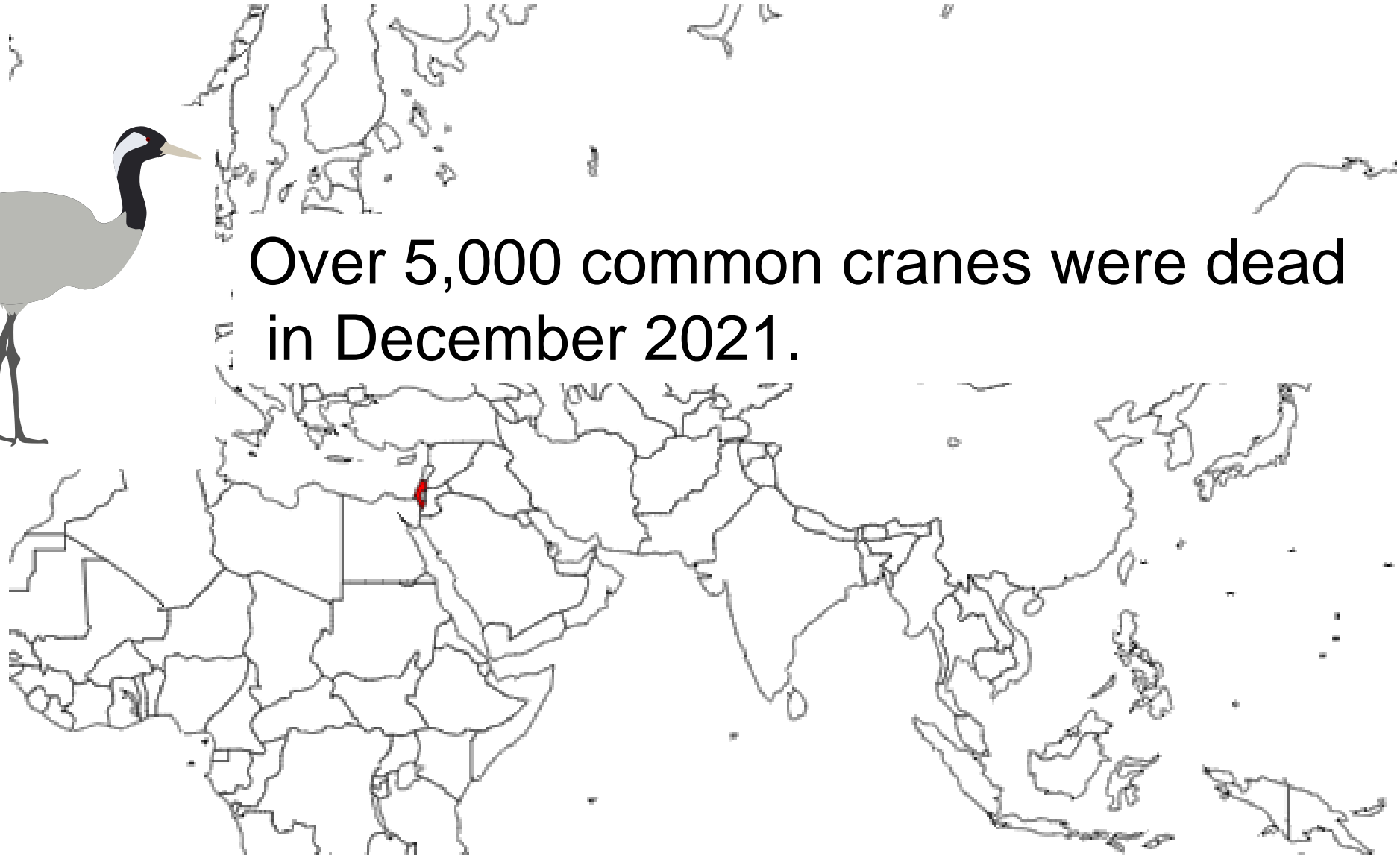
Poultry farm

This map shows a different section of the Izumi plain. It features a grid of agricultural fields and waterways. Several chicken silhouettes are overlaid on the map, indicating a poultry farm. The text '荒崎' (Arakaki) appears in two locations, '菜地' (Naidi) is in the center, and '東水流' (Toumizu) and '洗切' (Saiji) are on the right side.

H5 HPAIV caused mass death in Israel



Over 5,000 common cranes were dead
in December 2021.



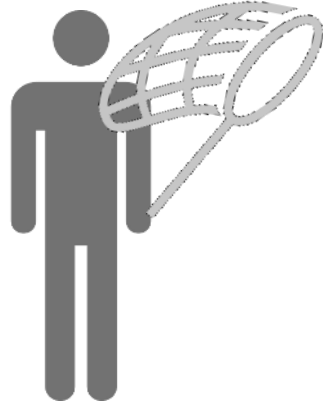
Topics

- H5 HPAI in endangered cranes
- H5 HPAIVs isolated from environment water
- Genetic characterization of H5 HPAIVs in the Izumi plain

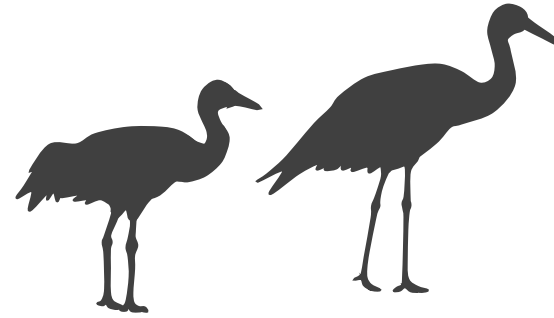
Topics

- H5 HPAI in endangered cranes
- H5 HPAIVs isolated from environment water
- Genetic characterization of H5 HPAIVs in the Izumi plain

