

Susceptibility of calves fed unpasteurized milk from cows experimentally infected with highly pathogenic avian influenza H5N1

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Abstract

Highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b genotype B3.13 was confirmed in a dairy cow in Texas on March 25, 2024, by the US Department of Agriculture (USDA) National Veterinary Services Laboratories (NVSL) in response to a multi-state investigation into milk production losses. The amount and duration of virus shed in milk from the inoculated mammary quarters point to milk as a critical source of virus spread within and between dairy herds. Pasteurization has been shown to inactivate the virus in milk, however, domestic cats consuming raw milk from affected cows have developed fatal systemic influenza infection, raising the concern for calves fed unpasteurized milk. Investigations on affected dairy herds demonstrated the presence of viral RNA in milk samples for up to 2 weeks before the appearance of clinical signs and diagnostic confirmation of infection. During this lag, unpasteurized milk may pose a risk to calves. Here, we sought to determine if H5N1 genotype B3.13 strain could be transmitted to calves fed unpasteurized milk from virus positive lactating cows. Nine Holstein calves of approximately 7 to 11 weeks old were fed 0.95 L of unpasteurized milk twice a day via bucket. Milk from two non-inoculated cows was fed to one negative control calf for four days. Infected milk was collected from two lactating Holstein cows following intramammary inoculation, early in infection and prior to seroconversion, and fed to four calves for four consecutive days. In addition, milk was collected from another two lactating Holstein cows, inoculated via the intramammary route, in mid-infection following seroconversion, pooled with milk from cows early in infection, and fed to four calves for four consecutive days. Following the 4 initial days, all nine calves were fed milk from non-inoculated cows. Calves fed infected milk without antibodies showed clinical signs including nasal discharge, mild fever, mild lethargy, loose stool and slightly increased respiratory effort for 5–6 days. Viral RNA was consistently detected in nasal swabs from 4/4 calves from 2 to 4 DPI, with detection persisting after calves were transitioned to milk from non-inoculated cows. Infection was confirmed by viral RNA detected in nasal swabs, antigen and viral RNA detection in lung lesions, lymph nodes and pharyngeal tonsil, and seroconversion. Positive PCR results of the nasal swabs were significantly correlated (-0.55) with lethargy in the calves. Calves fed infected milk from cows shedding neutralizing antibodies were partially protected against disease and did not show clinical signs, only 1/4 calves had viral RNA in nasal swabs, and seroconversion was not observed. These data demonstrate that milk diverted from the human food supply in H5N1 positive dairy herds or from suspect cows should not be fed to calves without pasteurization. As characterization of HPAI in the dairy community continues the determination of routes of transmission is an essential first step to inform subsequent research on intervention and vaccination strategies.

Summary

Calves were fed unpasteurized milk from cows infected with highly pathogenic avian influenza virus and became infected. Virus was detected in nasal swabs from the calves. Symptoms were mild and included nasal discharge, loose stool, fever, and lethargy. Lung lesions and enlarged lymph nodes were observed at necropsy. Infection was further confirmed by the detection of diagnostic antibodies 10 days after inoculation. Unpasteurized milk should not be fed to calves. Symptoms were mild, look like many other

calfhood diseases, and could be easily overlooked. Calves are often moved off farm and could be a source of farm-to-farm transmission. As follow up, additional calves were fed milk from cows later in infection that were shedding both virus and antibodies in the milk. The antibodies provided partial protection against infection, with only 1 of 4 (25%) calves becoming infected, compared to 4 of 4 (100%) of calves becoming infected from drinking infected milk without antibodies.

Introduction

Highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b genotype B3.13 was confirmed in a dairy cow in Texas on March 25, 2024, by the US Department of Agriculture (USDA) National Veterinary Services Laboratories (NVSL) in response to a multi-state investigation into milk production losses.¹ Viruses of the goose/Guangdong lineage in the hemagglutinin clade 2.3.4.4b had previously been detected in the United States (U.S.) in 2021 following widespread dispersion across Asia and Europe. H5N1 viruses in this clade were detected across the U.S. in mortality events in wild bird species and wild mammals^{2,3}. Additionally, outbreaks occurred in numerous commercial and backyard poultry premises, leading to culling to control the spread⁴. Subsequently, other outbreaks in dairy cattle were rapidly identified in Texas as well as other U.S. states⁵. As of December 20, 2024, 875 cases were confirmed in 16 states⁶.

