

**Animal and Plant Health Agency**

# **Report on a cross-sectional survey for the detection of HPAI H5N1 in dairy cattle in Great Britain (not including Northern Ireland)**

## **Executive summary**

In March 2024, several cases of highly pathogenic avian influenza (HPAI) H5N1 in dairy cattle were confirmed in the USA. United States Department of Agriculture (USDA) carried out testing of pasteurised milk sold at grocery stores and detected fragments of viral ribonucleic acid (RNA), although this was reported as viral particles in the popular press, sparking concerns in the Competent Authorities of multiple countries.

The findings of the preliminary outbreak assessment and Human Animal Infections and Risk Surveillance (HAIRS) group risk assessment conducted in Great Britain (not including Northern Ireland) were that risk to the general public of the same strain circulating in the UK in either birds or cattle is not only very unlikely but is also a very low impact. However, due to the high level of uncertainty and knowledge gaps at the time, there was a need to provide reassurance that there was not silent circulation of avian influenza virus in Great Britain dairy herds.

A cross-sectional survey was carried out over a period of six weeks from May to June 2024, testing 508 bulk milk samples from 455 dairy farms distributed across England, Scotland and Wales. All samples were negative to HPAI H5N1 real time polymerase chain reaction (RT-PCR) tests.

## **Background**

On the 25 March 2024, the USA reported the detection of Highly Pathogenic Avian Influenza virus (HPAIV) H5N1 from dairy cattle in two dairy farms in Kansas and Texas. Before then, HPAIV H5N1 clade 2.3.4.4b had not been detected in domestic cattle (*Hu et al., 2024*), only through experimental infection, despite multiple detections in wild and farmed mammalian species during the peak of the outbreak in different regions ([Bird species affected by H5Nx HPAI \(fao.org\)](https://www.fao.org/zh/topic/diseases/news/detail/c?id=1328932)).

The origin of infection in cattle is undefined although environmental pressure from wild birds is high and practises of utilising composted poultry litter as cattle feed and bedding are all potential methods that might have driven the initial infection event. Currently, however, infection from wild birds to cattle is proposed as the key source

for the initial incursion, based on sequencing of wild bird strains from November 2023 when this reassortant was first detected.

Following initiation of infection in cattle, the virus has then been spread across multiple states through cattle movement. Where infectious cattle have been translocated to new farms, the primary mechanism of transmission between cattle is proposed to be facilitated mechanical transfer through milking machinery, equipment, and staff. This route is considered most likely due to the fact that very high titres of virus have been reported in milk from infected cows, often only one quarter of the udder is affected suggesting that the virus cannot readily cause systemic infection if the initial route of exposure and infection is via milking machinery.

However, low levels of viral RNA have been reported in nasal swabs although the significance of that remains to be determined. Spread of infection can also occur through fomite transfer and may also contribute to dissemination of infectious virus from dairy to dairy and from dairy premises to poultry premises, peridomestic birds and mammals (USDA, 2024b).

The genotype that cattle are infected with in the US is one of over 100 different genotypes of H5N1 detected in the Americas. This particular genotype, B3.13 is thought to have emerged during late 2023 following a reassortment event between a B3.7 virus and a low pathogenicity North American virus. Prior to the emergence of the B3.13 genotype in cattle it was not a dominant genotype and had only been associated with a handful of cases in avian and non-bovine mammalian cases.

Other genotypes of this virus have been associated with multiple cases of spill-over into non-bovine mammalian hosts, including wild mammals and neonatal goats (CDC, 2024; USDA, 2024b). Since the emergence of B3.13 in cattle, there has been an increase in cases associated with mortalities in wild birds and domestic cats (Burrough et al., 2024), in Colorado, Minnesota, Ohio, Oklahoma, Texas, New Mexico and Michigan.

**H5N1-infected cattle cases in the US have been either subclinical or clinical with virus predominantly found in milk and mammary tissue regardless of clinical disease status.** In cattle, clinical signs may include a drop in feed consumption and rumination; clear nasal discharge; and subsequent acute drop in milk production (with colostrum-like milk or no milk produced in severe cases). There may also be abnormal faeces, lethargy, dehydration, and fever. (USDA, 2024c). Clinical disease typically lasts for between 10 days and 2 weeks although longer term shedding profiles remain to be investigated. Affected cattle recover with supportive treatment, with little or no directly attributed mortality (AVMA, 2024).

