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October 16-17, 2025

H5N1 virus invades the mammary glands of dairy cattle through 'mouth-to-teat' transmission

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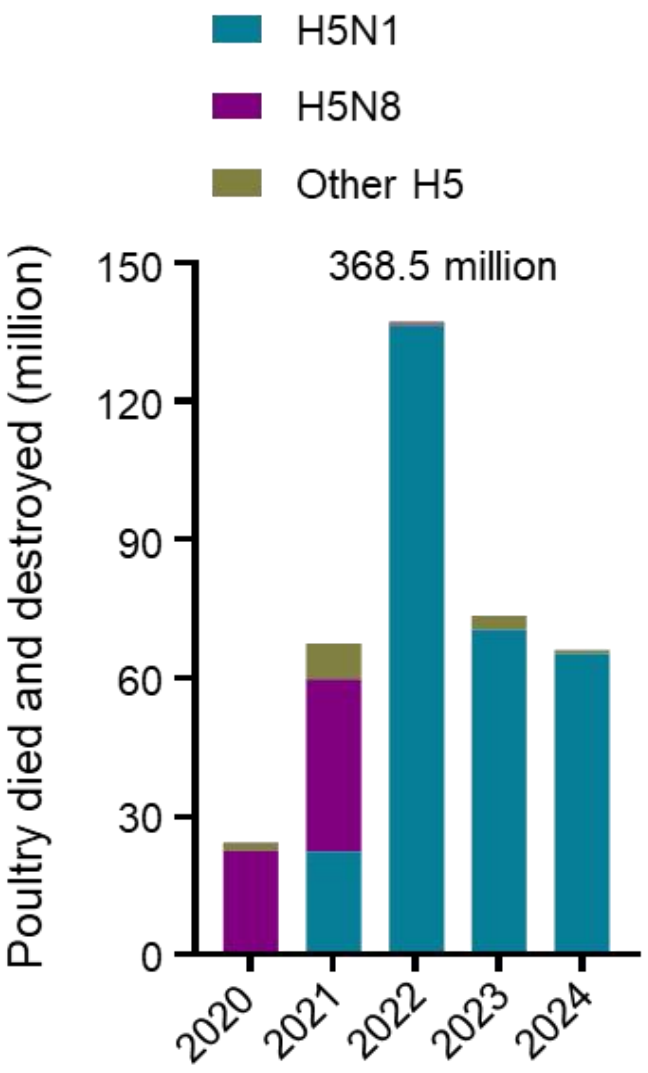
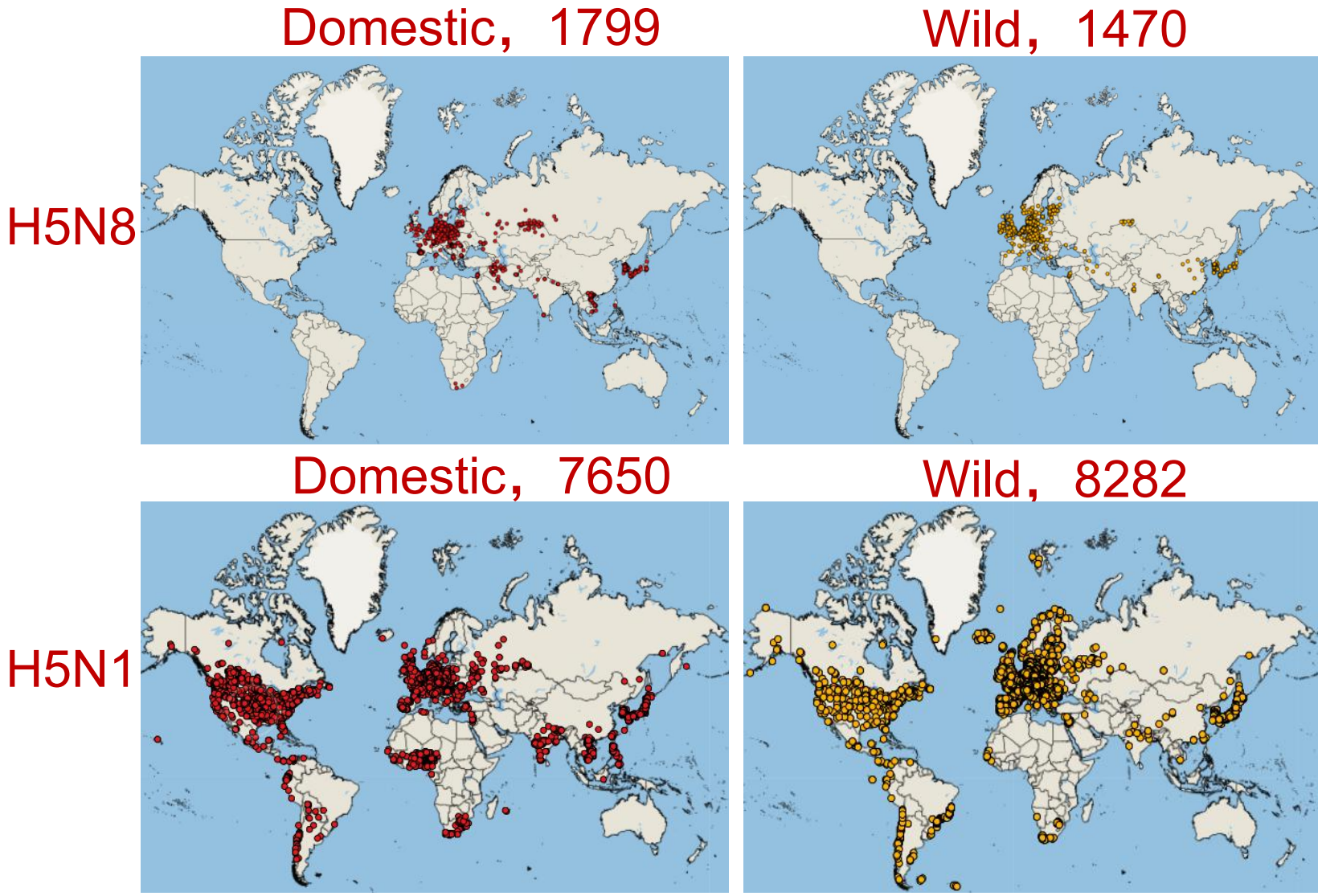
Outbreaks caused by clade 2.3.4.4b H5N1 viruses





More than 400,000 mammals died from the infection caused by the 2.3.4.4b H5N1 viruses since 2020

Finland, Raccoon dog

Finland, Fox

US, Dairy cattle

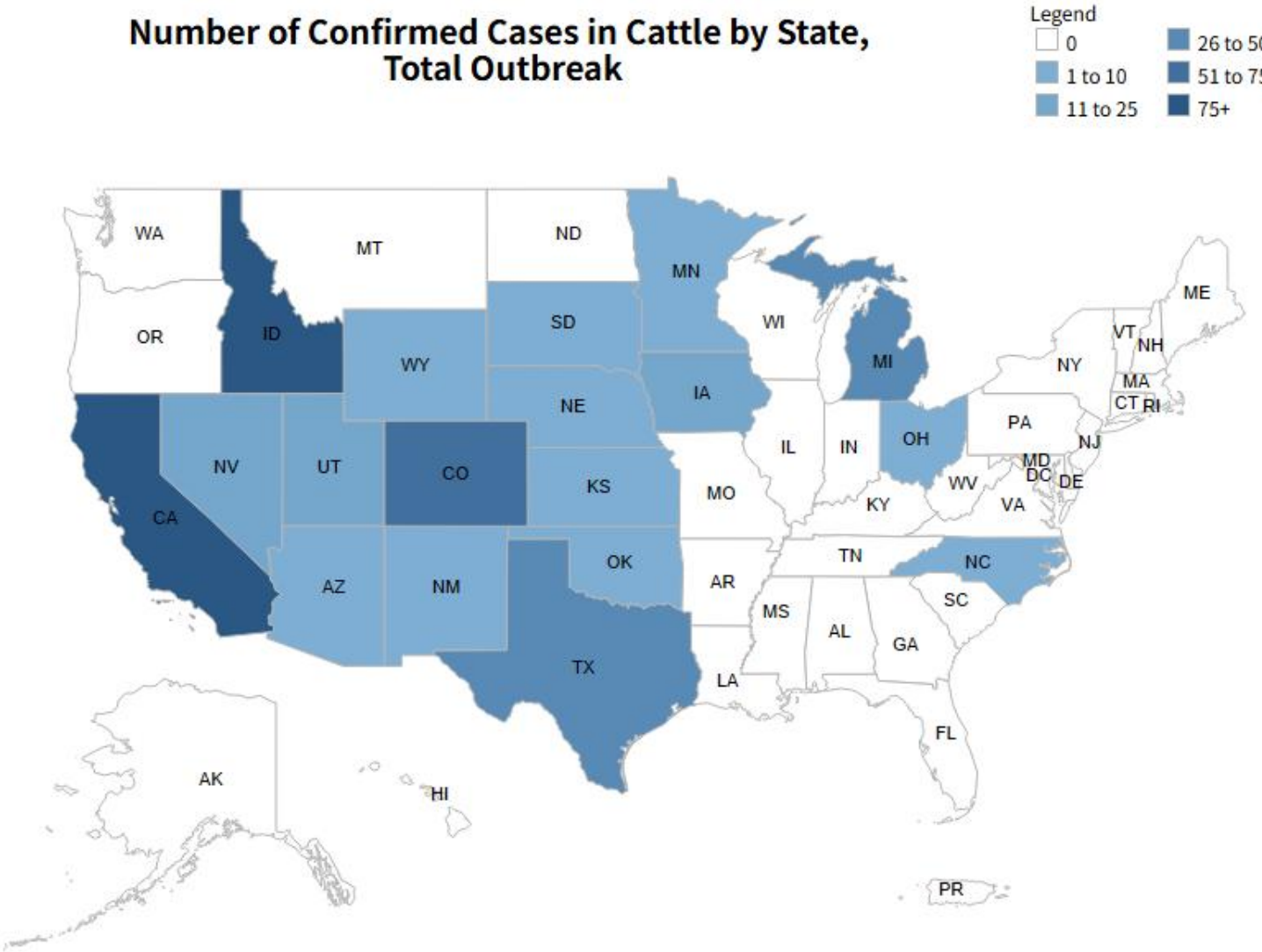
Finland and Spain, Mink

Chile and Peru, Seal and Sea lion

US, Goat

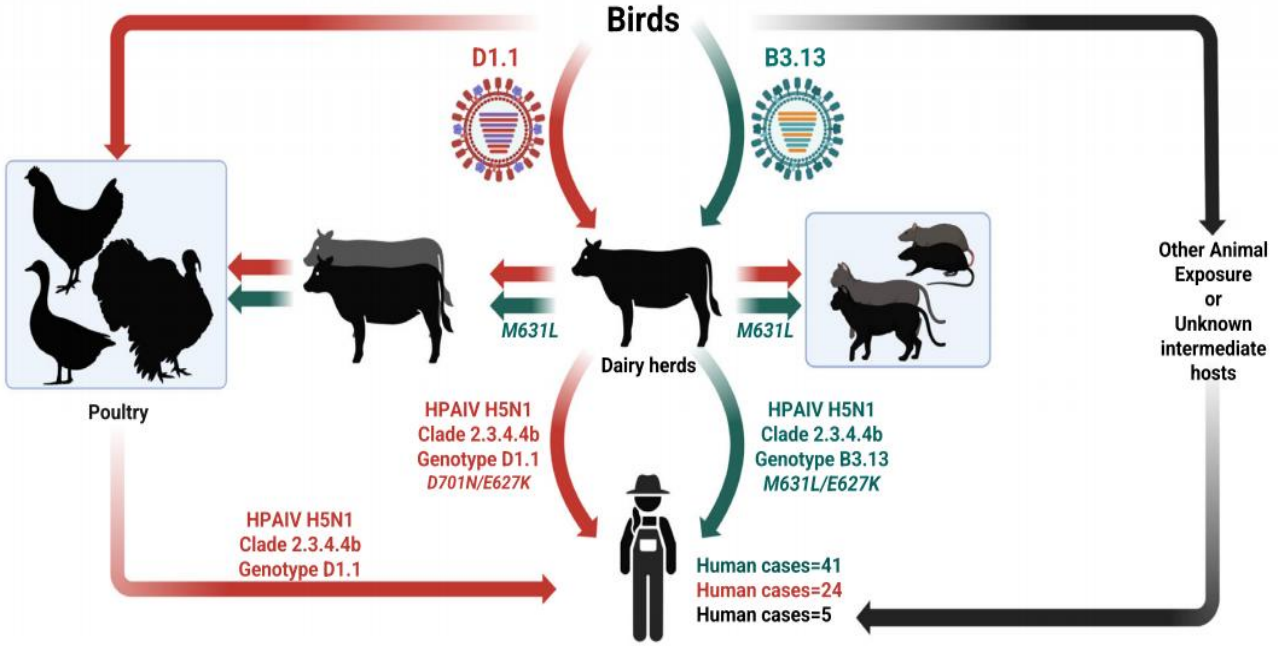
H5N1 virus infection in cows in the US (10/10/2025)