The typical case description of clinical cows includes a decrease in milk production and noticeable clinical signs such as thick yellow milk with flecks and/or clots. Infectious virus and viral RNA have been consistently detected in milk from affected cows^{7,8}. The amount and duration of virus shed in milk from the inoculated mammary quarters point to milk as a critical source of virus for potential spread within and between dairy herds⁹. Domestic cats consuming raw milk from affected cows developed fatal systemic influenza infection⁸. Although pasteurization has been shown to inactivate the virus in milk¹⁰, epidemiological investigations on affected dairy herds demonstrated the presence of viral RNA in milk samples for up to 2 weeks before the appearance of clinical signs and diagnostic confirmation of infection⁸. During this lag, unpasteurized milk may pose a risk to calves. Additionally, some dairies and calf ranches continue to feed unpasteurized non-saleable milk, despite recommendations to pasteurize waste milk fed to calves^{11,12}. Here, we sought to determine if H5N1 genotype B3.13 strain could be transmitted to calves fed unpasteurized milk from virus positive lactating cows and whether the presence of neutralizing antibodies in milk provided any protection from infection to the calves.

Materials and Methods

Animal inoculations

All animal work was carried out in a BSL3-Ag facility in compliance with a protocol approved by the Institutional Animal Care and Use Committee of the USDA Agricultural Research Service (ARS) National Animal Disease Center (NADC). Four adult, Holstein lactating cattle free of influenza A virus and

antibody were obtained from Iowa State University and moved into BSL-3 containment as part of a larger study described elsewhere. Following an acclimation period, cows were inoculated via the intramammary route as described in Baker *et al.*⁹ with the modification of 1 ml of 1×10^2 (right front quarter), 10^4 (left front quarter), or 10^6 (left rear quarter) TCID₅₀/ml A/dairy cattle/Texas/24-008749-002/2024. Milk from these cows was used to inoculate calves as described below.

Eight Holstein calves, approximately 7 to 11 weeks old, born on the NADC campus, were moved into BSL-3 containment and allowed to acclimate for two days. One additional calf remained in routine production conditions as a negative control and fed milk from healthy cows. Calves were fed 0.95 L of unpasteurized milk twice a day (AM and PM) via bucket throughout the studies. In Group One, 4 calves were given milk pooled from two cows during 1 through 4 days post inoculation (DPI). In Group Two, 4 calves were given 0.95L of milk from two in mid-infection following seroconversion (15–19 DPI), spiked with 187.5 ml of milk from a cow early in infection (3 DPI) to keep viral load as similar as possible to Group One. After day four of each study, all calves were fed raw milk obtained from non-inoculated cows housed at NADC's own dairy herd. The negative control calf was fed non-inoculated milk throughout the course of the study.

The presence of virus RNA from a pool of each day's milk fed from inoculated cows in Groups One and Two was confirmed by an influenza A virus reverse transcription quantitative real-time PCR (RT-qPCR), as described below. The presence of antibodies in the milk fed to calves in Group Two were confirmed individually in each inoculated cow, as described below.

Clinical evaluation and sample collection

Behavior (lethargy, response to stimulus such as personnel entering the pen, eating and drinking), respiratory effort, rectal temperatures, and clinical samples were monitored and/or collected daily from calves for 6 days post-inoculation (DPI). Criteria for clinical scores used to assess dairy calves are shown in Extended Data Table 2. Ocular, nasal, and rectal samples were collected with FLOQ nylon swabs (Copan, Murrieta, CA) and placed into molecular transport media (PrimeStore MTM, Longhorn, Bethesda, MD). Blood was collected via jugular venipuncture and transferred into serum separator tubes. Tubes were centrifuged and serum was aliquoted. Saliva was collected with an absorbent pad (Super-SAL, Oasis Diagnostics, Vancouver, WA). All samples were taken prior to feeding milk each day.