AIV surveillance in cranes in the Izumi plain

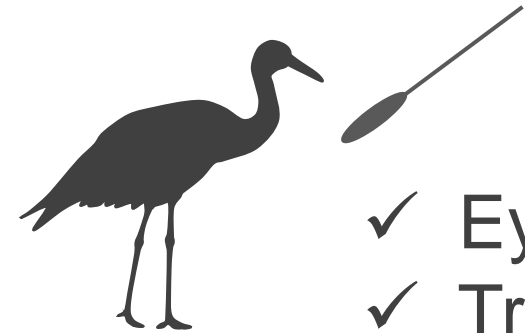


Retrieving



Debilitated and dead cranes

Municipal employees of Izumi City



- ✓ Eye swab
- ✓ Tracheal swab
- ✓ Cloacal swab



- ✓ AIV rapid test kit

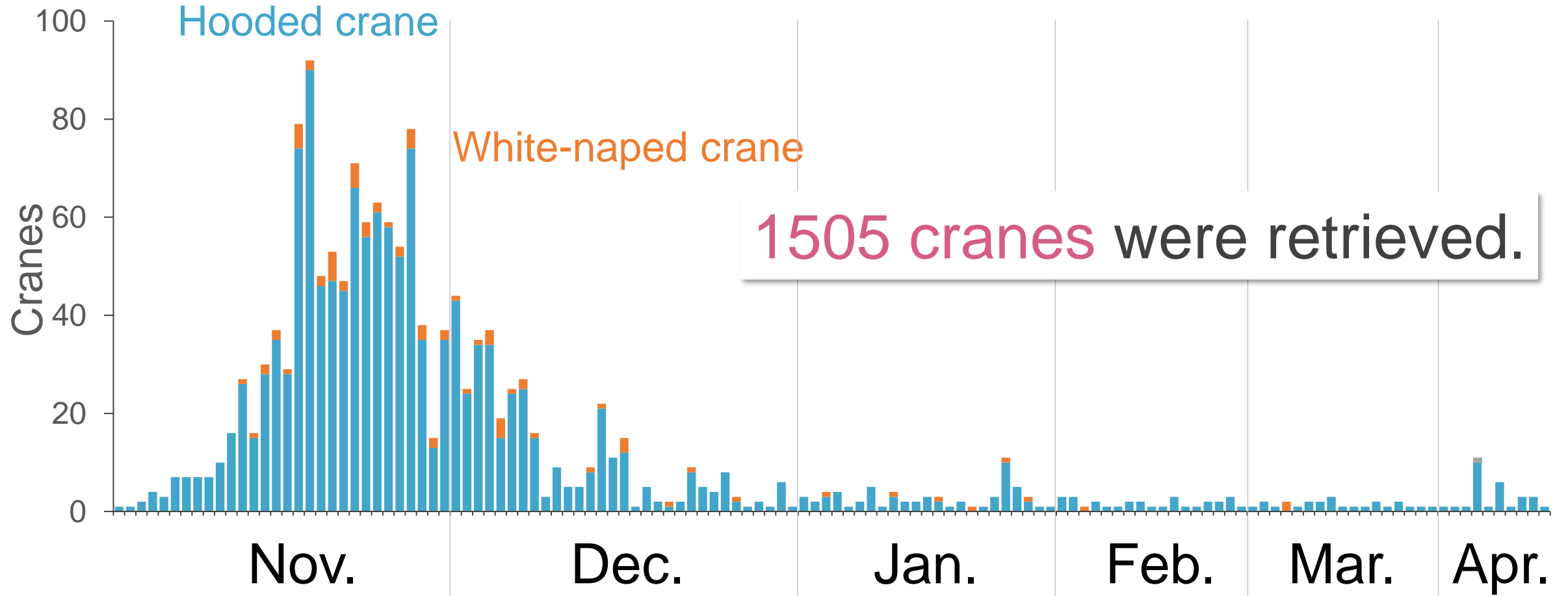


- ✓ AIV gene diagnosis
- ✓ Virus isolation
- ✓ Sequencing

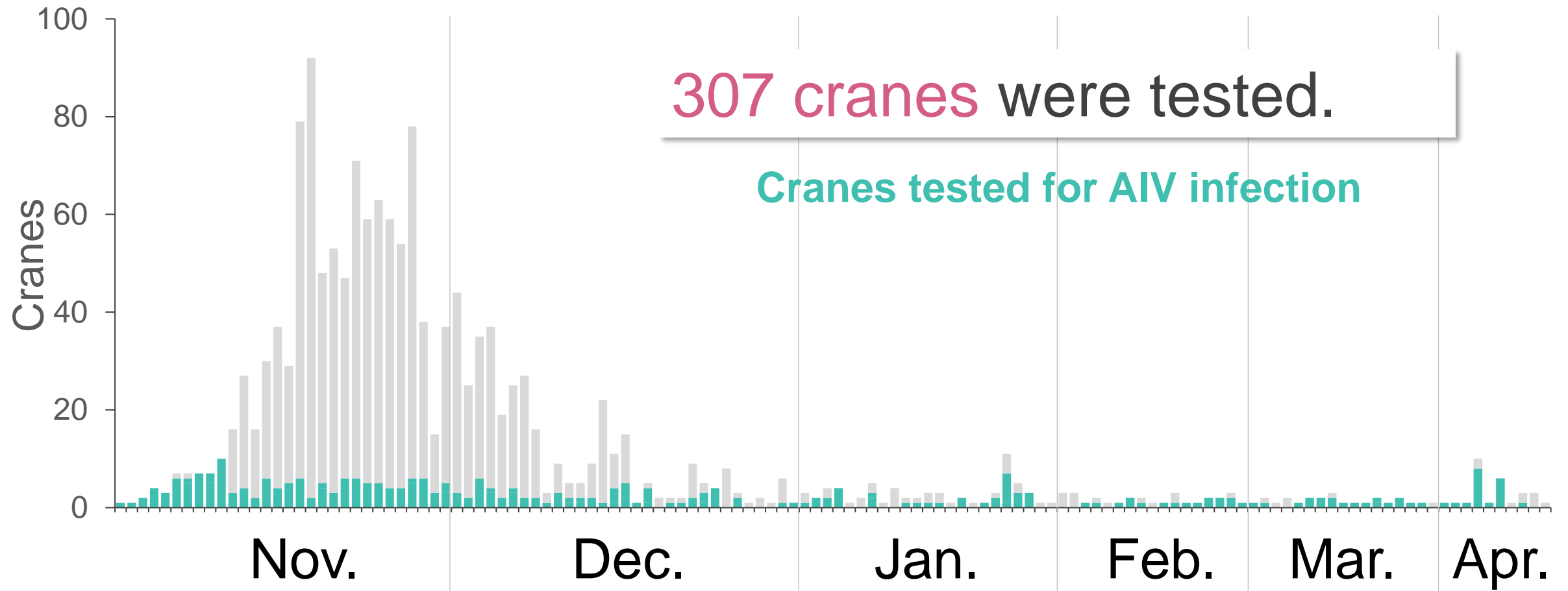
Kagoshima Univ.



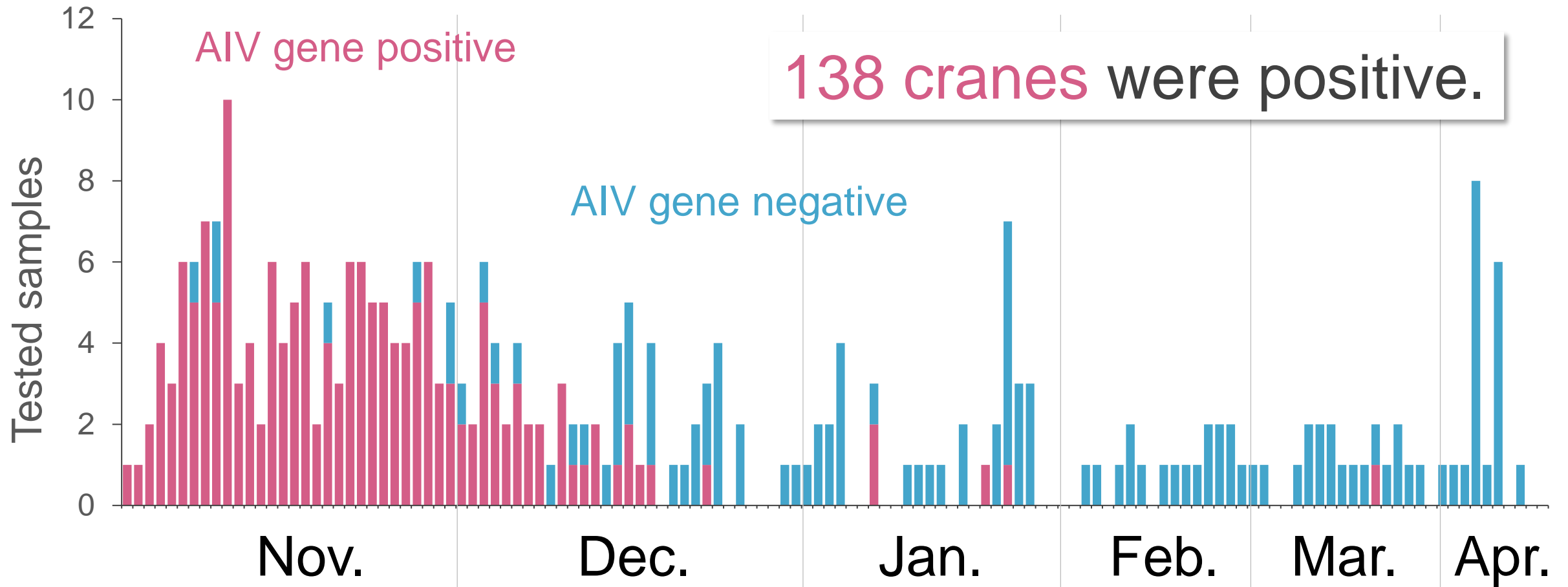
Number of cranes retrieved in the Izumi plain