- Cow H5N1 infection was reported on 1,081 farms across 18 states in the US



The most harmful characteristic of H5N1 influenza outbreaks in dairy cattle is the viral invasion of and damage to the mammary glands

The H5N1 viruses isolated from humans and cattle in the US have obtained several harmful mutations, posing a significant threat to global animal and public health



Two questions

1. How does the H5N1 virus get to the mammary glands?

2. Could vaccination prevent H5N1 virus infection and spread in cattle?

We performed **a large scale** of cattle study in the P3+ animal facility in HVRI

60 lactating dairy cattle

Four calves

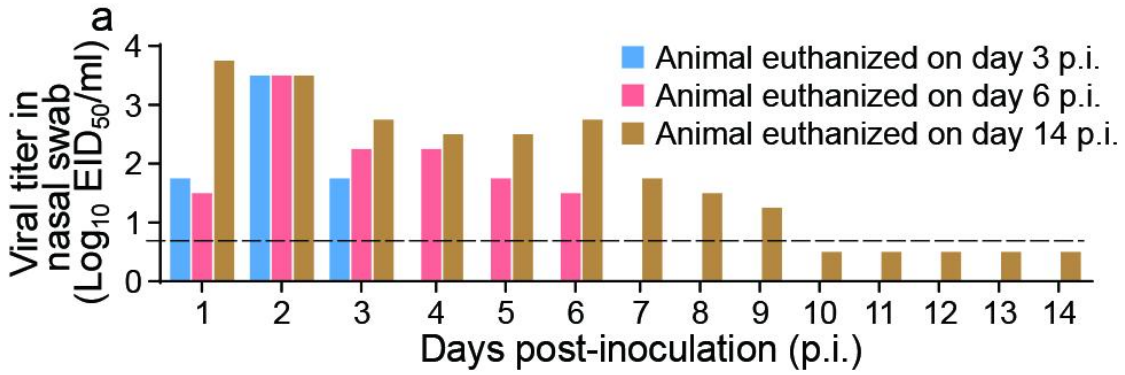
We tested three different H5N1 viruses in lactating cattle. For each virus, we infected three cattle with high dose of the virus intranasally, swabs and milk samples were collected daily, and tissure samples were collected from the animals that were euthanized on different days.

Day 3.p.i.

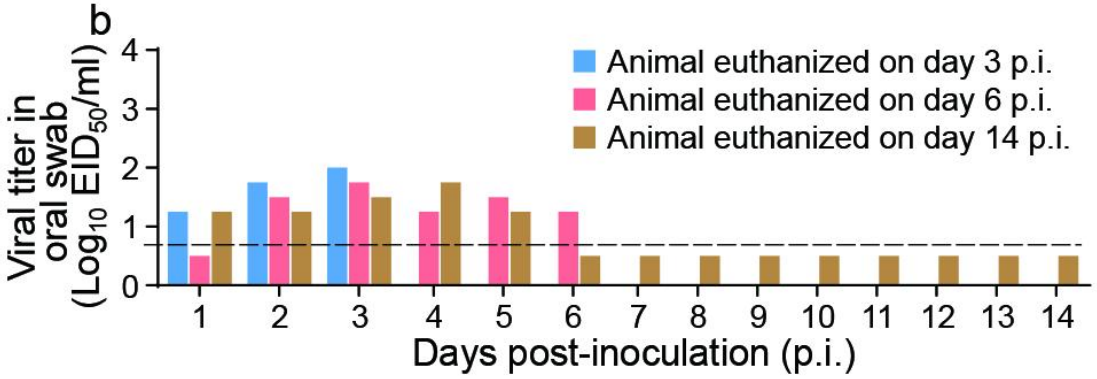
Day 6 p.i.

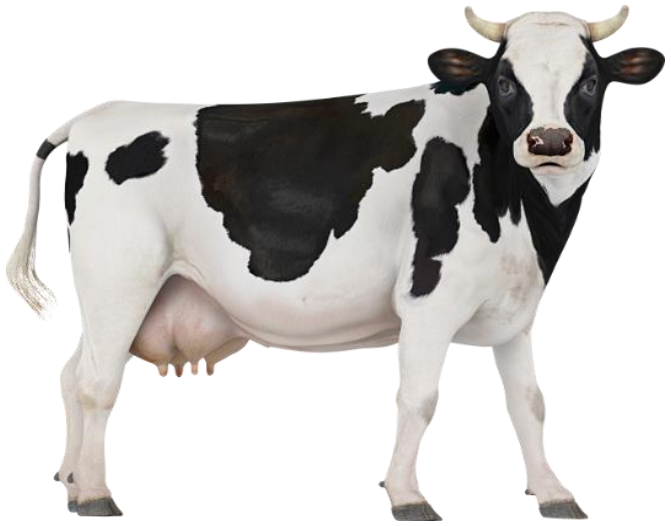
Day 14 p.i.

Nasal swab

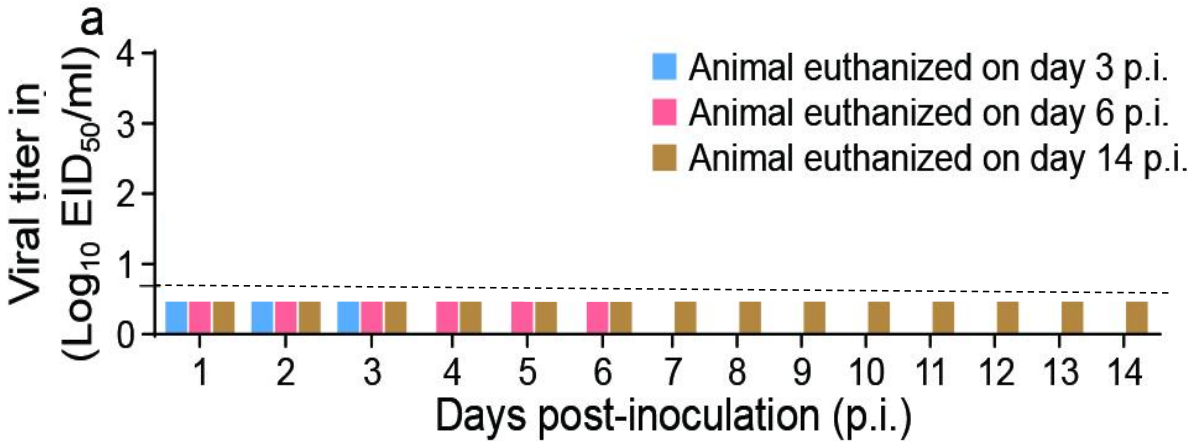


Oral swab

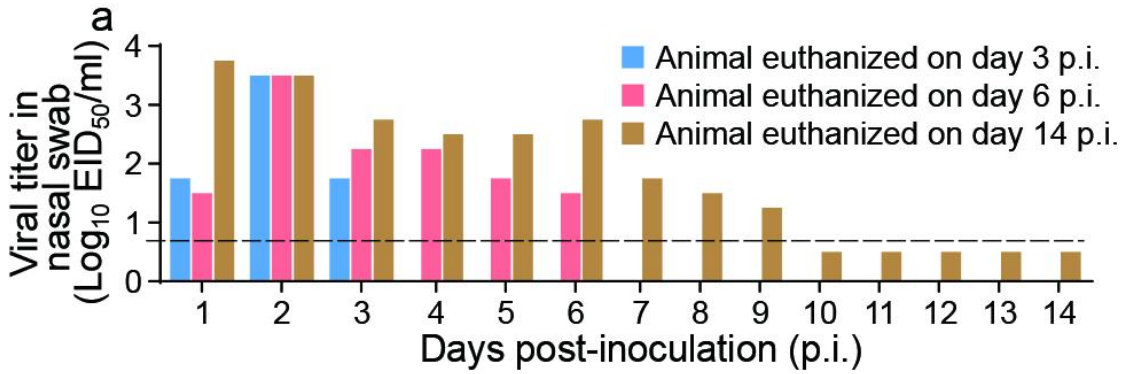




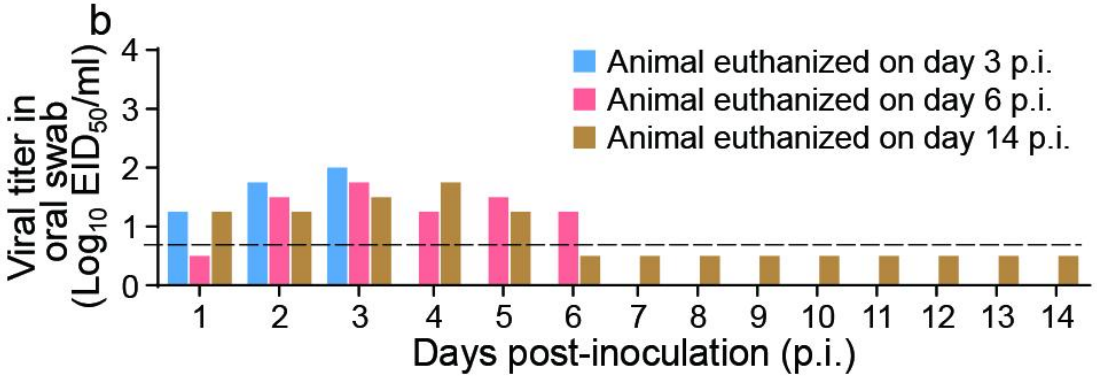
No virus in milk, urine, or rectal swabs



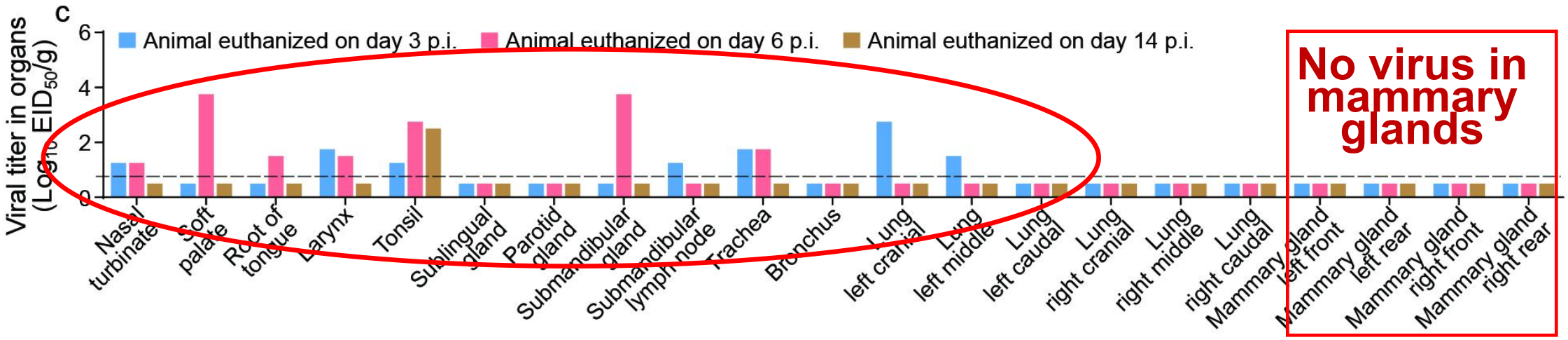
Nasal swab



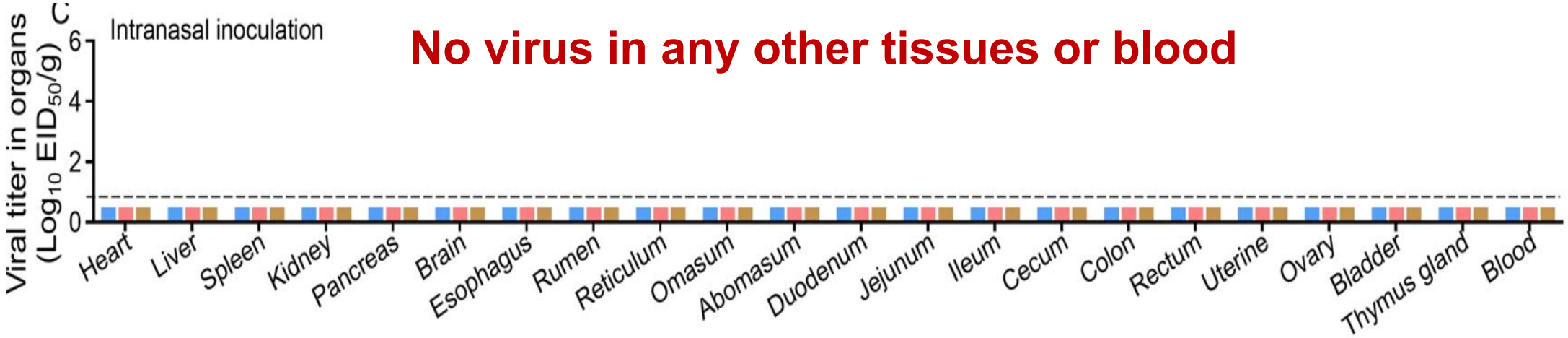
Oral swab



Virus was detected in the tissues collected from mouth and respiratory tract, but not in any other tissues