Virus detection in clinical samples

Milk pooled for feeding, swabs (ocular, nasal, and rectal), serum, saliva, and tissue samples were tested using an influenza A virus (IAV) reverse transcription quantitative real-time PCR (RT-qPCR) kit, as previously described¹³. Briefly, viral RNA was extracted from milk, swabs, serum, and saliva using the MagMAX™-96 Viral RNA Isolation Kit (ThermoFisher Scientific, catalog #AMB18365) following manufacturer's instructions. RNA extraction from tissues was processed through the spin procedure of

the MagMAX™-96 for Microarrays Total RNA Isolation Kit (ThermoFisher Scientific, catalog #AM1839). Extracted product was subjected to RT-qPCR using the VetMAX-Gold SIV Detection kit (Life Technologies, catalog # 4415200). Ct values less than 35 were considered positive while Ct values greater than 38 were considered negative. Ct values 35–38 were considered suspect.

Antibody Detection

Seroconversion was determined using a blocking ELISA to detect antibodies to the nucleoprotein (NP) (Influenza A Ab, IDEXX, Westbrook, Maine), according to the manufacturer instructions with a 1:5 starting dilution for serum. The cut-off sample to negative (S/N) optical density (O.D.) ratio was ≤ 0.6 . Serum samples were also tested using a competition multi-species ELISA to detect antibodies against the hemagglutinin H5 (ID Screen® Influenza H5 Antibody Competition 3.0 Multi-Species, Innovative Diagnostics, Grabels, France), according to the manufacturer instructions. Samples were positive when the percentage of test sample to kit negative control ratio (S/N) was ≤ 0.4 .

H5 specific hemagglutination inhibition (HI) was conducted as described previously^{14,15}, with an H5 clade 2.3.4.4b hemagglutinin gene from A/Bald eagle/FL/W221340P/2022 engineered to be low pathogenicity (kindly provided by Dr. Richard Webby, St. Jude's Children's Research Hospital) for the calf sera and the homologous challenge strain for the cow sera. Prior to HI, serum samples were heat inactivated at 56° C for 30 minutes, treated with receptor destroying enzyme (Hardy Diagnostics, Santa Maria, CA), and adsorbed with 100% rooster red blood cells for 60 minutes to remove nonspecific hemagglutinin inhibitors and natural serum agglutinins. A reciprocal HI titer ≥ 40 was considered positive. Virus neutralization assays with milk from inoculated cows for the Group Two feeding were conducted on the London line of Madin-Darby Canine Kidney (MDCK) cells, as described previously in Baker *et al.*⁹

Macroscopic and microscopic evaluation

All cattle were humanely euthanized by a veterinarian via sedation with a mixture of xylazine (Drecha, Overland Park, KS) and ketamine (VetOne, Boise, ID) intramuscularly, followed by intravenous administration of pentobarbital sodium (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI). Two inoculated calves from each milk feeding group and the negative control calf were necropsied at 6 DPI while the remaining two inoculated calves from each group were necropsied at 13 DPI. At the time of necropsy, the thoracic cavity, abdominal cavity, nasal cavity, and cranium (longitudinal section) underwent macroscopic evaluation. Paired fresh and formalin-fixed tissues for RT-qPCR and microscopic evaluation, respectively, were collected (Extended Data Tables 3 and 4). Formalin-fixed tissues were processed routinely for microscopic evaluation. Additional fresh samples included tracheal swab, urine, feces and rumen, omasum, abomasum, and reticulum contents.

Immunohistochemistry (IHC) targeting the nucleoprotein (NP) antigen of Influenza A virus (IAV) was performed as previously described with the inclusion of a positive and negative tissue control on tissues from calves necropsied at 6 DPI¹⁶. A representative section was selected for IHC from the ethmoids, nasal turbinates, and tracheas. IHC was performed on all sections of lung, tonsil, and lymph nodes. Additional tissue sections with RT-qPCR detection and/or histologic lesions were also evaluated by IHC for the detection of NP antigen to confirm a causal link between IAV and the lesion(s).

Statistical Analysis

Pearson correlation coefficients between Ct values for nasal swabs and clinical observations for all timepoints were calculated using the package Hmisc v 5.3c in Rstudio 2002.02.1 = 461 "Prairie Trillium". Correlations were considered significant at $P < 0.05$.

Results

Viral RNA and neutralizing antibodies in milk

We confirmed the presence of viral RNA in milk fed to calves twice per day used to inoculate calves in this study, for both Group One and Group Two calves (Extended Data 1). All cows were confirmed to be free of influenza antibodies by NP ELISA prior to inoculation. The 2 cows providing milk to calves in Group Two each had serum HI titers of 1:320 and milk VN titers of 1:40 on 13 DPI before feeding began on 15 DPI.