Number of cranes retrieved in the Izumi plain

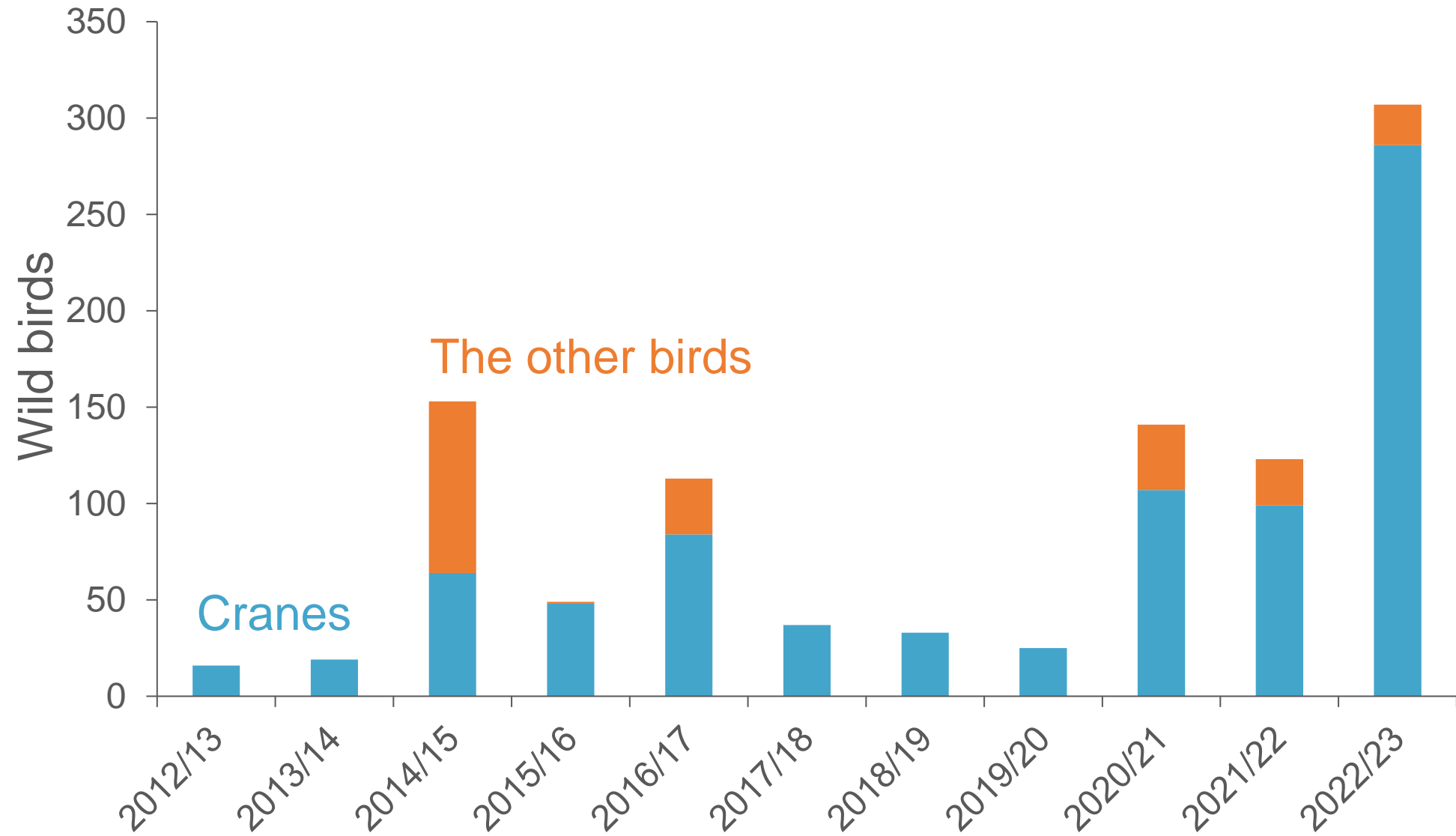


Number of cranes tested for AIV gene

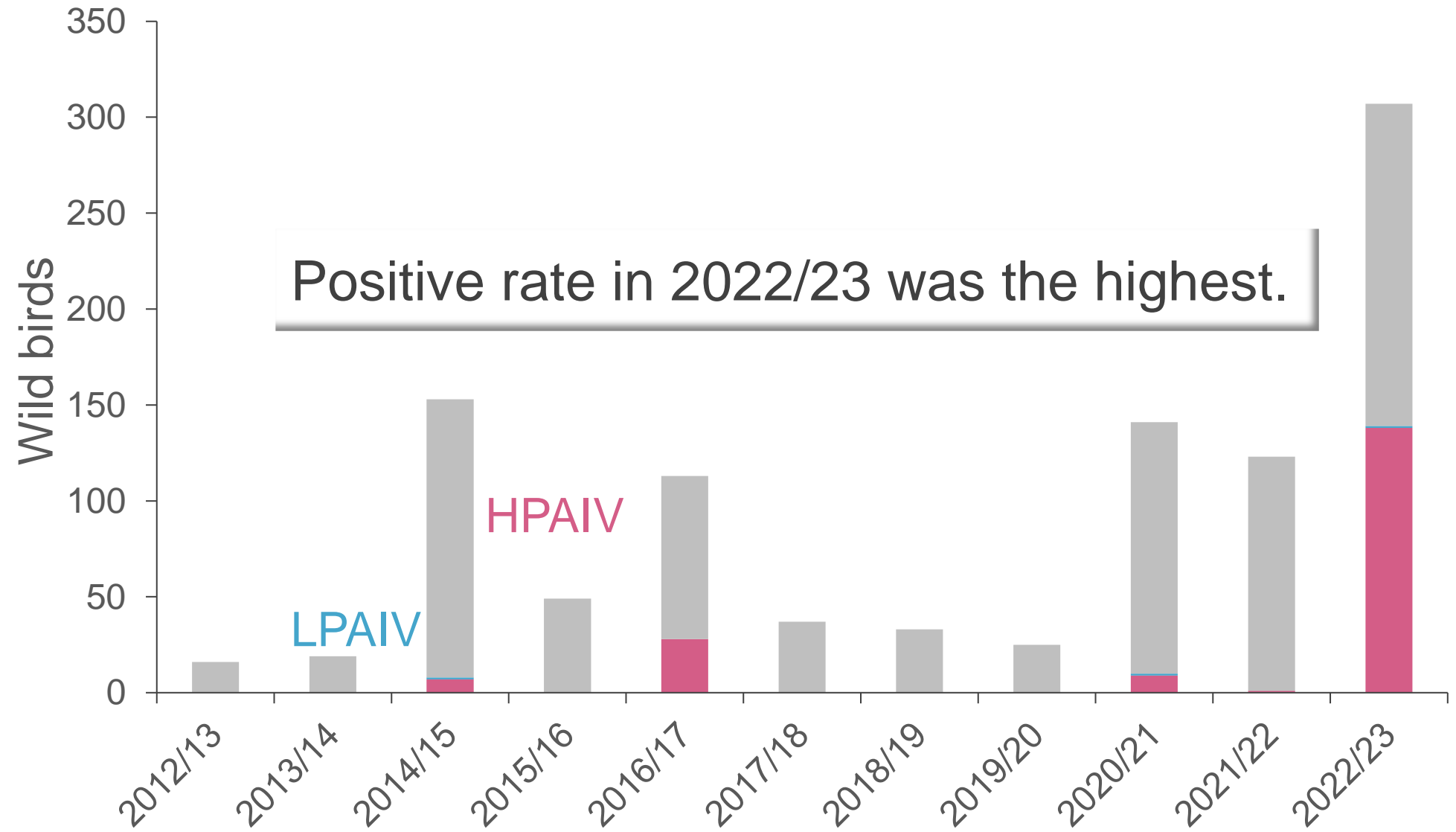


HPAIV circulated among cranes in early winter.

Number of wild birds tested for AIV gene since 2012



Number of wild birds tested for AIV gene since 2012



Topics

- H5 HPAI in endangered cranes
- H5 HPAIVs isolated from environment water
- Genetic characterization of H5 HPAIVs in the Izumi plain

AIV surveillance

Natural reservoirs



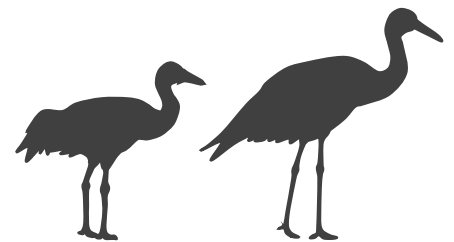
Wild ducks

AIV replicates in intestine

AIV surveillance using **fecal samples** is golden standard.

Collecting the fecal samples is time-consuming
→ it is not appropriate for weekly surveillance.

environment water would be alternative methods for AIV surveillance.

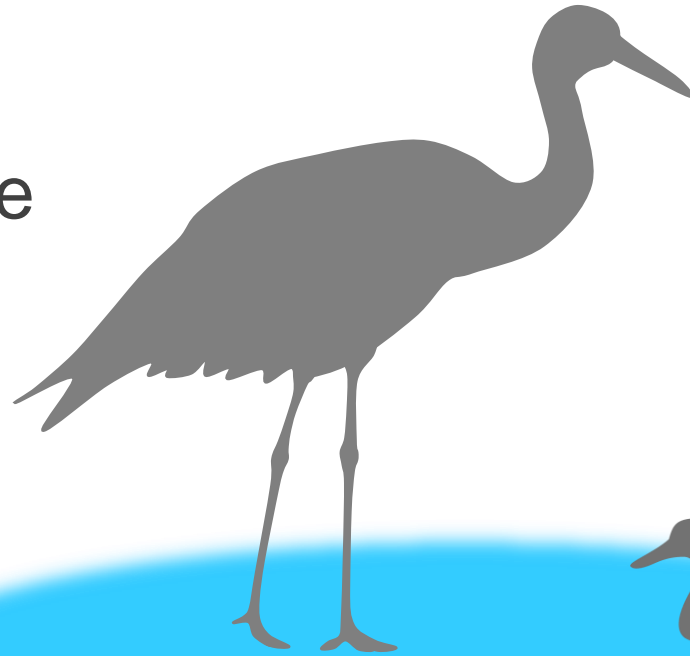


AIV surveillance using crane roost water in the Izumi plain



Conducting AIV isolation from crane roost water since 2012.