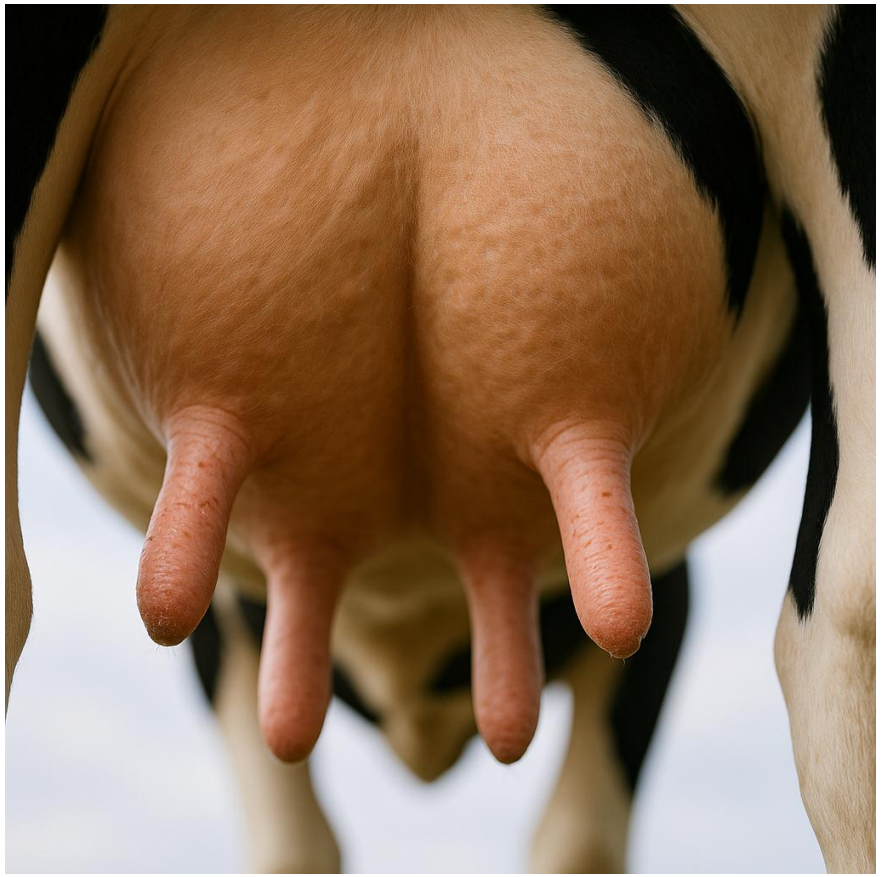


No virus in mammary glands

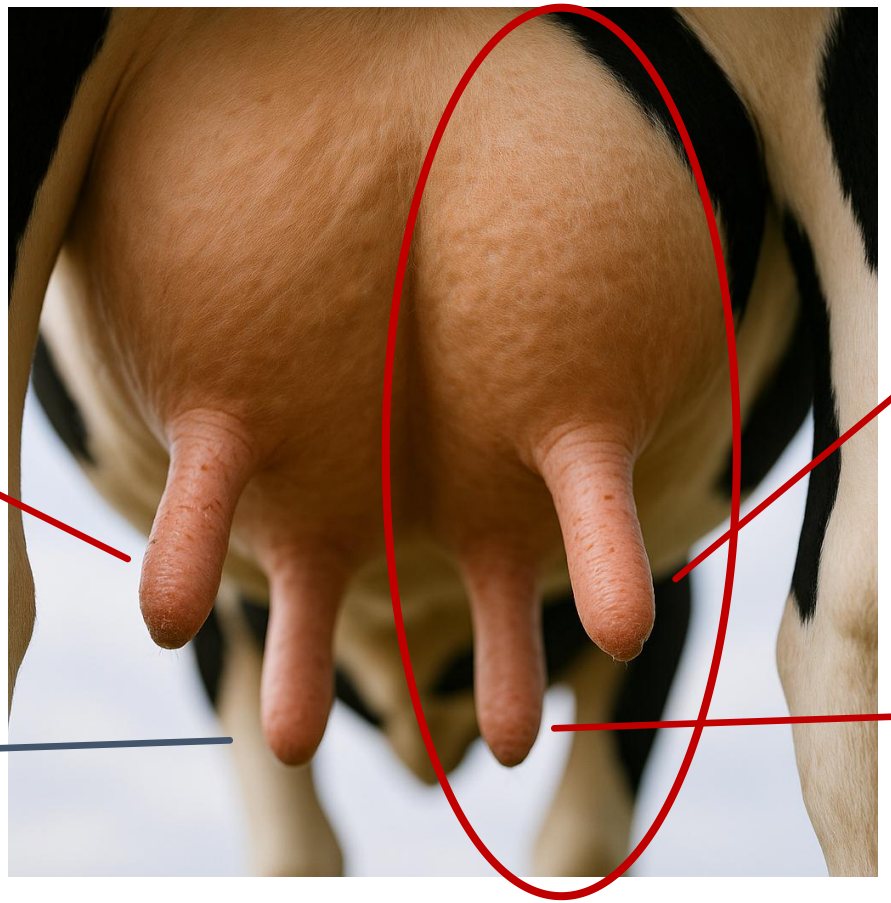


No virus in any other tissues or blood

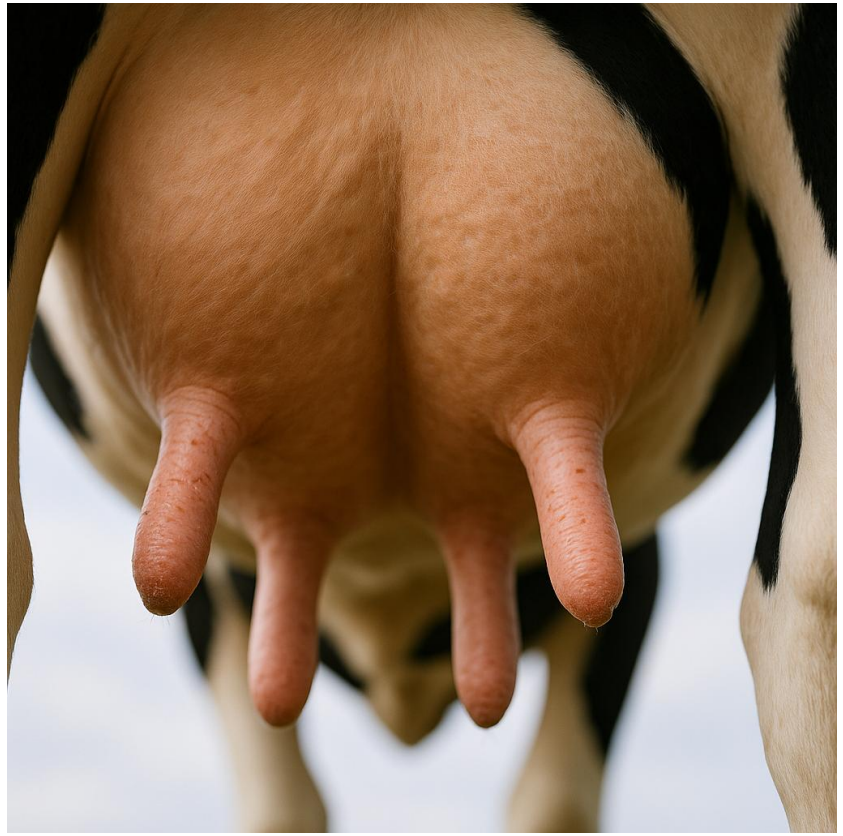
Viruses infected intranasally can only replicate in tissues of the mouth and respiratory tract of the cattle, none of the viruses can migrate to the mammary glands.



Mammary glands are highly susceptible to the H5N1 virus, as 10 EID_{50} of the virus could establish infection



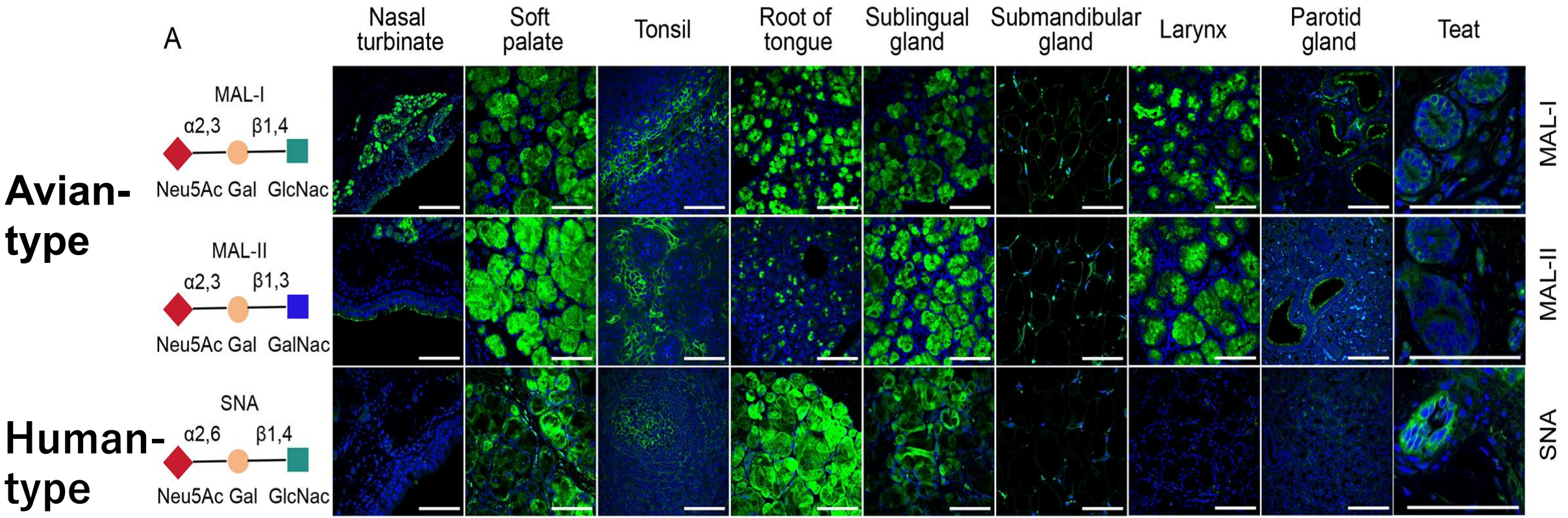
Mammary glands are highly sesceptible to the H5N1 virus; however, the virus only replicates in the inoculated mammary glands, **but cannot migrate to the neighboring mammary glands.**

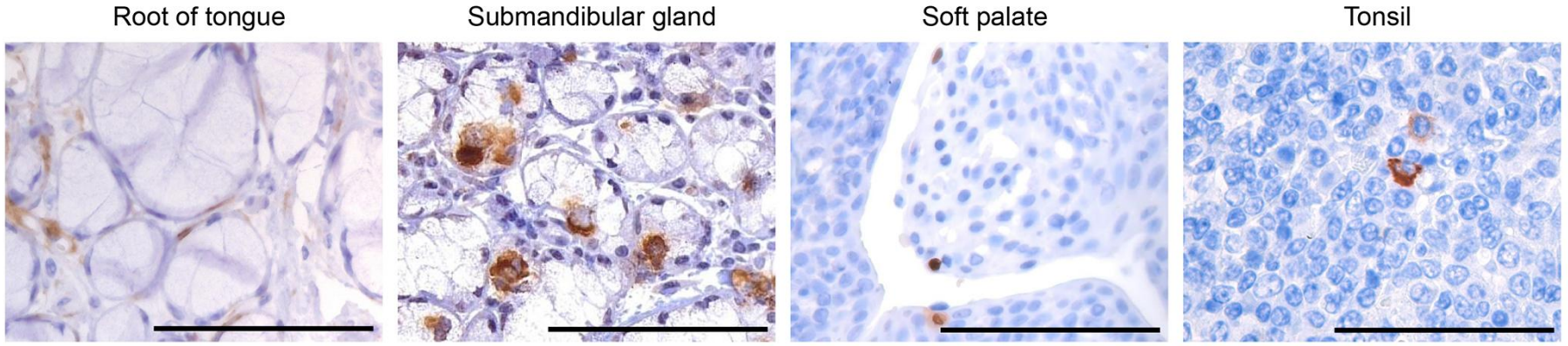


The H5N1 virus is capable of replication in mammary gland tissue only upon **“direct inoculation”.**

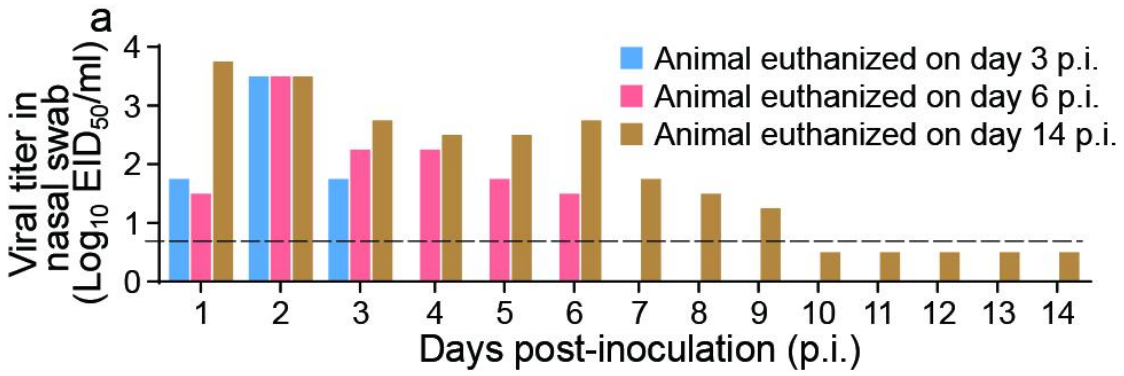
How could this **“direct inoculation”** occur in nature?