Clinical observations

The control calf consuming milk from non-inoculated cows did not develop any clinical signs of disease during the study. However, mild lethargy and slightly increased respiratory effort were observed in all 4/4 calves in Group One consuming unpasteurized milk from antibody negative experimentally inoculated cows beginning 1 DPI (Fig. 1). By 2 DPI, loose stool and nasal discharge were observed in 3/4 calves. These signs continued intermittently and resolved by 6–7 DPI in inoculated calves. Elevated rectal temperatures, greater than 39.0°C, were observed beginning on 2 DPI in 4/4 calves, as shown in Table 2, and remained elevated in two calves (2410 and 2414) through 5 DPI with the highest temperatures (40.6°C) recorded on 4 DPI. Temperatures were not recorded for Group One on 1 DPI due to thermometer failure and were also not recorded for the two calves necropsied at 6 DPI.

For Group Two calves, no clinical signs were observed other than occasional, intermittent loose stool. Calf 2958 had loose stool on 5 and 6 DPI, calf 2947 had loose stool on 4 and 6 DPI, and calf 2957 had loose stool on 4 DPI. Elevated rectal temperatures were not observed in 2 calves.

Viral RNA detection by RT-qPCR

All samples collected from the negative control calf were negative for IAV by RT-qPCR (Fig. 3). However, in Group One, nasal swabs from 4/4 calves were positive (Ct < 35) starting on 2 and 3 DPI. The lowest

average Ct values (highest viral load) were observed at 2 DPI (Ct 31.5) and 3 DPI (Ct 31.7). At 4 DPI nasal swabs were positive for 3/4 calves and by 5 DPI only 2/4 calves had positive nasal swabs; nasal swabs from the other two calves were in the suspect range (Ct > 35 < 38). Ocular swabs remained in the suspect range for 4/4 calves on DPI 2 and DPI 4. At 3 DPI one calf (2410) had a positive ocular swab (Ct = 33.9) while the ocular swabs from the other three calves remained in the suspect range. By 5 DPI all ocular swabs were negative. No fecal swabs or serum samples were positive at any timepoint. Only 2/4 saliva samples were positive (Ct < 35), one for calf 2140 on 1 DPI and the other for calf 2417 on 3 DPI. There was a strong negative correlation (-0.55) between Ct values from the nasal swabs and behavioral scores in the calves, indicating that as Ct values dropped, and viral load increased, calves became more lethargic.

For calves in Group Two, nasal swabs were negative in 3/4 calves at all timepoints analyzed. In the remaining calf (2950), Ct values were in the suspect range on 1–2 DPI (Ct = 35.9 and 36.3, respectively) and positive on 4–5 DPI (Ct = 33.9 and 33.6, respectively). All other samples collected in Group Two calves (saliva, serum, and ocular and fecal swabs) were negative in 4/4 at all timepoints analyzed.

In the necropsy samples, viral RNA was detected within and outside the respiratory tract tissues of Group One calves (Extended Data Table 5). In Group One calves necropsied at 6 DPI viral RNA was detected Ct value < 35 in multiple tissue types including multiple lung lobes, the palatine tonsils and all four stomach compartments (rumen, reticulum, omasum, and abomasum) of both calves. At 13 DPI viral RNA was also detected at a Ct value < 35 in the lung lobes and reticulum in one of the two calves in Group One. In Group Two, viral RNA was detected only in Calf 2950 necropsied at 6 DPI. Viral RNA was detected outside the respiratory tract of Calf 2950 in the retropharyngeal and tracheobronchial lymph nodes.

Macroscopic and microscopic evaluation Histologic evaluation and IHC results by calf and tissue are summarized in Extended Data Table 5. Two calves (2410 and 2414) from Group One were necropsied at 6 DPI. Minimal multifocal obstructive atelectasis was present in multiple lung lobes in both calves necropsied at 6 DPI (2410 and 2414) and in one of the calves necropsied at 13 DPI (2411; Fig. 4A and 4B; Extended Data Fig. 1; Extended Data Table 5). Gross pulmonary lesions were not observed in the control calf. Lymphadenomegaly was present in the medial retropharyngeal and mandibular lymph nodes of both calves as well as in the parotid and mesenteric lymph nodes in calf 2410 and tracheobronchial lymph node of calf 2414 at 6 DPI (Fig. 4B). Within the mesentery of 2410 adjacent to the mesenteric lymph node there was a focal area of hemorrhage. Macroscopic evaluation of the remaining tissues was unremarkable.