Crane



Wild duck

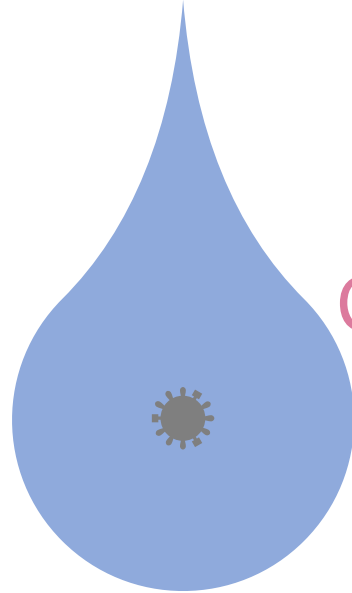


Crane roost water

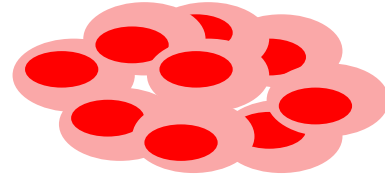
AIV isolation from crane roost water



Crane roost water
(14 samples/week)



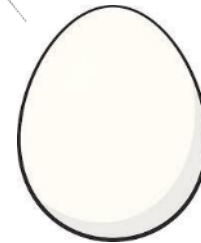
Mixing with chicken red
blood cells



Concentration



Inoculation

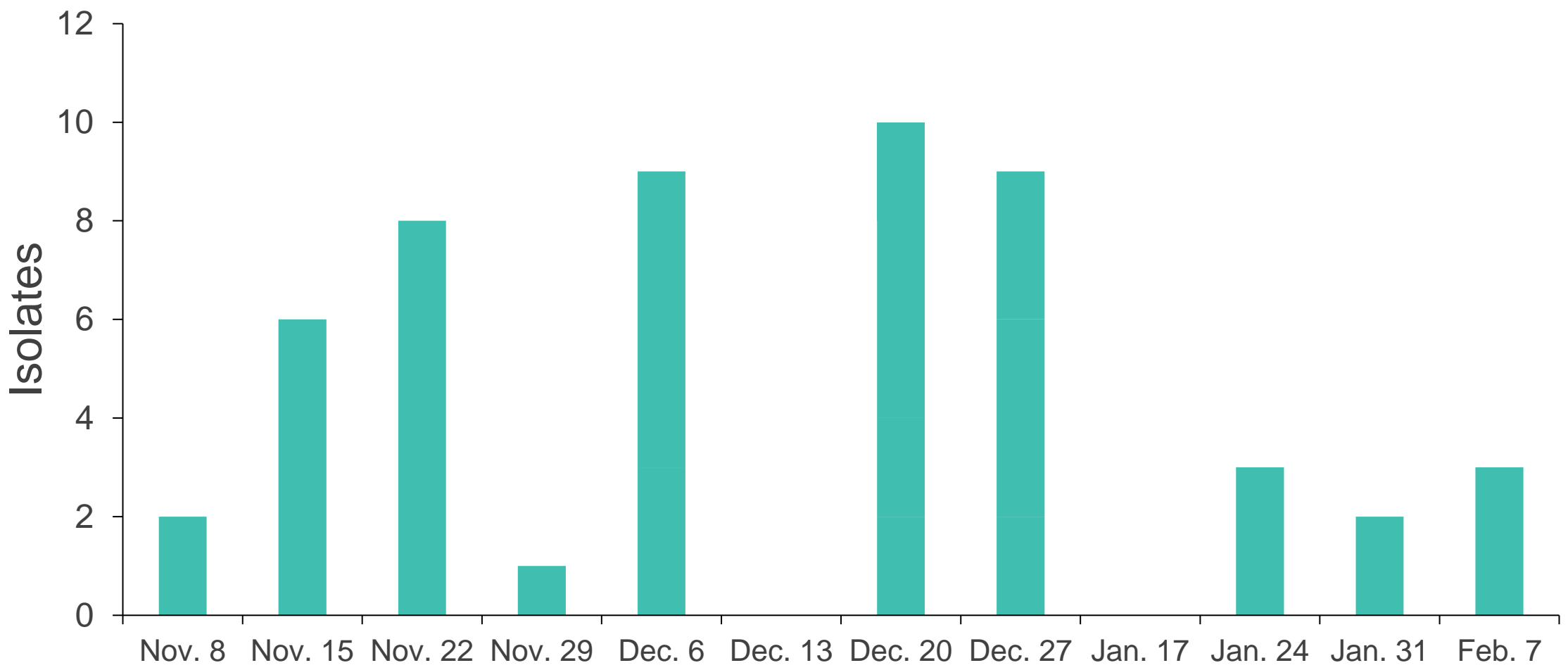


embryonated chicken eggs
(4 eggs/sample)

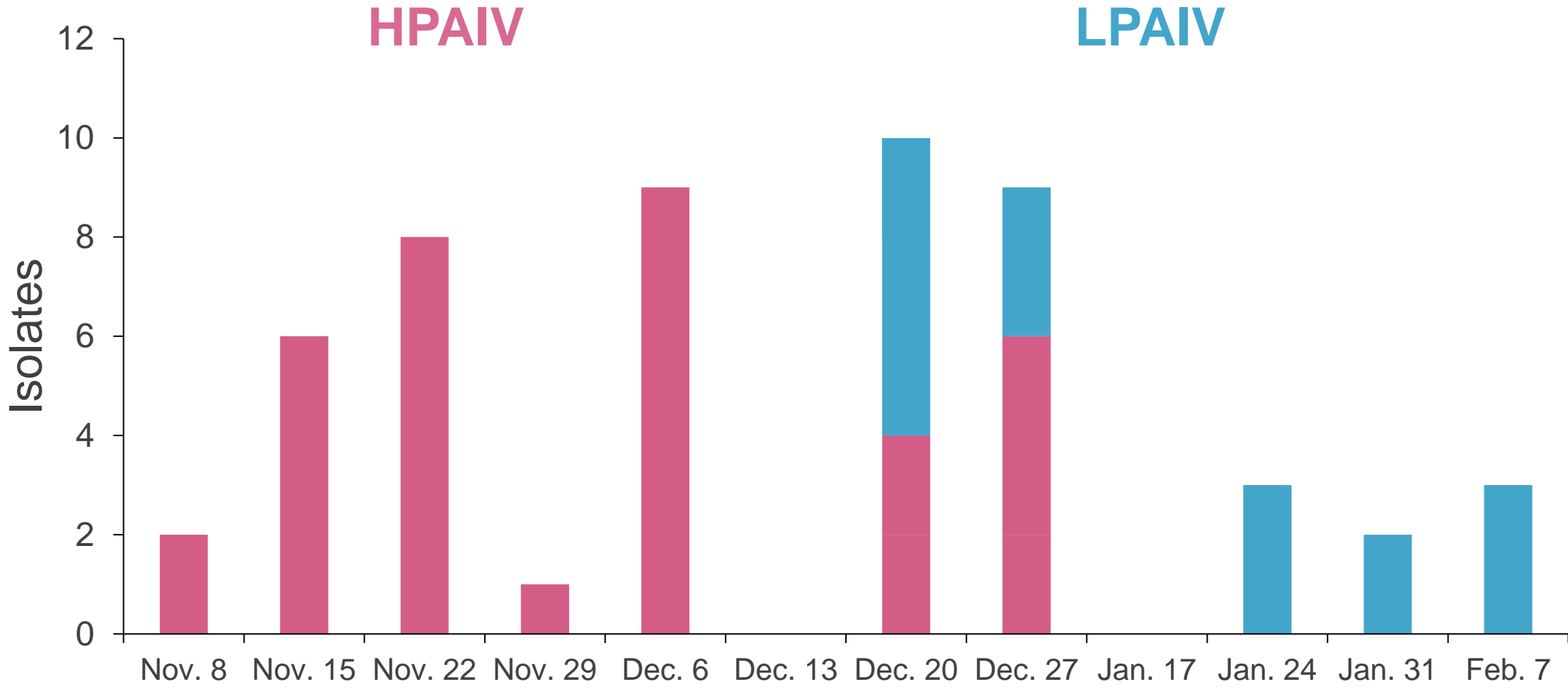
AIVs from crane roost water during the 2022/23 winter season