Oral tissues of cattle express both avian-type and human-type sialic acid receptors

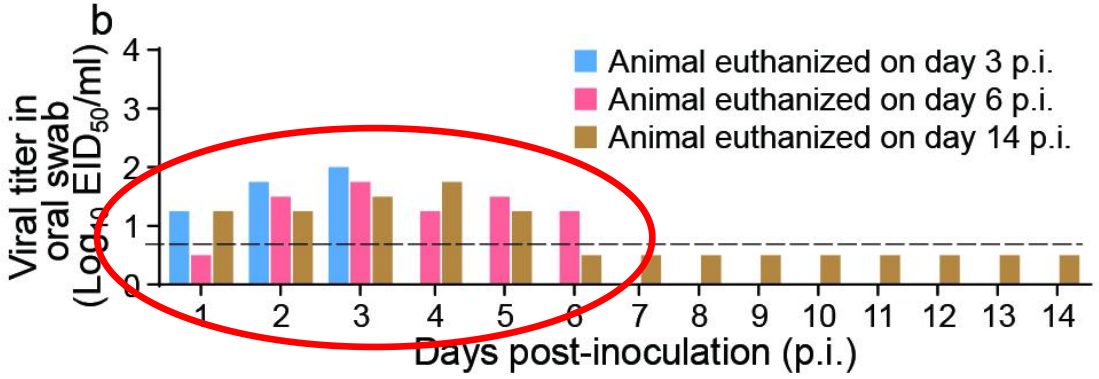




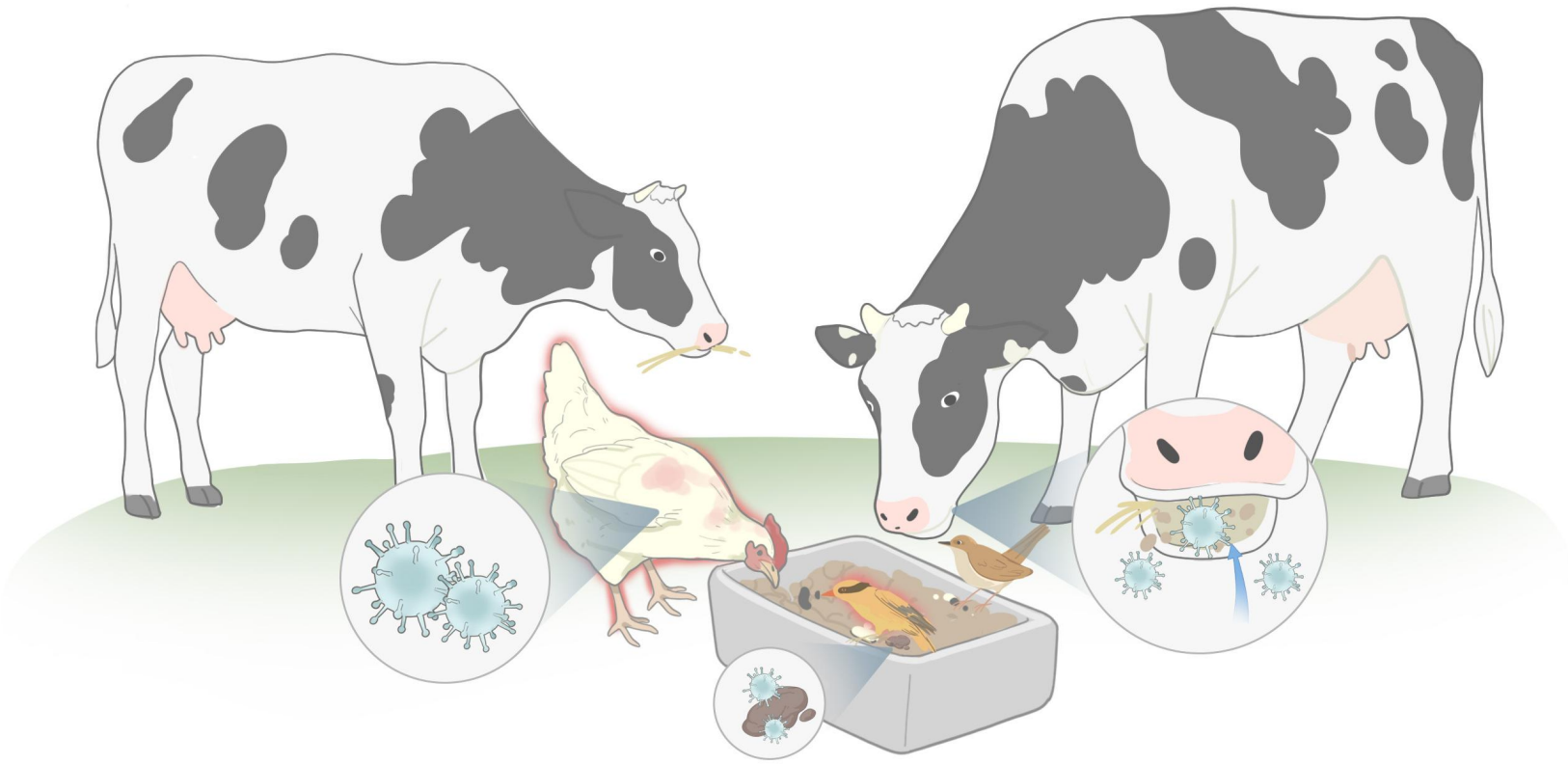
Nasal swab



Oral swab



The receptor exprssion in mouth supports influenza virus infection through contaminated feed or water



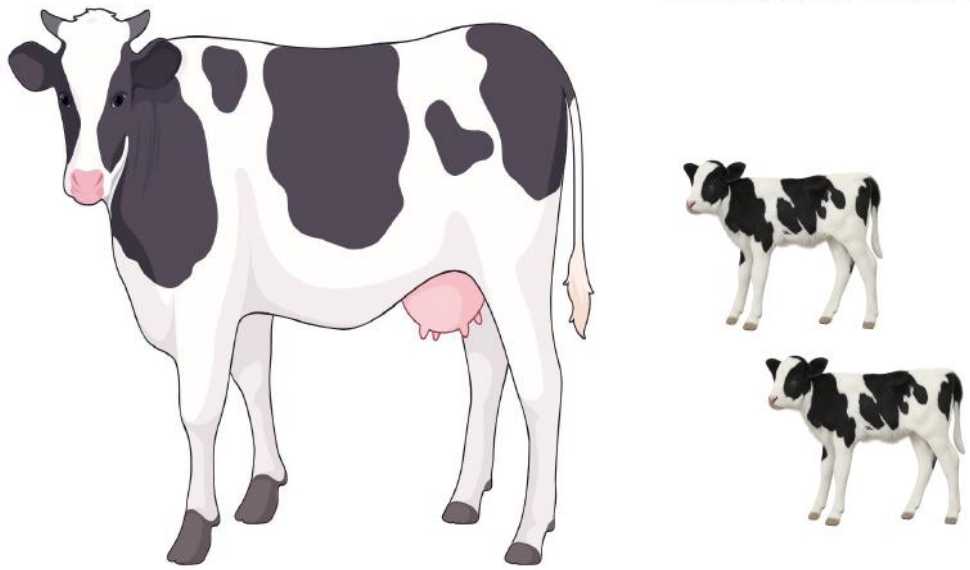
We speculated that ‘mouth-to-teat’ transmission may be the route by which the H5N1 virus infects the mammary glands of dairy cows.