Minimal to mild histologic lesions were noted in the lung sections from Calf 2410 and 2414. Lesions included bronchiolitis obliterans, luminal cellular debris in bronchioles with adjacent atelectasis, and perivascular lymphocytic infiltrates (Fig. 5). IAV antigen was detected by IHC in the luminal debris of a single bronchiole in the middle lung lobe of Calf 2414 (Fig. 5). Mild to moderate parafollicular hyperplasia was observed in multiple lymph nodes (Extended Data Fig. 2). IAV antigen was detected by IHC in the cytoplasm and nucleus of leukocytes in the retropharyngeal lymph node, mandibular lymph

node, parotid lymph node, and tracheobronchial lymph node of both calves necropsied on DPI 6 (Fig. 6; Extended Data Fig. 3). IAV antigen was detected by IHC in the pharyngeal tonsil (epithelial layer and subjacent leukocytes) and palatine tonsil (leukocytes) of Calf 2414 (Fig. 7A and 7B). Mild, multifocal erosion with loss of goblet cells of the conjunctiva was also observed from Calf 2410 that had an ocular swab with positive RT-qPCR, but IAV was not detected by IHC.

Two calves (2950 and 2958) from Group Two were necropsied at 6 DPI. Minimal multifocal obstructive atelectasis was present in multiple lung lobes in one calf (2958) necropsied at 6 DPI and in one of the calves necropsied at 13 DPI (2957). Lymphadenomegaly was not observed and macroscopic evaluation of the remaining tissues was unremarkable. Influenza A virus nucleoprotein antigen was detected by IHC in one tissue (retropharyngeal lymph node) in one calf (2950) (Extended Data Fig. 4)

Serological responses

All calves were negative for NP ELISA antibody in serum prior to inoculation and at 6 DPI for both groups of calves. The two remaining calves from Group One were positive on 10 DPI using a sample/negative (S/N) ratio cut-off of ≤ 0.6 . The sham inoculated negative control remained seronegative as expected (data not shown). At 13 DPI, the calves from Group One continued to be positive for NP antibody and became positive for H5 ELISA antibody and HI antibody, as shown in Table 3. The two remaining calves from Group Two were negative at both 10 DPI and 13 DPI.

Discussion

This manuscript describes the first model of experimental infection of calves fed raw milk from cows inoculated with H5N1 genotype B3.13 strain. Seroconversion confirmed exposure from this route of transmission. Additionally, calves developed clinical signs including nasal discharge, mild fever, mild lethargy, loose stool and slightly increased respiratory effort for 5–6 days, had RT-qPCR positive samples, and lesions consistent with an active influenza A virus infection. Signs of clinical disease were mild and may not be recognized or attributed to HPAI under field conditions with other environmental or health stressors. Viral RNA was consistently detected in nasal swabs from all four calves from 2 DPI through 4 DPI, with continued detection after calves were transitioned to being fed milk from non-inoculated cows. In comparison, virus was only intermittently detected in saliva samples (only two positives) with no positive saliva samples detected after calves were fed milk from non-inoculated cows. Although possible that virus RNA detection in saliva was residual virus from infected milk consumption, samples were collected in the morning prior to the AM feeding to reduce this possibility. Ct values of the nasal swabs were significantly correlated (-0.55) with lethargy in the calves. As Ct values dropped and viral load increased, calves became more lethargic.

This work also demonstrates that the presence of neutralizing antibody in the milk can impact H5N1 transmission, as we reduced, but did not completely prevent, the transmission of H5N1 genotype B3.13 to calves. No clinical signs of disease nor seroconversion were observed in these calves (Group Two). In 3/4 calves we did not detect any viral RNA or antigen in any samples collected ante- or post-mortem.

However, viral RNA was detected in nasal swabs from one of the four calves on DPI 4 and DPI 5 and IAV antigen was detected by IHC in the retropharyngeal lymph node of the same calf on 6 DPI. The lack of seroconversion supports the observation that transmission was prevented in the 2 calves that survived to 13 DPI, but additional timepoints are warranted in future studies.