HPAIV	LPAIV	Mixed	Total
24	14	15	53
H5N1	H3N8 and H10N6		

Isolates from crane roost during the 2022/23 winter season

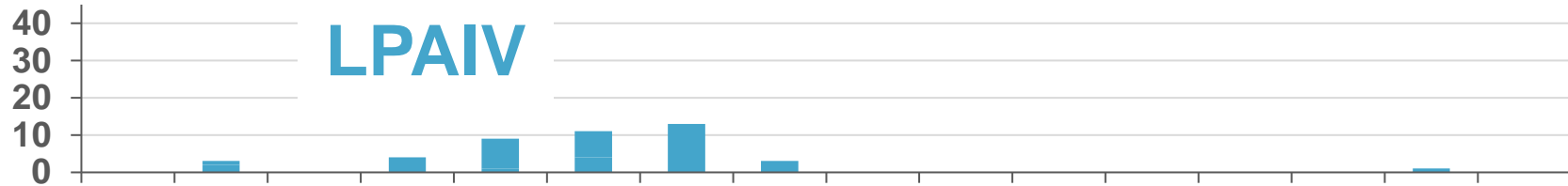


Isolates from crane roost during the 2022/23 winter season

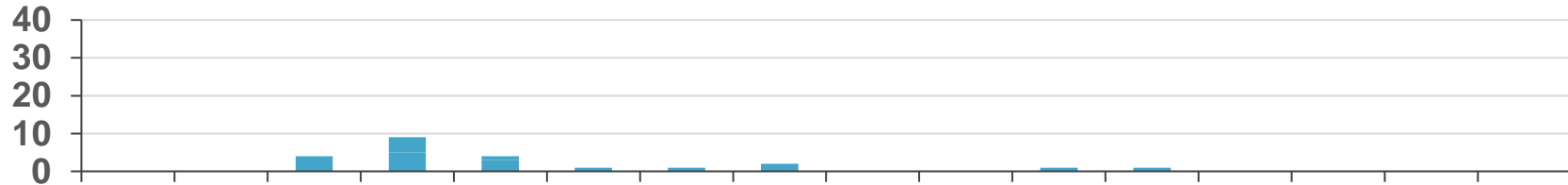


Isolates from crane roost water since 2018

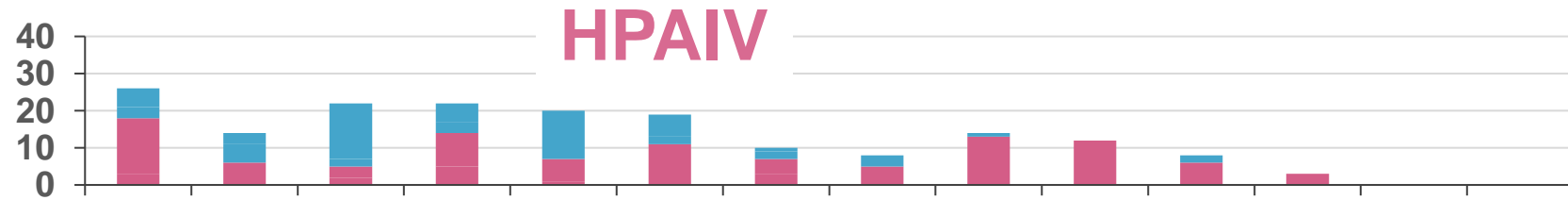
2018/19



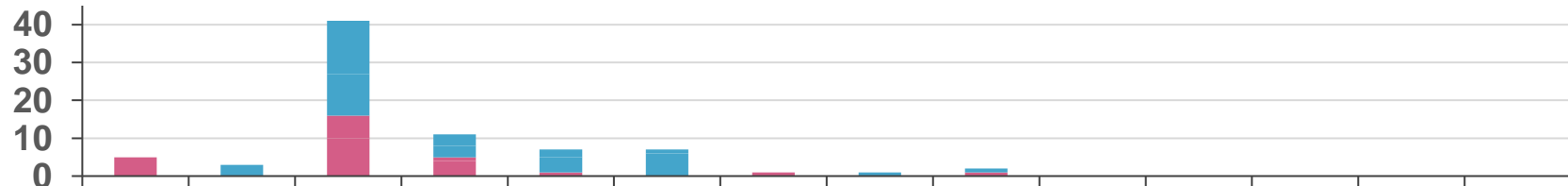
2019/20