## Methodology and results

The clinical presentation in the US with very high titres of virus being detected in milk meant that unpasteurised bulk milk was the priority sample for assessment within the UK. Furthermore, bulk milk tank testing allows all the lactating cows in a herd to be

tested from one sample. The APHA National Reference Laboratory (NRL) for avian influenza undertook an assessment of frontline molecular assay performance on both pasteurised and unpasteurised milk samples to confirm that the sensitivity and specificity of the RT-PCR test for the detection of viral nucleic acid would not be hampered by the fatty composition of milk.

Contact was made with the National Milk Records (NMR) company, via contacts made during the FSA Path Safe programme, to explore the feasibility of accessing milk samples from their customers; these are dairy processors that regularly send samples to NMR for testing. Several of these processors agreed to participate in this surveillance programme and voluntarily sacrificed 1ml of their monthly sample, that was sent by NMR to the APHA NRL for testing. A data sharing agreement was signed between Defra and NMR, to ensure compliance with GDPR.

The sampling plan was developed by APHA Animal Health and Welfare Advice team. The agreed objective of this surveillance was to be able to detect, with a 99% confidence, at least one infected farm if the between-herd disease prevalence was at least 1% (a frequently used threshold to prove absence from a contagious exotic disease and deemed appropriate in this context). The minimum sample size was calculated to be 453 dairy farms, considering a sampling frame of nearly 7,000 dairy farms. It was recognised that larger farms will have more than one bulk milk tank, therefore a milk sample from each tank would be submitted for testing.

The allocation of samples per geographical region at ITL2 was done by NMR veterinary leads on a 'probability proportional to size' (PPS) basis, this means more samples were allocated to those regions with a higher number of dairy farms. Sample collection started on the week commencing 20th May 2024, with 80-100 samples delivered at APHA Weybridge every Thursday morning for same-day testing. The samples were submitted with information about the geographical origin at ITL1 level to ensure anonymity. A letter of consent explaining the objective and terms of the survey was sent and signed by the processors who chose to participate. There were no other ethical issues to consider.

A total of 455 farms were tested. Results were reported every Thursday evening to chief veterinary officers (CVOs) and policy makers in UK. Table 1 provides a summary of the total number of samples per ITL1 region.