Macroscopic and/or microscopic lesions were observed in the lung, lymph nodes, and conjunctiva. IAV antigen was detected by IHC in the lymph nodes of the head and respiratory tract (Calf 2410 and 2414), tonsils (Calf 2414), and one lung section in the luminal debris of a single conducting airway affected by bronchiolitis (Calf 2414). The presence of IAV antigen in the nuclei of leukocytes in lymph nodes is consistent with IAV replication in these cells and suggests the possibility of immune trafficking of infected cells that could infect other cells in other tissues.^{16,17} Detection of IAV antigen in the epithelial layer of the pharyngeal tonsil suggests this may be an additional site of IAV replication in cattle and requires further investigation. The presence of erosive conjunctivitis in Calf 2410 aligns with the positive RT-qPCR ocular swab at 3 DPI; however, IAV was not detected by IHC. This may be a result of the time between detection by RT-qPCR and necropsy at 6 DPI. Conjunctivitis was not observed macroscopically.

Other work has shown mild respiratory disease and lung lesions with concurrent detection of IAV by IHC in yearling heifers experimentally inoculated with HPAI through an aerosol respiratory route⁹. However, the calves in the study reported here had consistent nasal swab detection over subsequent days and lower Ct values compared to aerosol inoculated heifers. Furthermore, IAV was detected by IHC in additional tissue types. These data, along with seroconversion of the Group One calves necropsied at 13 DPI, confirms infection from this route of inoculation. The minimal detection of IAV antigen within positive tissue sections in both studies may be a result of necropsy timing and the minimal macroscopic lesions for which to select histologic sections. Evaluating tissues at peak infection around 3 DPI may provide additional insights into viral pathogenesis and tissue and/or cellular tropism.

The clinical signs observed in calves including nasal discharge and loose stool are common in pre-weaned dairy calves and could easily be attributed to other etiologies under field conditions. Digestive and respiratory pathogens in dairy calves continue to be a major problem for dairy producers¹⁸ and account for over 80% of morbidity events in pre-weaned dairy calves (50.9% digestive, 28.1% respiratory, 5.4% digestive and respiratory)¹⁹. Furthermore, calves are often transported off farm, with 1 of every 10 operations in the U.S. raising dairy heifers off site, and most veal and dairy-beef calves transported to another facility for raising²⁰. Therefore, infected calves with mild clinical signs could be a source of farm-to-farm transmission.

Milk diverted from the human food supply in H5N1 positive dairy herds or from suspect cows should not be fed to calves without pasteurization. The continued transmission of HPAI in dairy cattle is an animal health crisis due to the associated morbidity, mortality, interspecies transmission events, and economic losses and is a public health challenge due to occupational exposure on dairy farms. The determination of routes of transmission and of mechanisms of protection are essential steps to inform subsequent research for the development of successful intervention strategies.

Declarations

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Tables

Tables 2 and 3 are available in the Supplementary Files section.