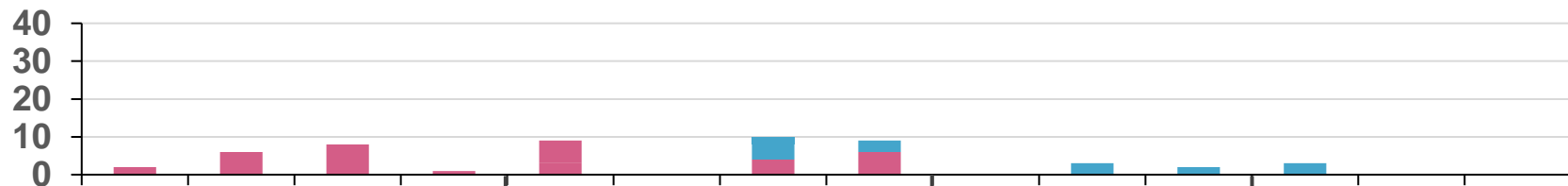
2020/21



2021/22

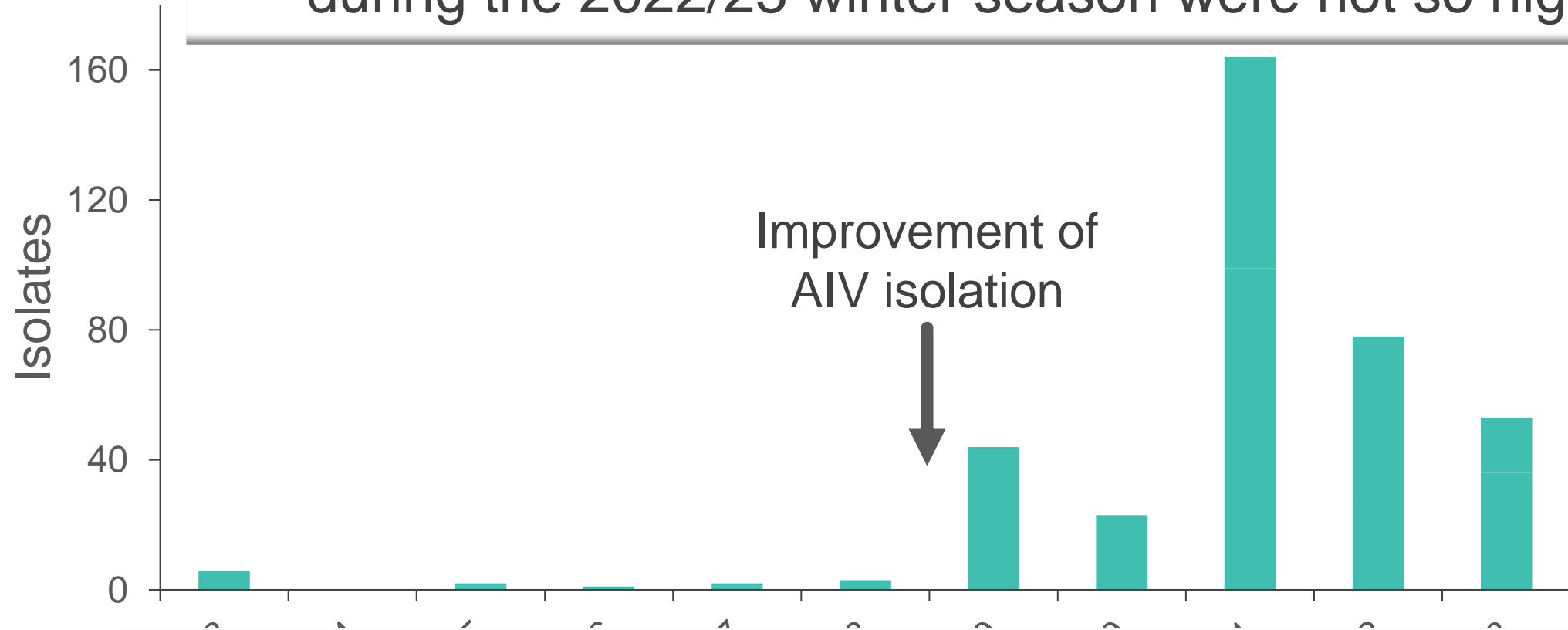


2022/23



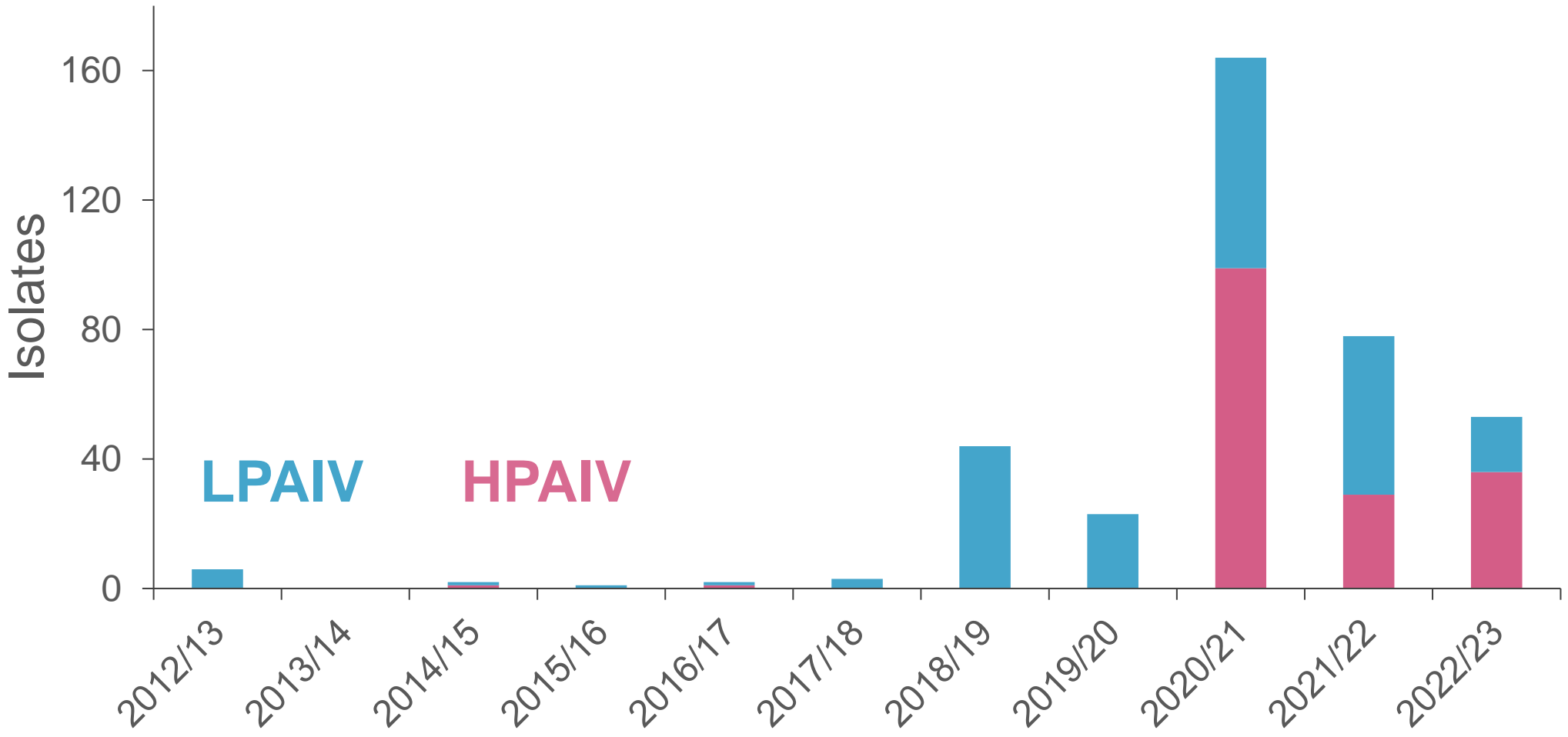
Isolates from crane roost water since 2012

The number of AIV isolates from the crane roost water during the 2022/23 winter season were not so high.



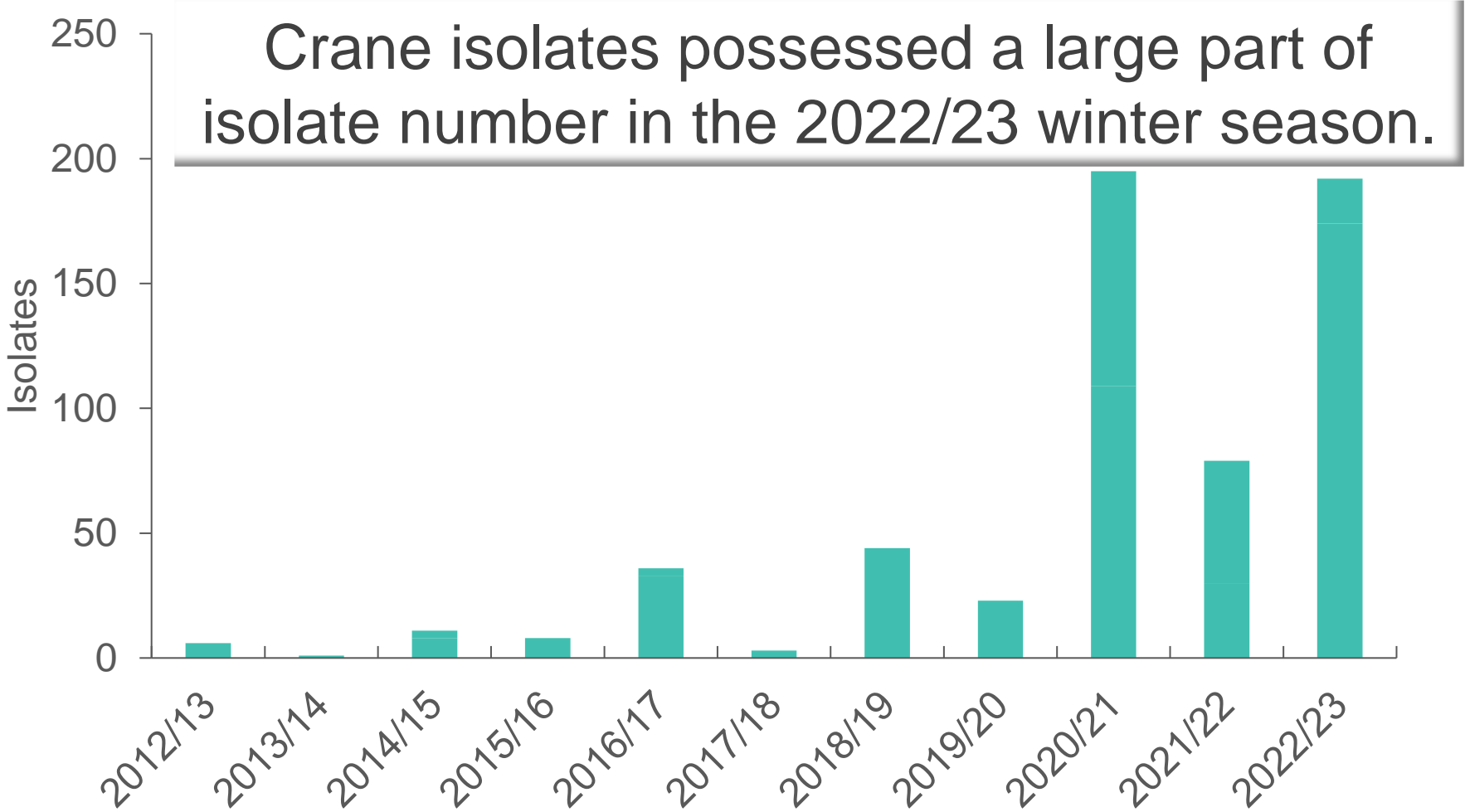
Viral shedding in feces in the 2022/23 winter season would be relatively low.