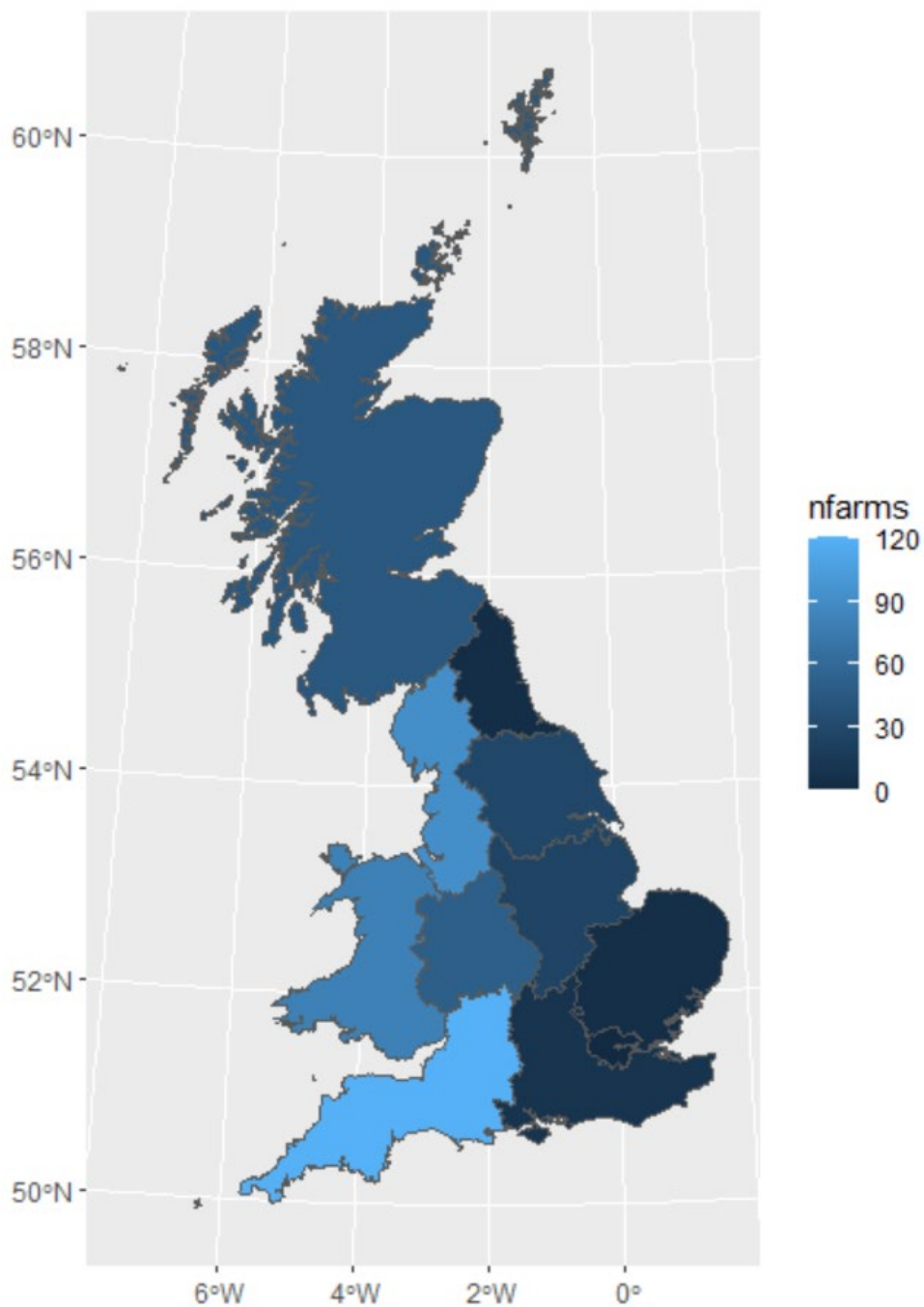
**Table 1**

Number of farms and number of samples tested per ITL1 in GB.

<b>Region</b>	<b>Number of farms tested</b>	<b>Number of samples</b>
<b>East England</b>	4	4
<b>Scotland</b>	44	54
<b>Wales</b>	80	89
<b>West Midlands – England</b>	50	57
<b>Yorkshire and the Humber (England)</b>	28	29
<b>South West (England)</b>	120	131
<b>North West (England)</b>	92	104
<b>North East (England)</b>	3	3
<b>South East (England)</b>	9	9
<b>East Midlands (England)</b>	25	28
<b>Total</b>	455	508