Figures

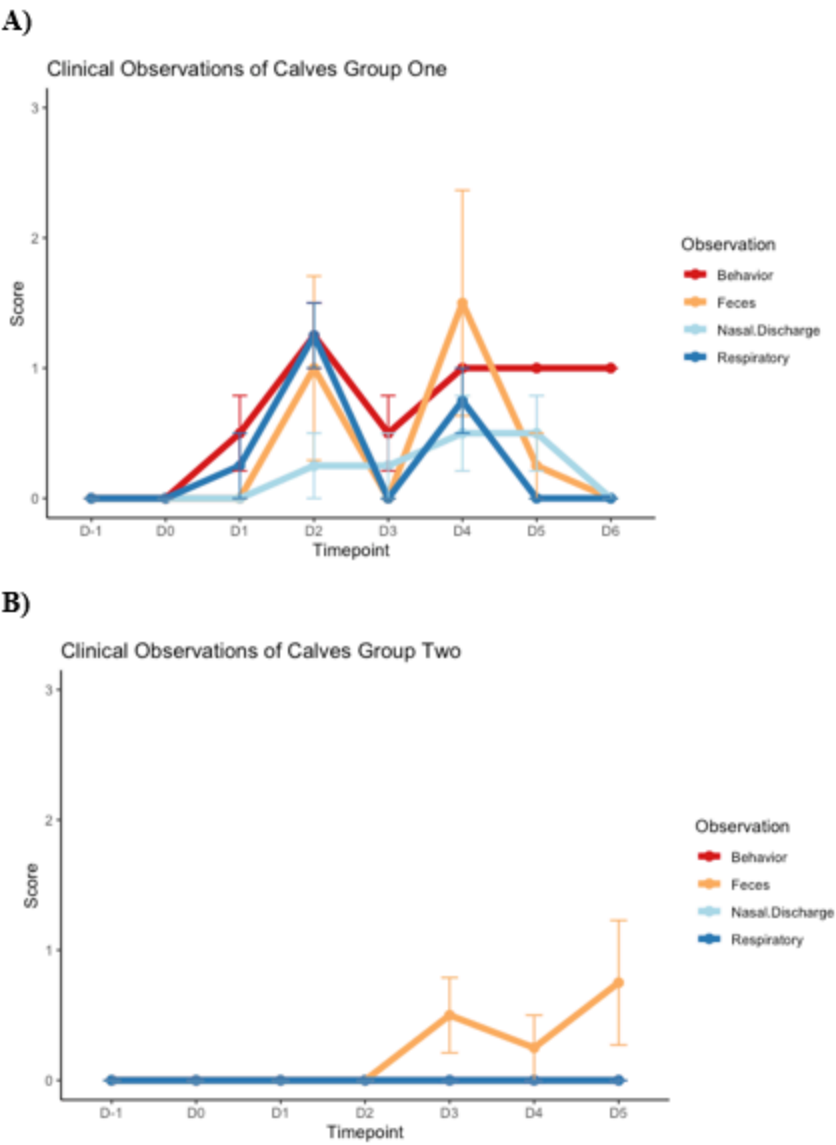


Figure 1

A) Clinical observations of Group One calves fed milk from experimentally inoculated cows shedding virus but not antibody in the milk. B) Clinical observations of Group Two calves fed milk from experimentally inoculated cows post seroconversion with virus and antibody in the milk. Criteria for clinical scores used to assess dairy calves are shown in Extended Data Table 2

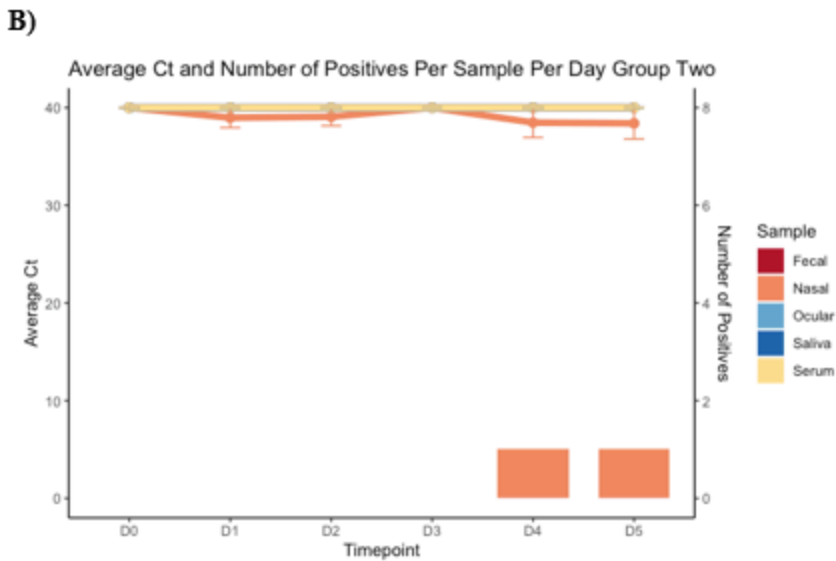
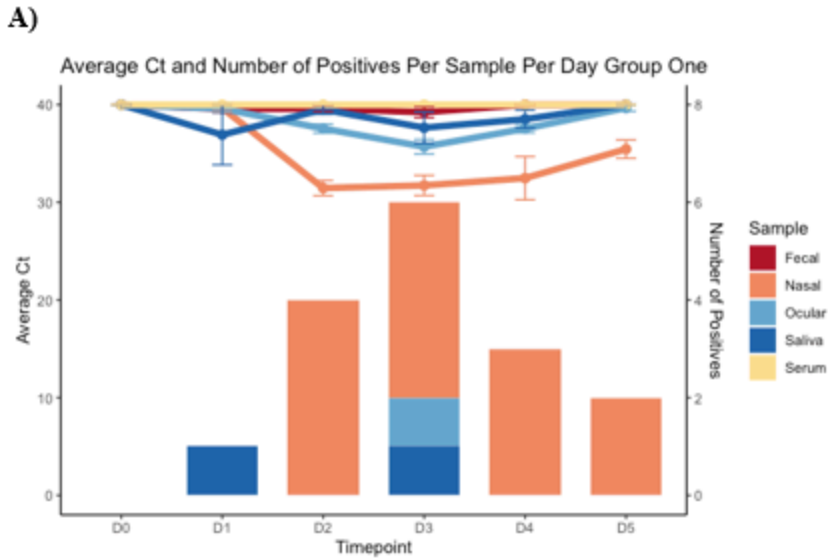