Isolates from crane roost water since 2012



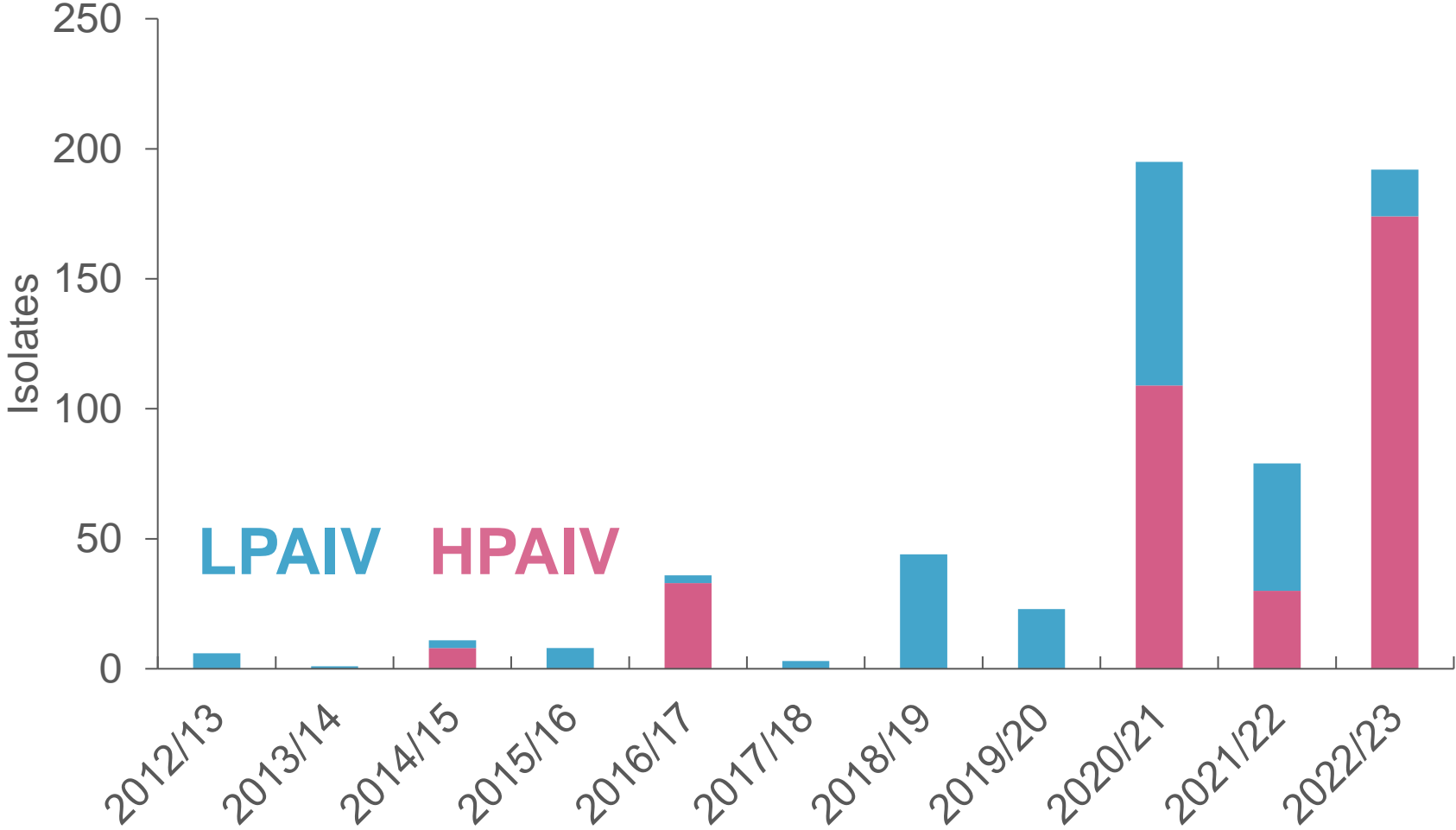
AIVs in the Izumi plain since 2012

Total isolates from both wild birds and crane roost water



AIVs in the Izumi plain since 2012

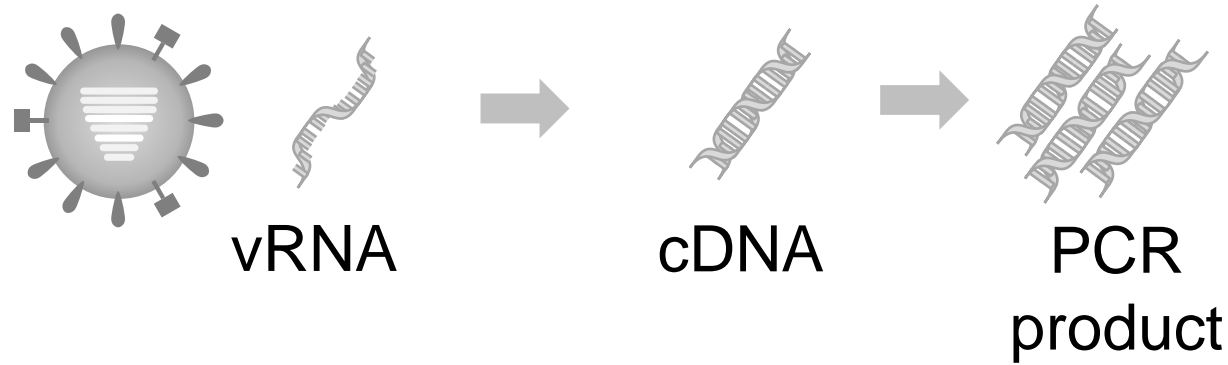
Total isolates from both wild birds and crane roost water



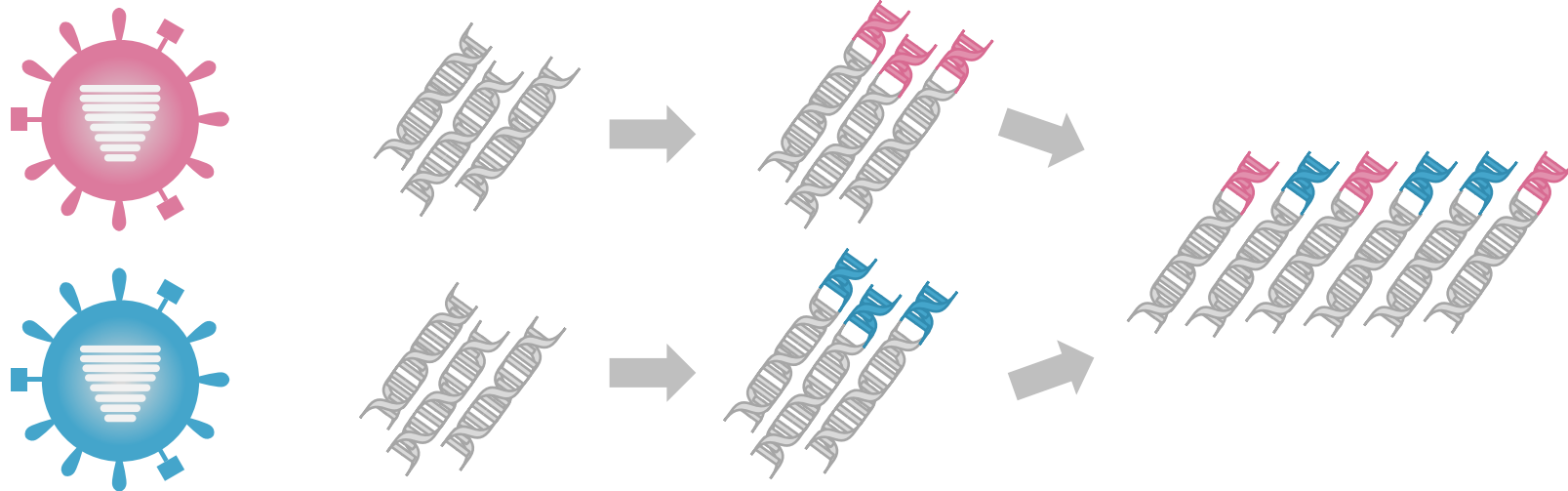
Topics

- H5 HPAI in endangered cranes
- H5 HPAIVs isolated from environment water
- Genetic characterization of H5 HPAIVs in the Izumi plain

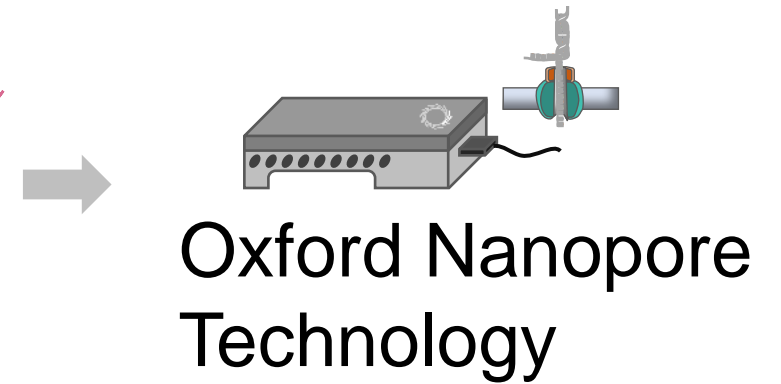
Full genome sequencing by using nanopore sequencer