Lactating cattle often ‘steal milk’ through self-nursing

Lactating cattle also ‘steal milk’ through cross-nursing

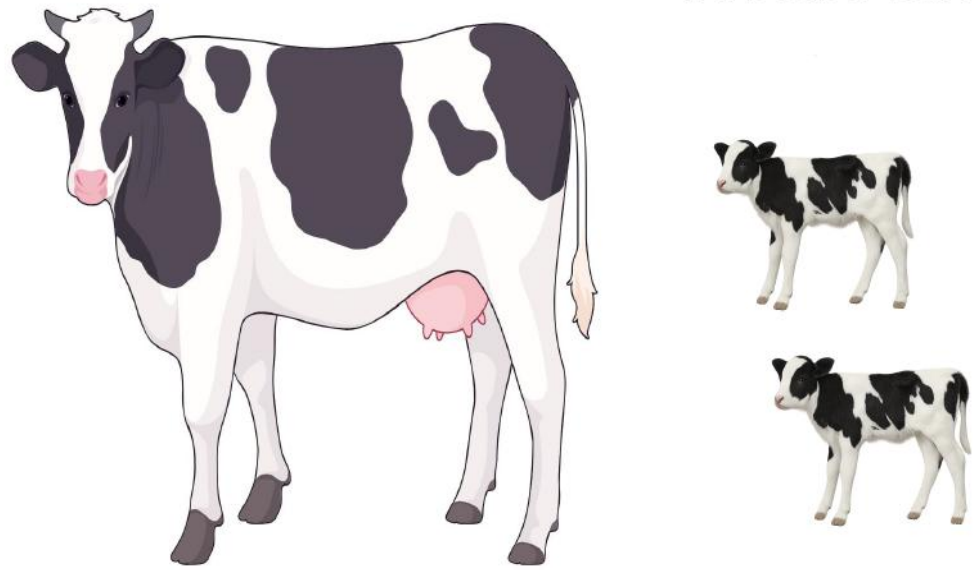
Lactating cow

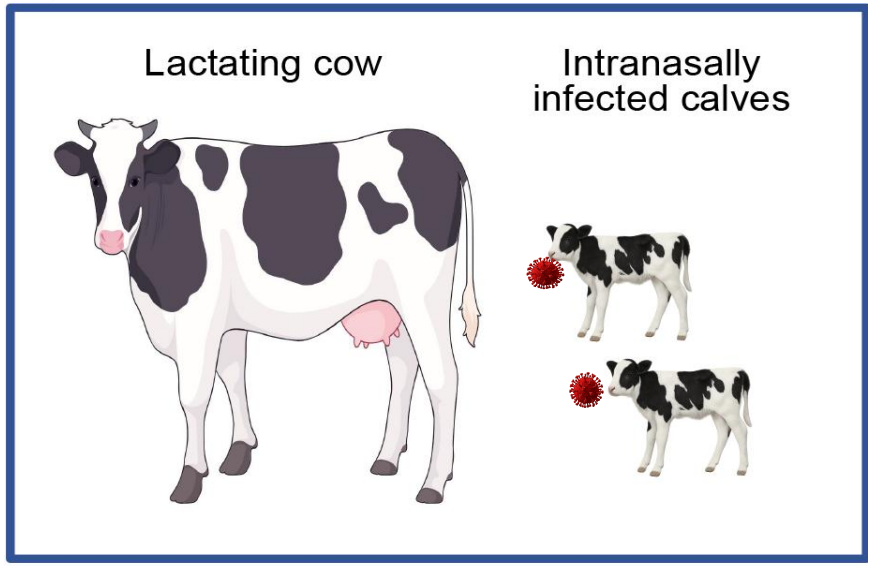
Intranasally infected calves



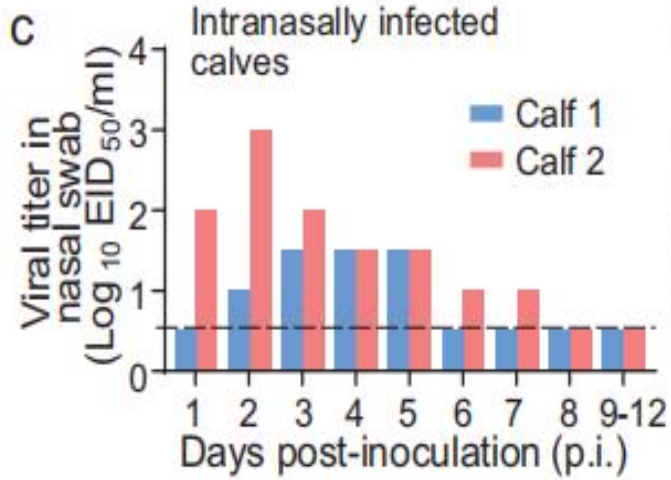
Lactating cow

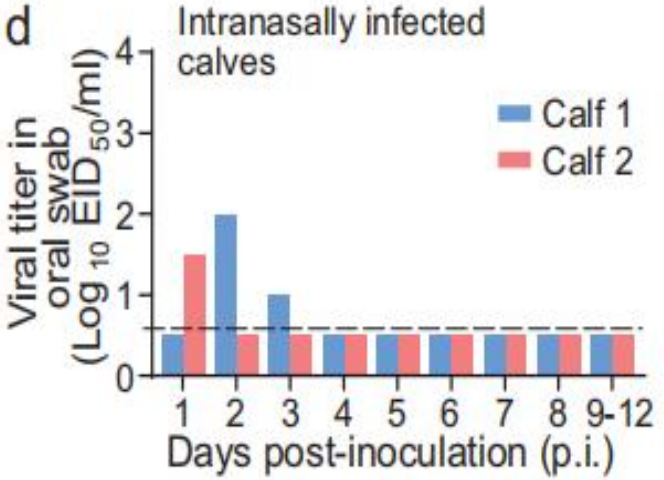
Orally infected calves

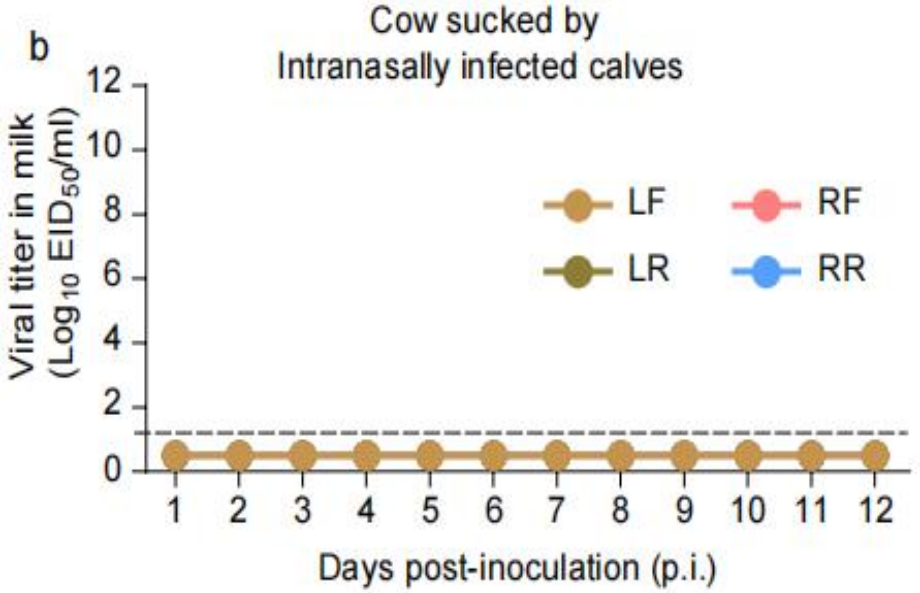


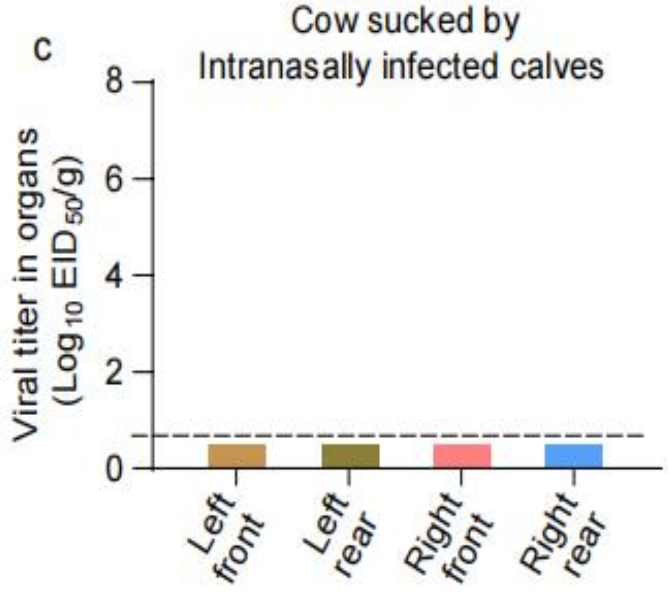


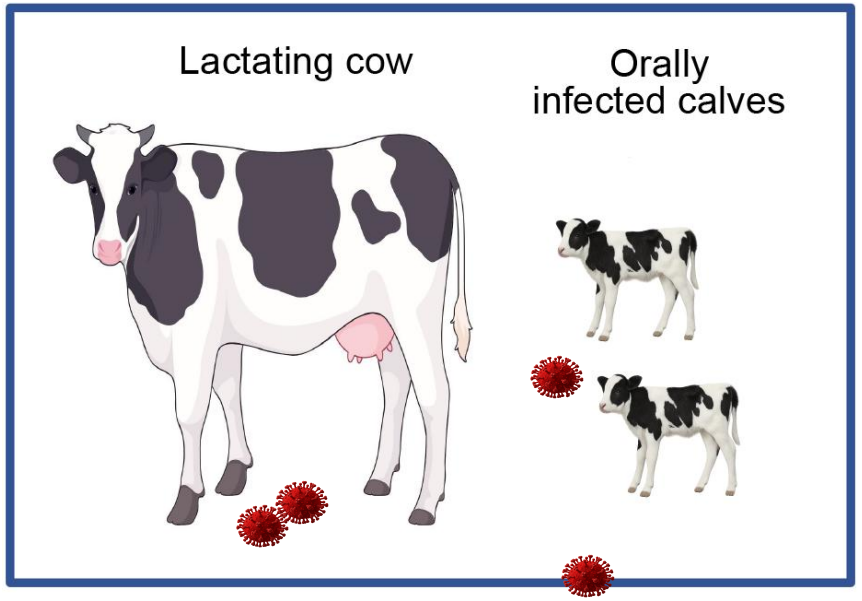
Transmission did not occur

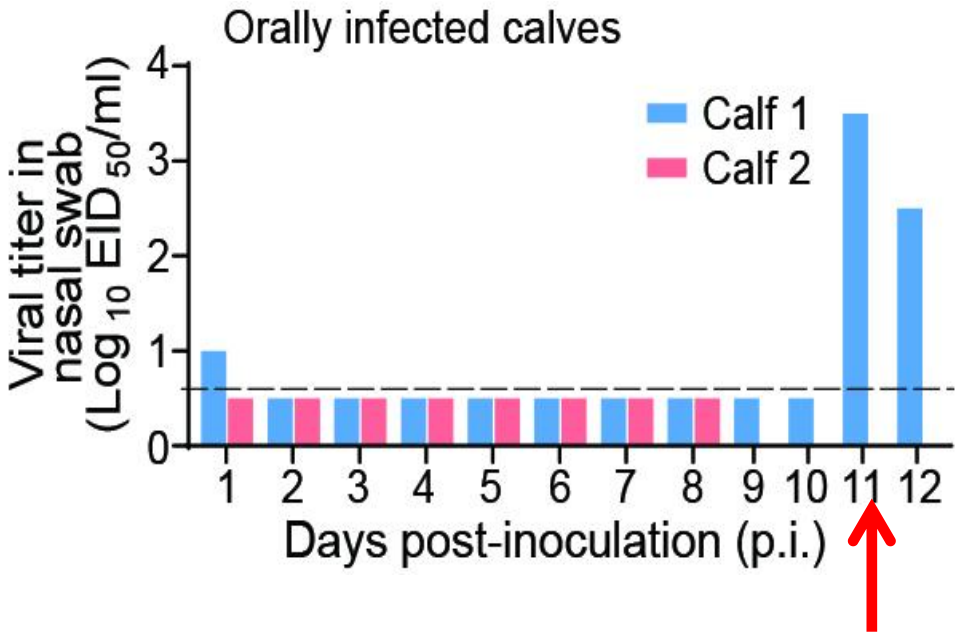


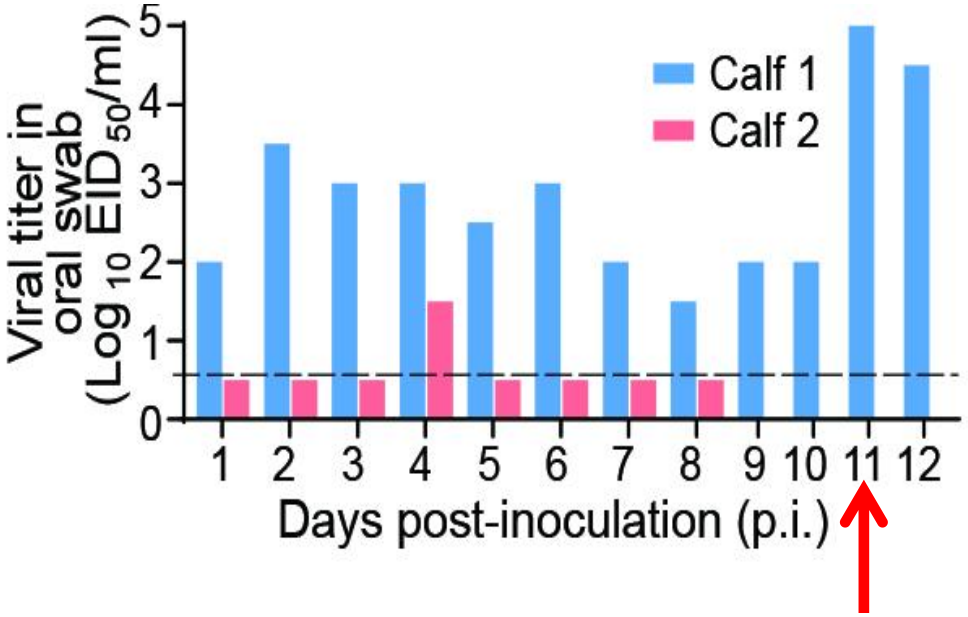




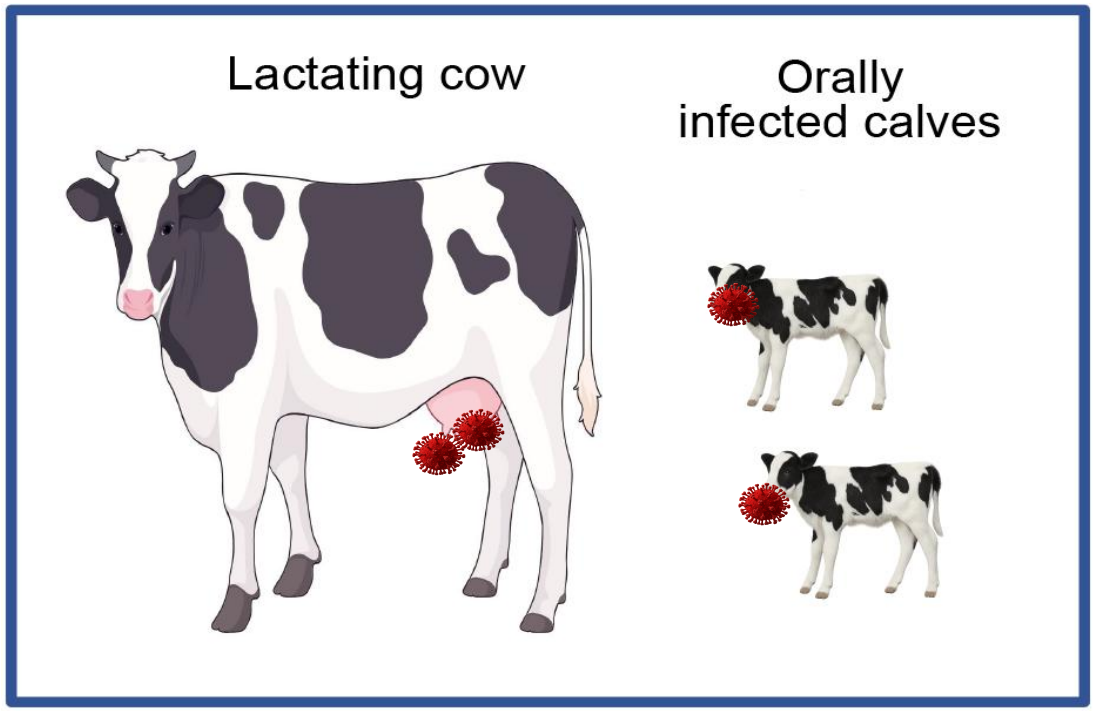


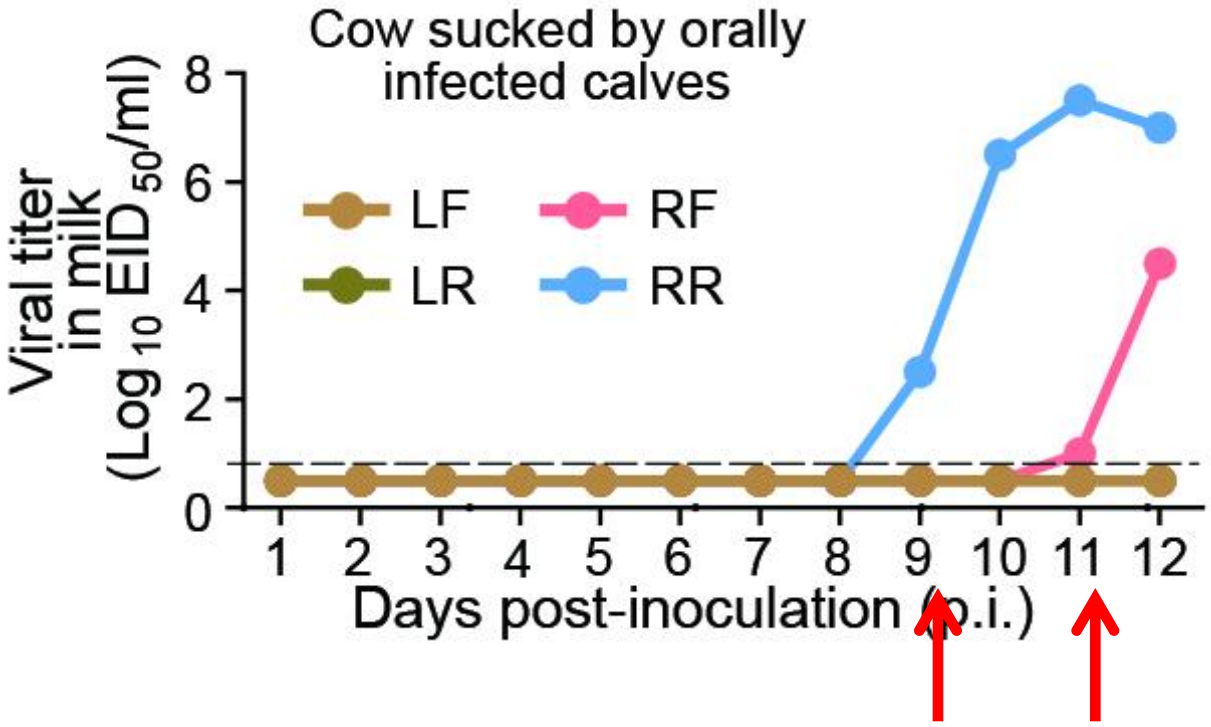

Lactating cow
Orally infected calves


Orally infected calves
Calf 1
Calf 2
Viral titer in nasal swab (Log 10 EID 50/ml)