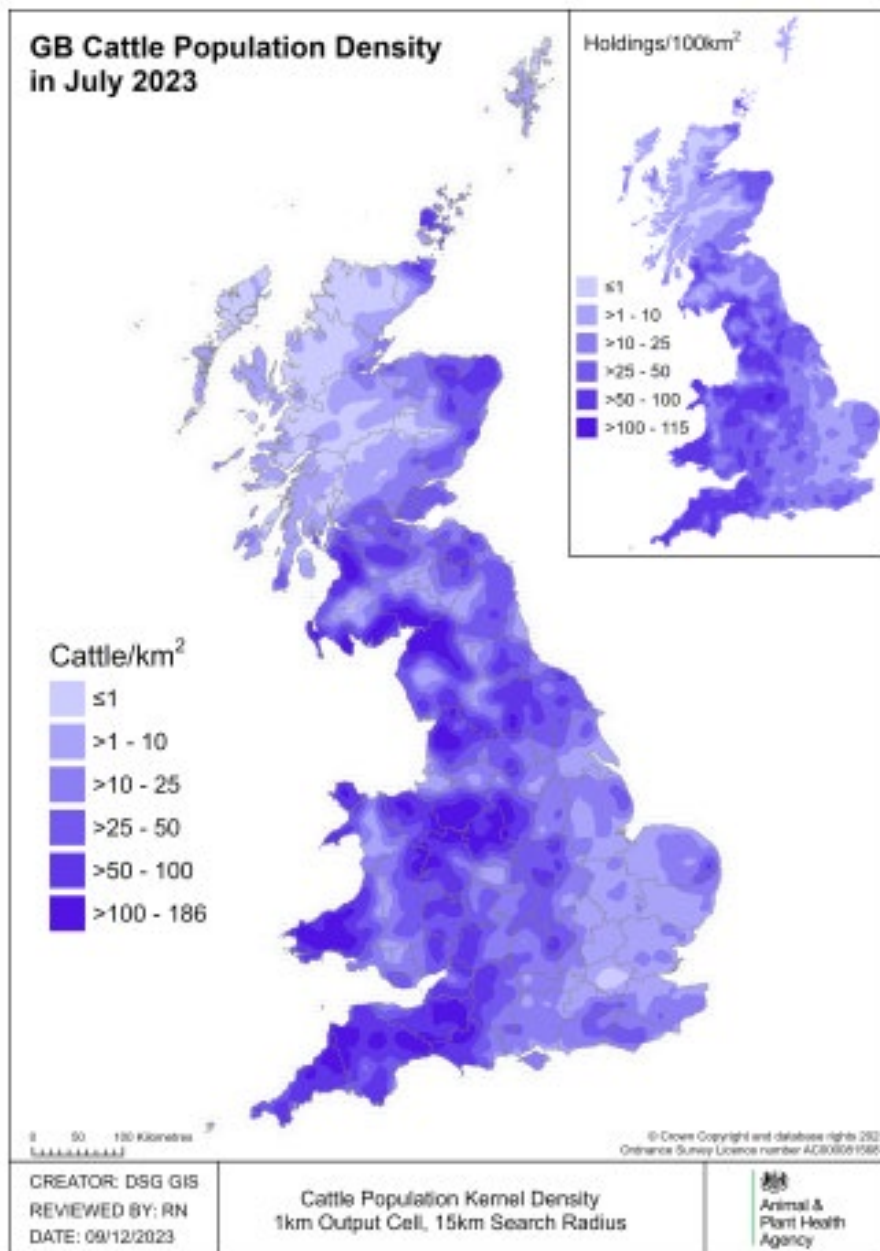
## Map 1

Numbers of farms tested per region at ITL1 level. Higher number of farms were tested in the South West and North West of England, and in Wales, reflecting the larger number of dairy farms in those regions.



## Map 2

Cattle population density as of July 2023, by CTS-recorded number of animals per square kilometre (inset: holding density). The highest densities of both beef and dairy population and holdings tend towards the west of Great Britain.



## Discussion and conclusions

The objective of this survey was to confirm that HPAI H5N1 was not present in dairy cows in GB during that period. At the time of designing the sampling plan, there was no evidence to support silent infection in Great Britain dairy cattle and extensive investigation into the genotypes of H5N1 HPAIV circulating in the UK and Europe had previously demonstrated that the virus causing cattle infection in the US had never been detected outside of those cases reported.

The UK had just submitted a self-declaration of freedom from HPAI to WOA. There had been a sharp reduction in the number of positive cases in wild birds (with only two positives in April the last case being in an apex predator (sparrowhawk) on the 5<sup>th</sup> April), and there was no indication of increased mastitis cases in dairy cattle sent to APHA Veterinary Investigation Centres for which an etiological agent had not been identified (only 1 case out of 27 was not attributed to a known pathogen between April and May, of 3/53 between February and March). However, there was a need to provide reassurance to Public Health Authorities and to the public that there was no HPAIV circulating in cattle in Great Britain.

While some other countries chose to test retail pasteurised milk to detect traces of viral nucleic acid, Defra and Devolved Administrations, in consultation with APHA, decided to test raw milk taken directly from dairy processors, using a highly specific PCR test for HPAI H5 viruses, which had been proven to detect very low viral levels in spiked milk samples.

Participation in this surveillance was entirely voluntary and this might have introduced bias in the selection of samples; however, the processors who agreed to participate represented over 80% of NMRs supplying farms, therefore reducing the impact of bias. Testing was carried out over a 6-week period, from 23 May to 27<sup>th</sup> June 2024. A total of 508 bulk milk samples from 455 dairy farms were tested, all with negative results to HPAI H5N1 RT-PCR tests. The sampling plan was designed to have a 99% probability of detecting at least one infected farm if the disease prevalence at national herd level was at least 1%; given that all samples tested negative, it can be concluded that either disease was not present or, in the unlikely event that it was present, it was at a prevalence below 1% at herd level.

The study successfully showed that the sampling process was quick to put in place, cost effective, highly specific and could be used for further sampling and assessment if needed when or if the risk level rises. Anonymised sampling is not always ideal, as it sacrifices traceability for speed, but in this situation, it was considered the most cost-effective activity to provide assurance to the public health bodies and the general public that HPAI was not present in the Great Britain dairy herd.

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