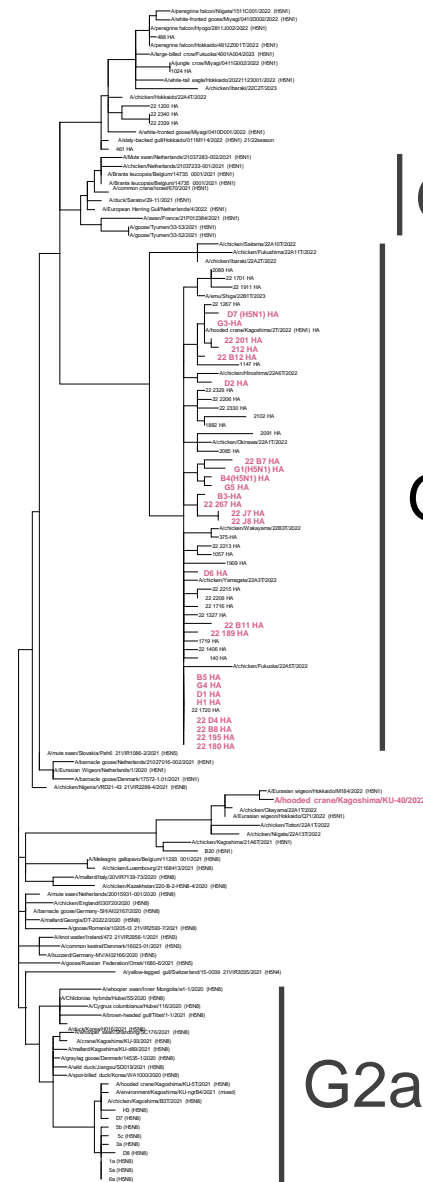
Amplification of each gene segment



Ligation with different barcode sequences



Genetic characterization of H5 HA gene



G2d: Northern Japan isolates in 2021-2023

G2e: European isolates in 2022-2023

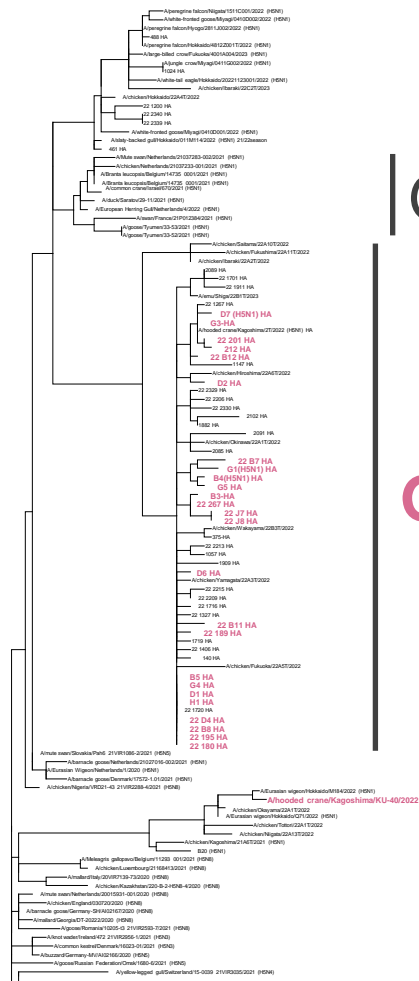
G2c: Japan/South Korea isolates in 2022-2023

G2b: Northern and Southern Japan isolates in 2021-2022

G2a: Southern Japan isolates 2020-2021

0.01

Genetic characterization of H5 HA gene



G2d: Northern Japan isolates in 2021-2023

G2e: European isolates in 2022-2023

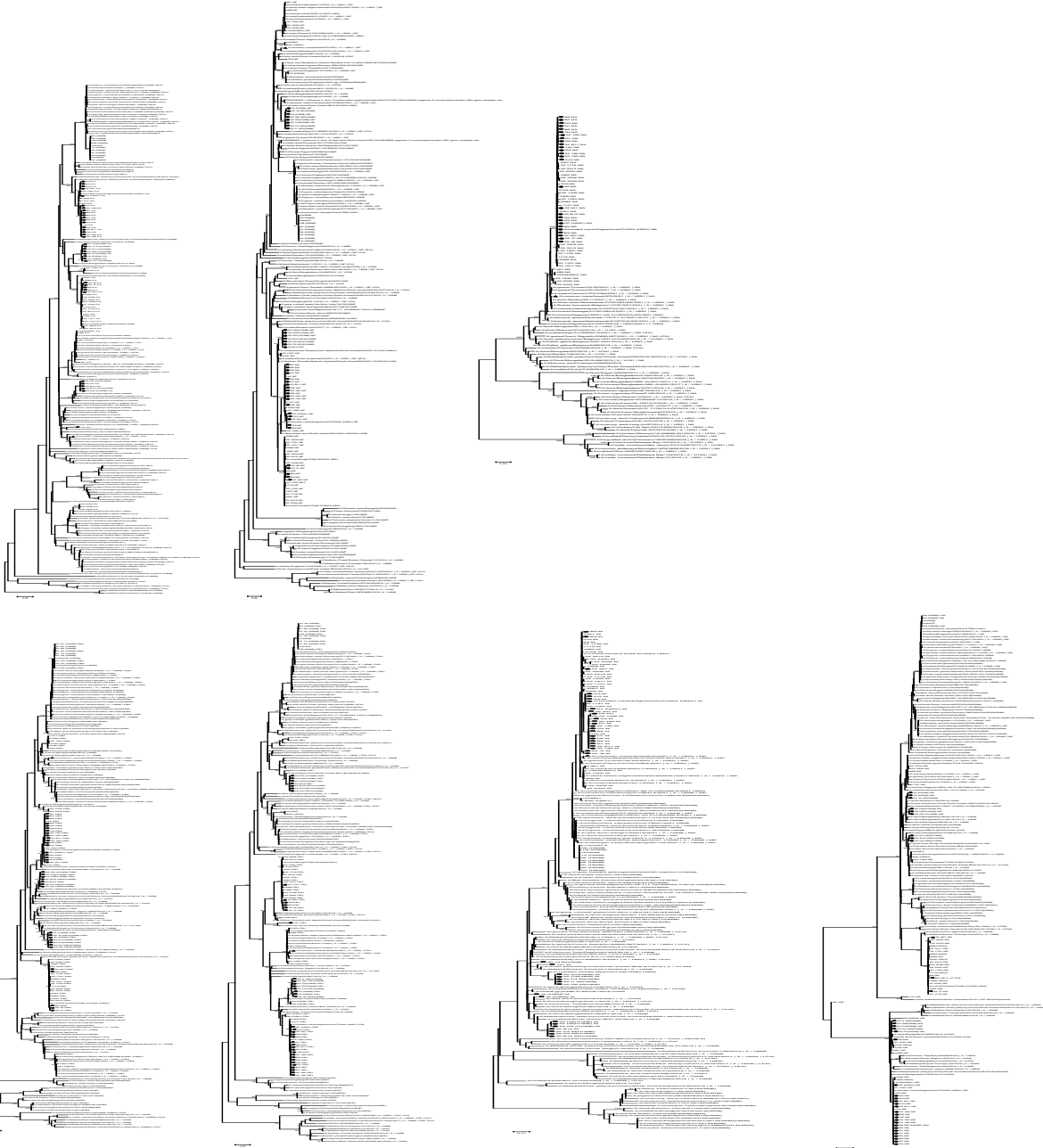
G2c: Japan/South Korea isolates in 2022-2023

G2b: Northern and Southern Japan isolates in 2021-2022

Izumi isolates belong to G2b and G2c

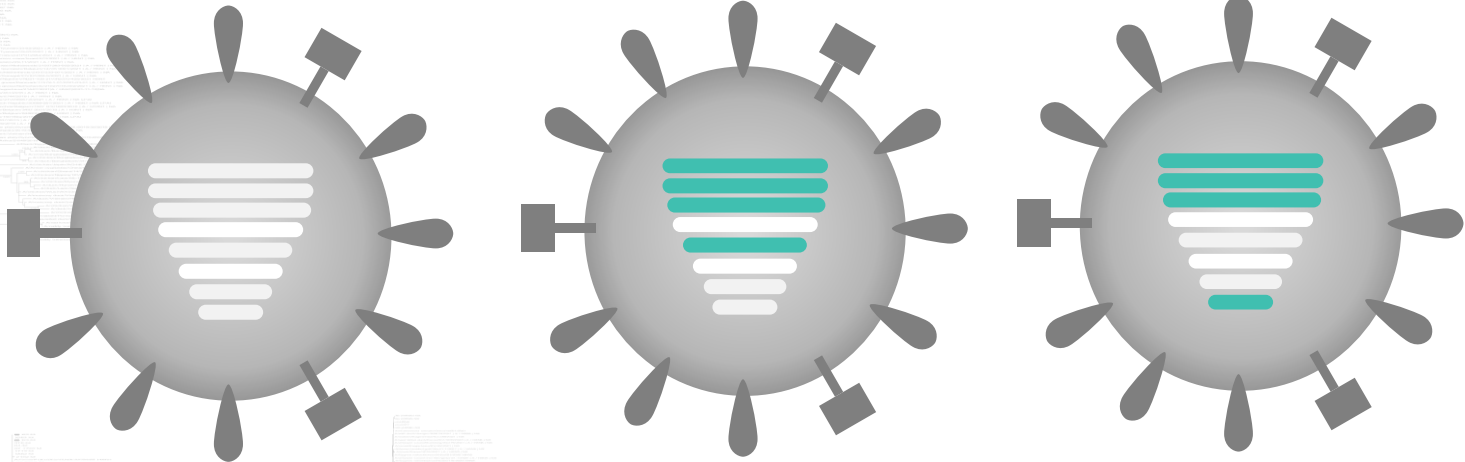
0.01

Genetic constellation of AIVs isolated from the Izumi plain



Genetic constellation of AIVs isolated from the Izumi plain

G2c



Three patterns of genetic constellation among G2c isolates

Summary

- The H5N1 HPAI outbreak occurred among endangered cranes in the Izumi plain.
- Number of AIVs isolated from crane roost water was relatively low.
- H5N1 HPAIVs might circulate among cranes via aerosol transmission.
- Multiple genotypes of H5 HPAIVs were invaded in the Izumi plain.

Acknowledgement

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Mrs. Nishi Natsuko

Mrs. Koyamada Donna

Ms. Saito Rara

Ministry of the Environment Kyushu Regional Environment Office

Izumi city crane museum

“Crane Park Izumi”

Thank you for your kind attention



Ozawa Lab.