0
1
2
3
4
1 2 3 4 5 6 7 8 9 10 11 12
Days post-inoculation (p.i.)


Calf 1
Calf 2
Viral titer in oral swab (Log 10 EID 50/ml)
0
1
2
3
4
5
1 2 3 4 5 6 7 8 9 10 11 12
Days post-inoculation (p.i.)

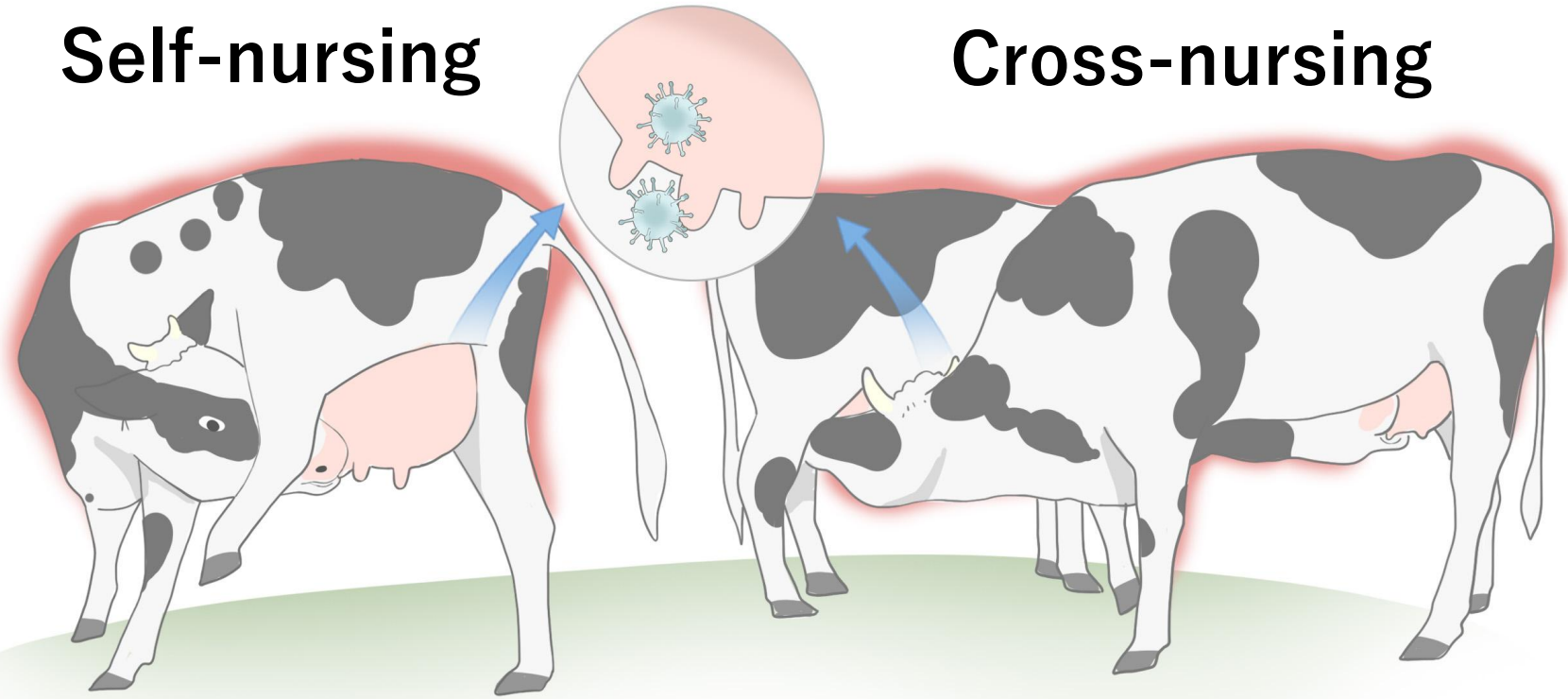
The milk from two mammary glands of the cow that was sucked by the orally infected calves became viral postive





Two questions

1. How does the H5N1 virus get to the mammary glands?



Two questions

1. How does the H5N1 virus get to the mammary glands?

2. Could vaccination prevent H5N1 virus infection and spread in cattle?

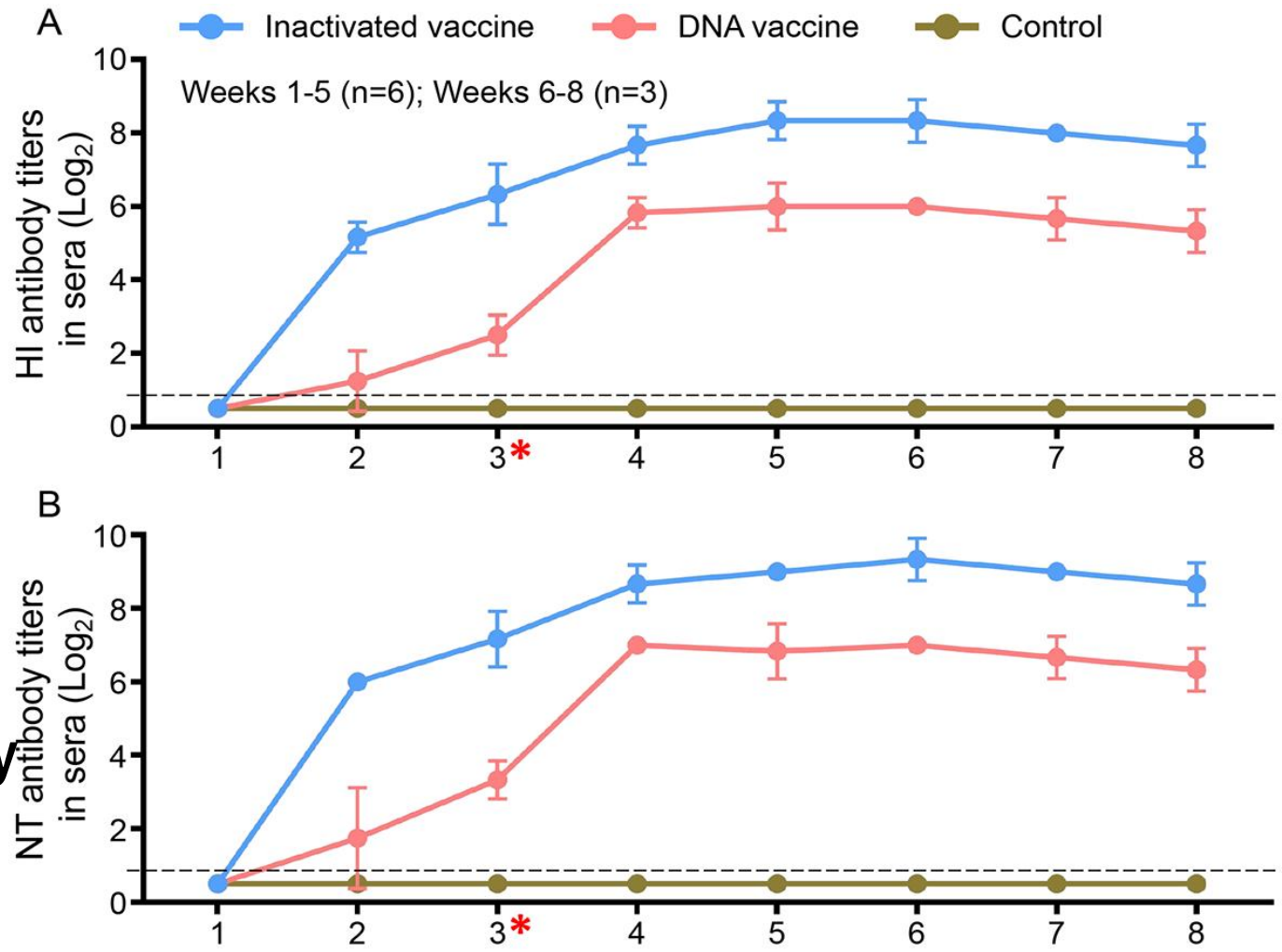
Vaccines developed in China

- Inactivated vaccine developed by reverse genetics
- Virus-vectored live vaccine: Newcastle disease virus vectored and duck enteritis virus vectored vaccines
- DNA Vaccine

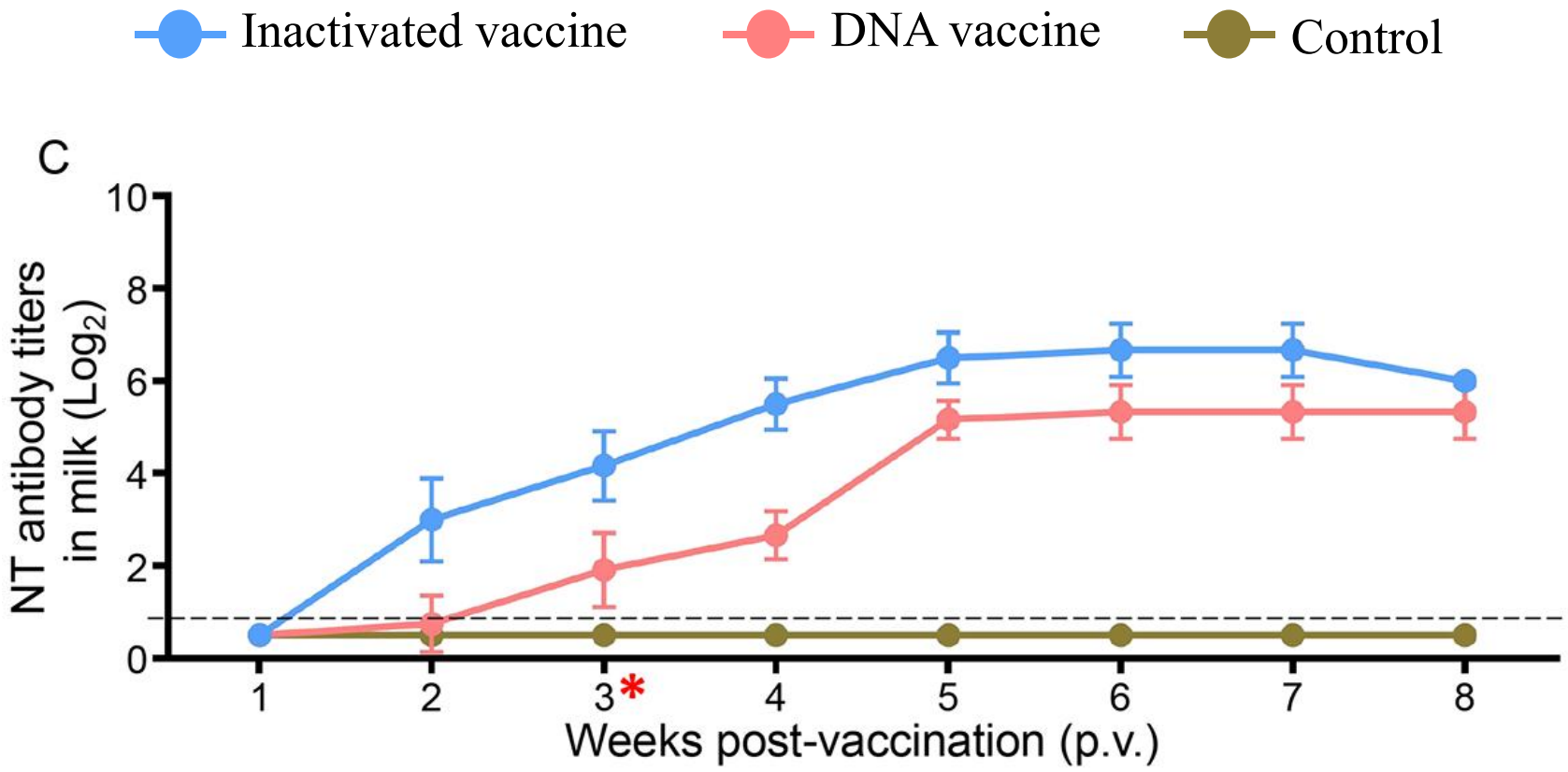
Both vaccines induced high level of HI and NT antibodies in serum of cattle

HI antibody

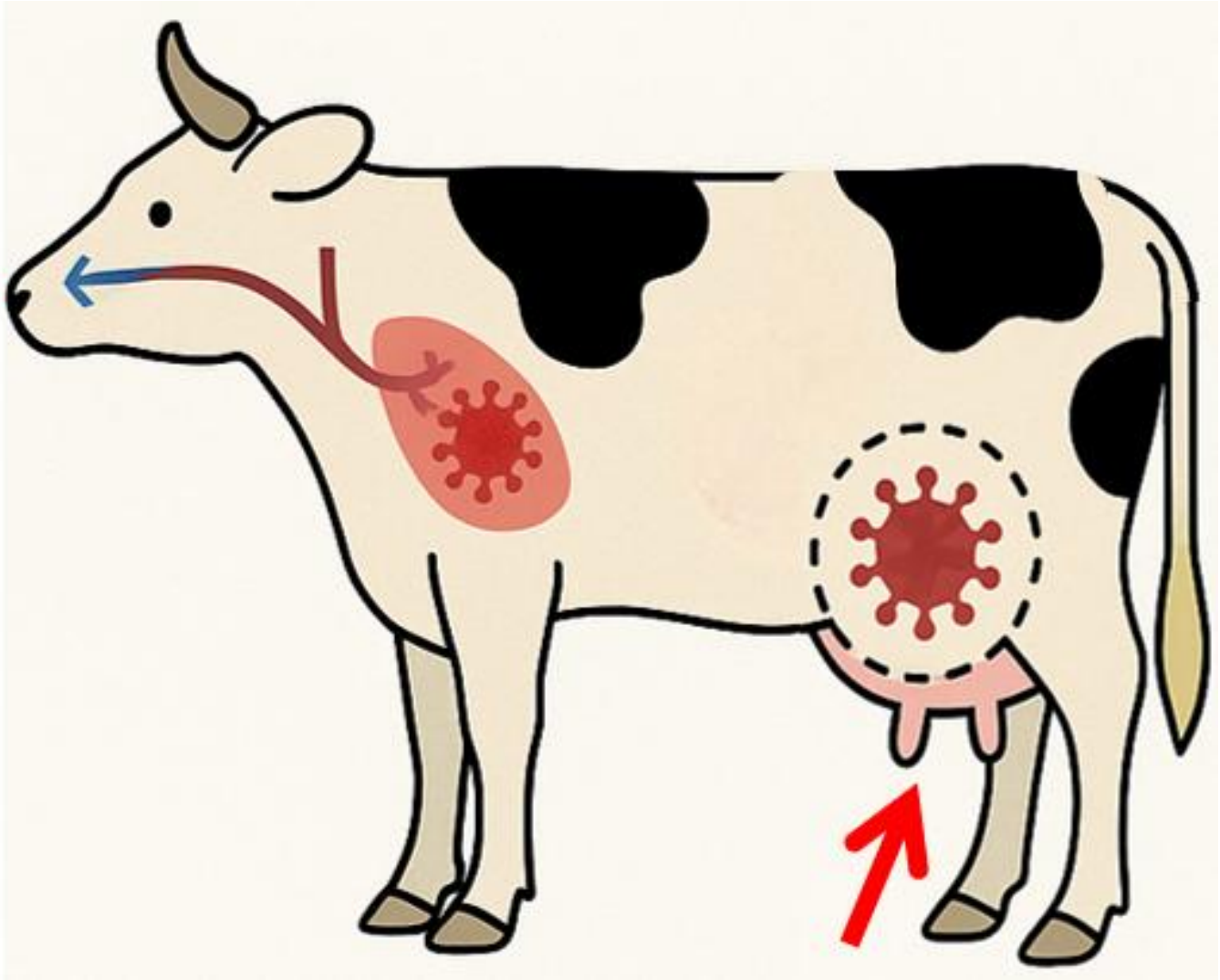
NT antibody



Both vaccines induced high level of NT antibody in milk of cattle

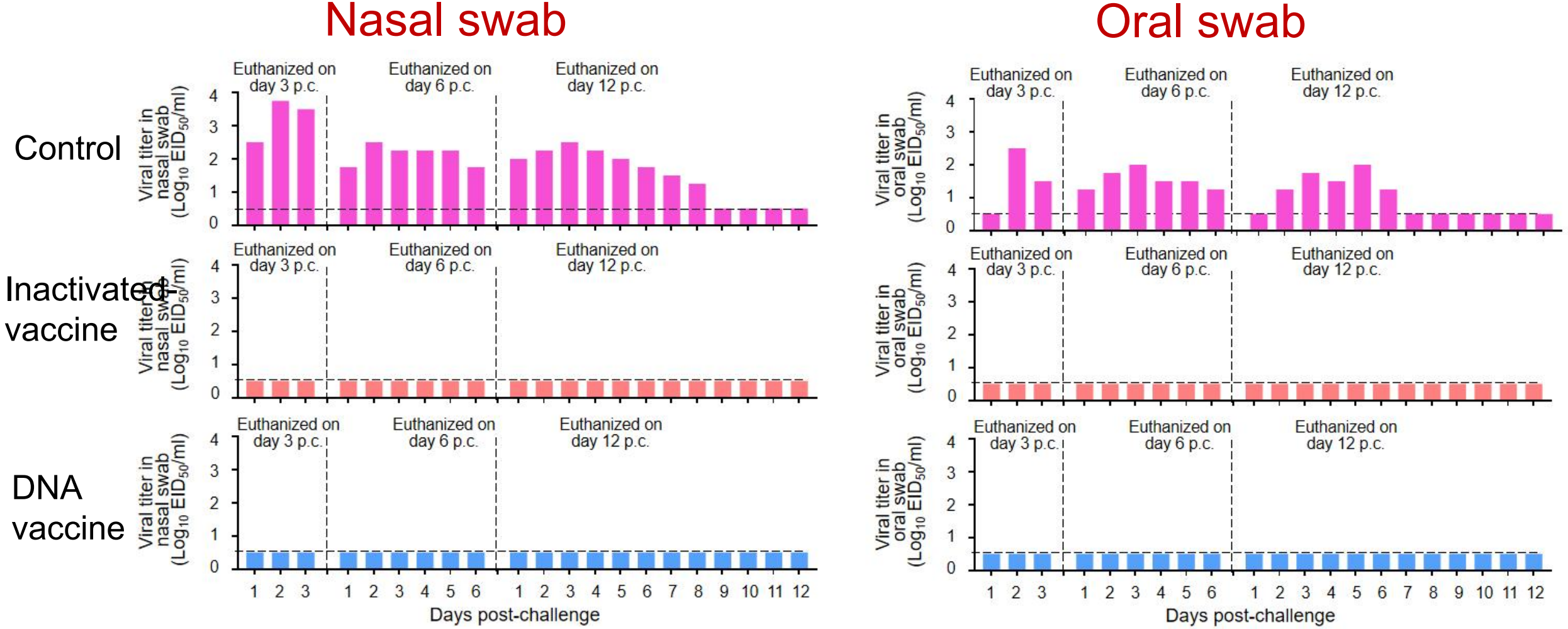


$2x10^6$ EID_{50}



$2X10^2$ EID_{50} to two different teats

Swabs and milk samples were collected for virus titration

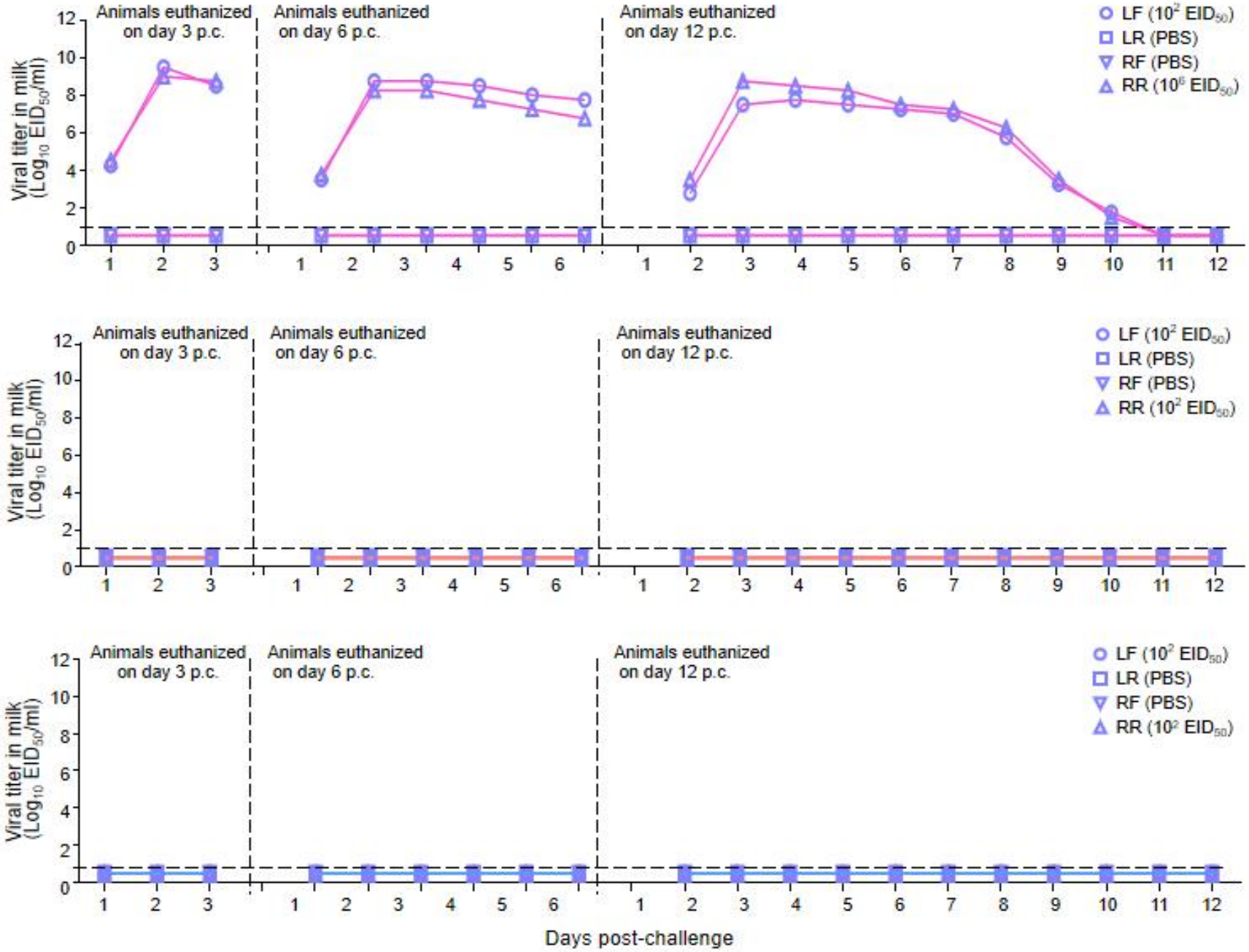


Milk

Control

Inactivated-vaccine

DNA vaccine

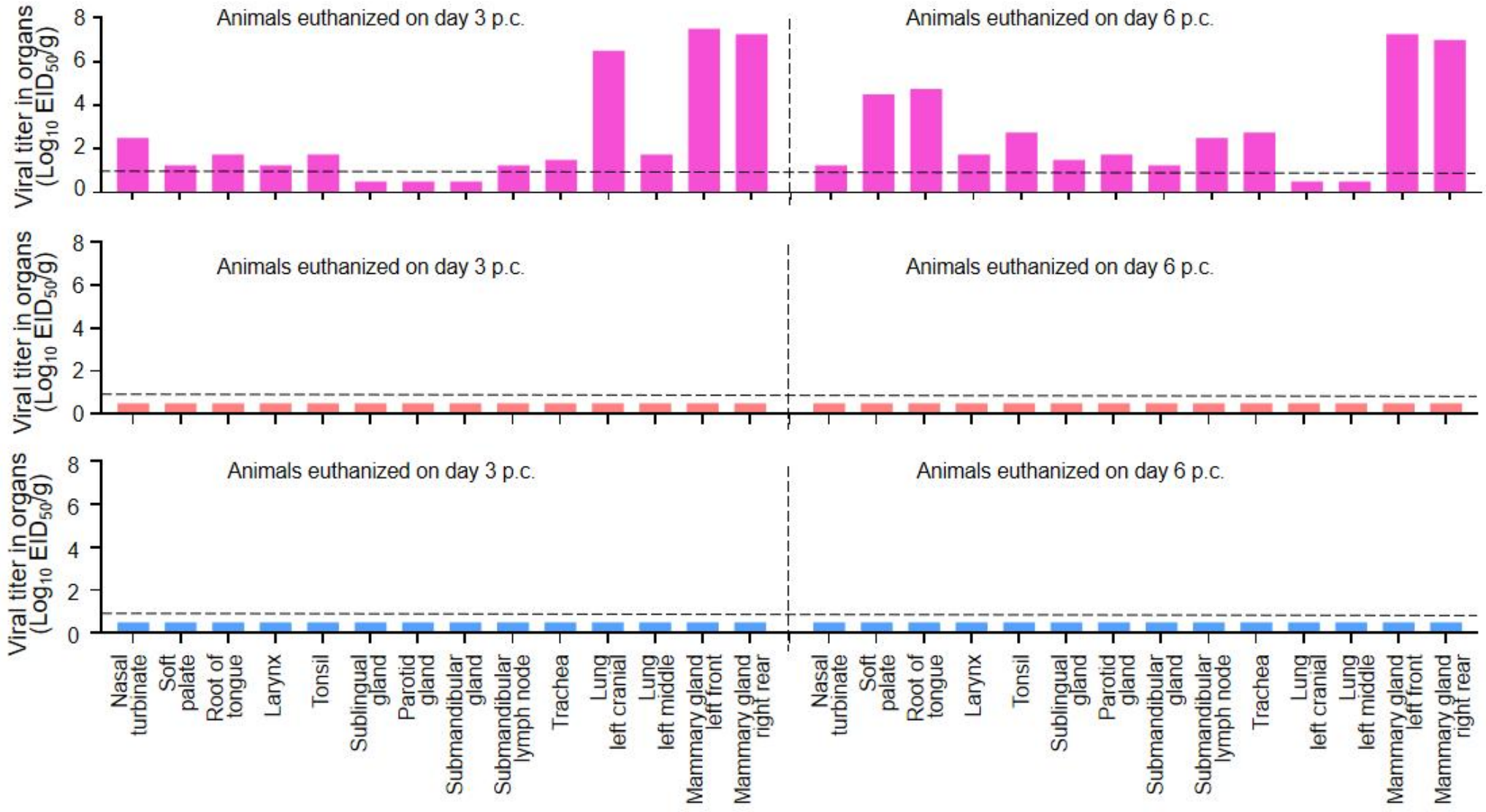


Virus was only detected in the oral and respiratory tract tissues of control cattle

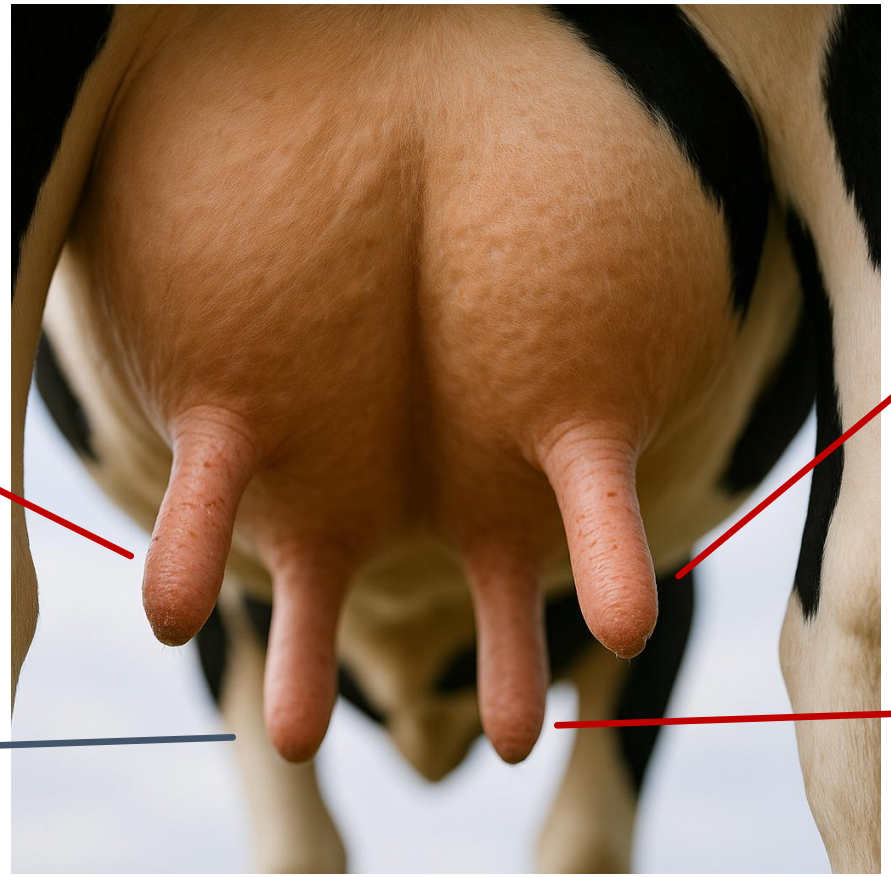
Control

Inactivated-vaccine

DNA vaccine



Could the cattle be protected against high dose of H5N1 virus challenge through the intramammary glands inoculation?

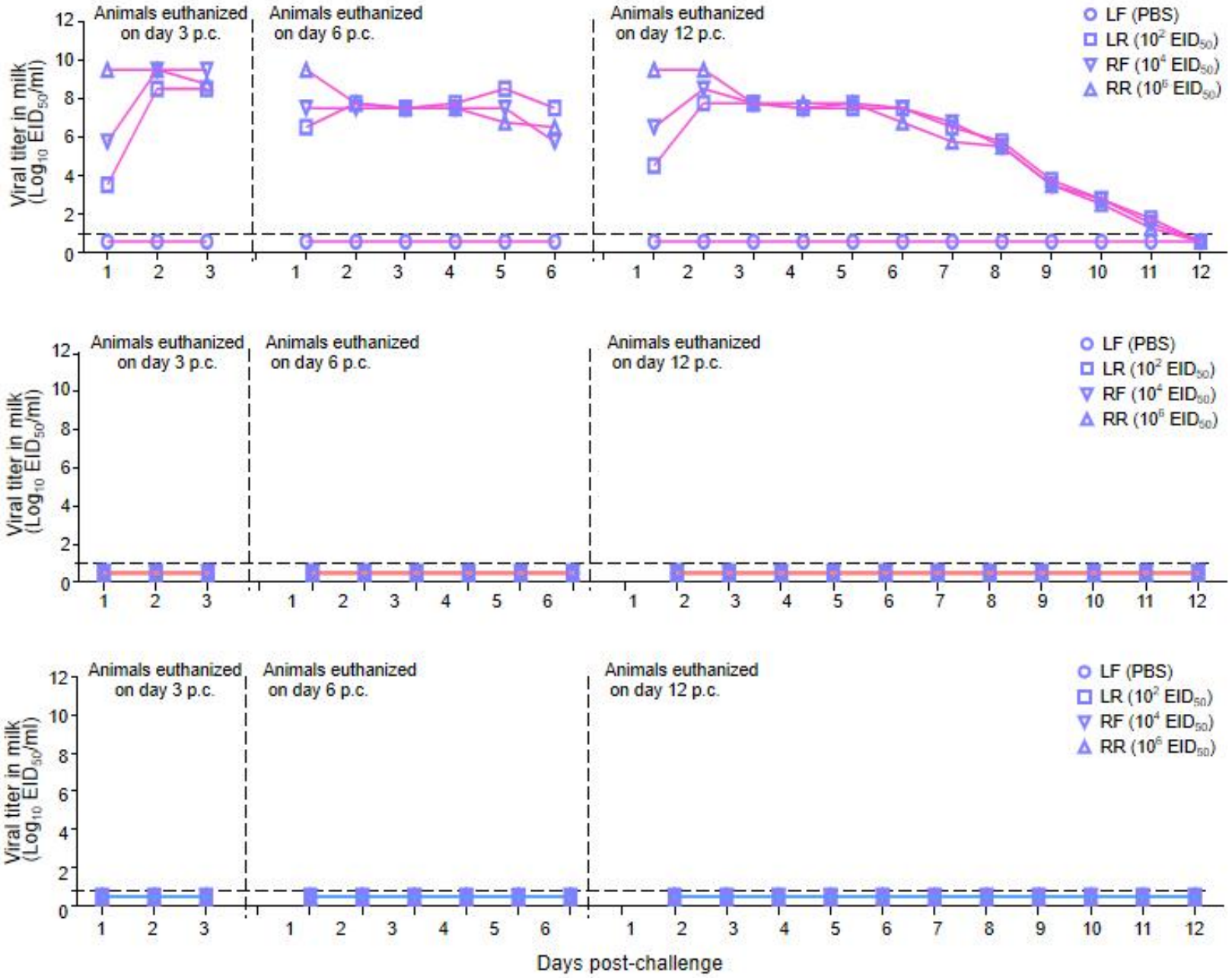


Milk

Control

Inactivated-vaccine

DNA vaccine



Two questions

1. How does the H5N1 virus get to the mammary glands?

2. Could vaccination prevent H5N1 virus infection and spread in cattle?

YES, COMPLETELY!

Conclusion

1. We demonstrated that H5N1 virus replicated in the mouths of cattle can be transmitted to their mammary glands during sucking, thus revealing how the H5N1 virus enters the mammary glands of cows in nature. (Milk-stealing cow management is important!)

2. Inactivated and DNA vaccines induce sterilizing immunity in cattle against high-dose intranasal and intramammary gland challenge with H5N1 virus.

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MICROBIOLOGY

H5N1 virus invades the mammary glands of dairy cattle through 'mouth-to-teat' transmission

Jianzhong Shi [1,†], Huihui Kong [1,†], Pengfei Cui [1,†], Guohua Deng [1,†], Xianying Zeng [1,†], Yongping Jiang [1,†], Xijun He [2], Xianfeng Zhang [2], Lei Chen [1], Yichao Zhuang [1], Yan Wang [1], Jinming Ma [1], Jiaqi Li [1], Yaping Zhang [1], Congcong Wang [1], Chen He [1], Jiaxin Yang [1], Jiongjie Li [1], Wenyu Liu [1], Jinyu Yang [3], Shenggang Mao [3], Jinxiong Liu [1], Pucheng Chen [1], Guobin Tian [1], Chengjun Li [1], Yuntao Guan [2], Zhigao Bu [1,2] and Hualan Chen [1,*]

Thank you for your attention!