Figure 2

Figure 3. Viral RNA detection in experimental samples. A) Average Ct values for calves in Group One from experimental samples in connected lines on the left y-axis and number of positive samples from each sample type per day in bars on the right y-axis. B) Average Ct values for calves in Group Two from experimental samples in connected lines on the left y-axis and number of positive samples from each sample type per day in bars on the right y-axis.

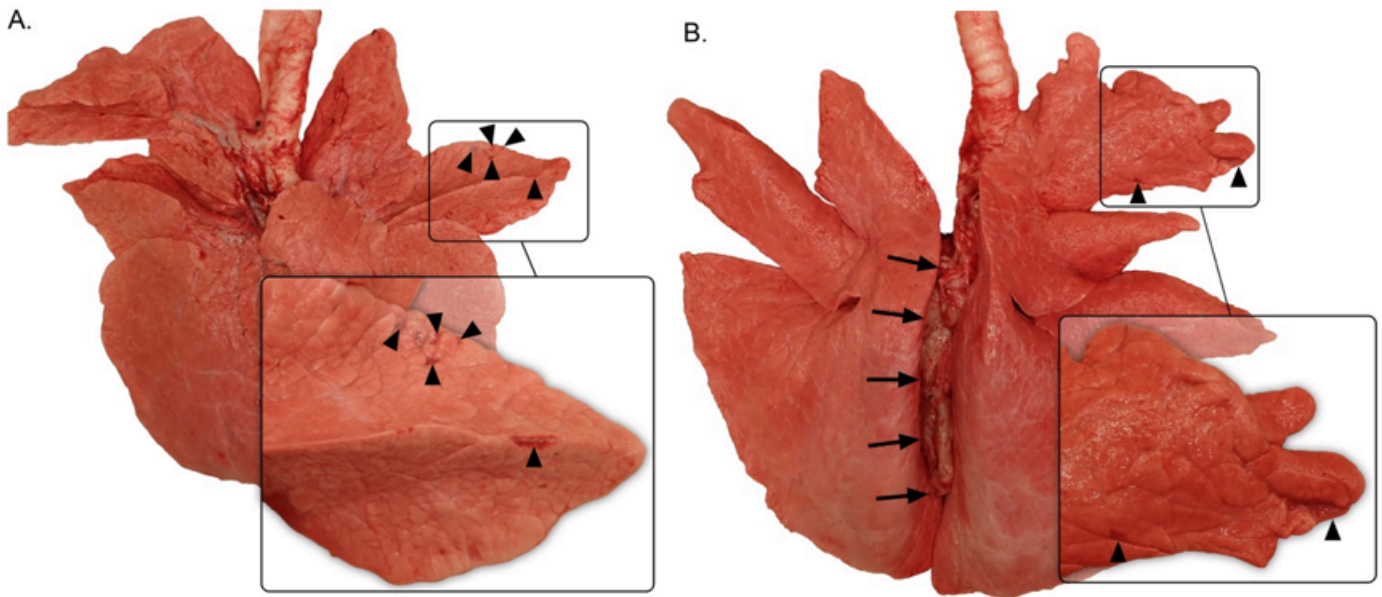


Figure 3

Figure 4. Lung lesions and lymphadenomegaly of Group One calves fed milk containing virus . Minimal multifocal obstructive atelectasis (arrowheads) consistent with influenza A virus at 6 dpi in calf 2410 (A) and 2414 (B). B) Lymphadenomegaly (arrows) of the mediastinal lymph node in calf 2414.

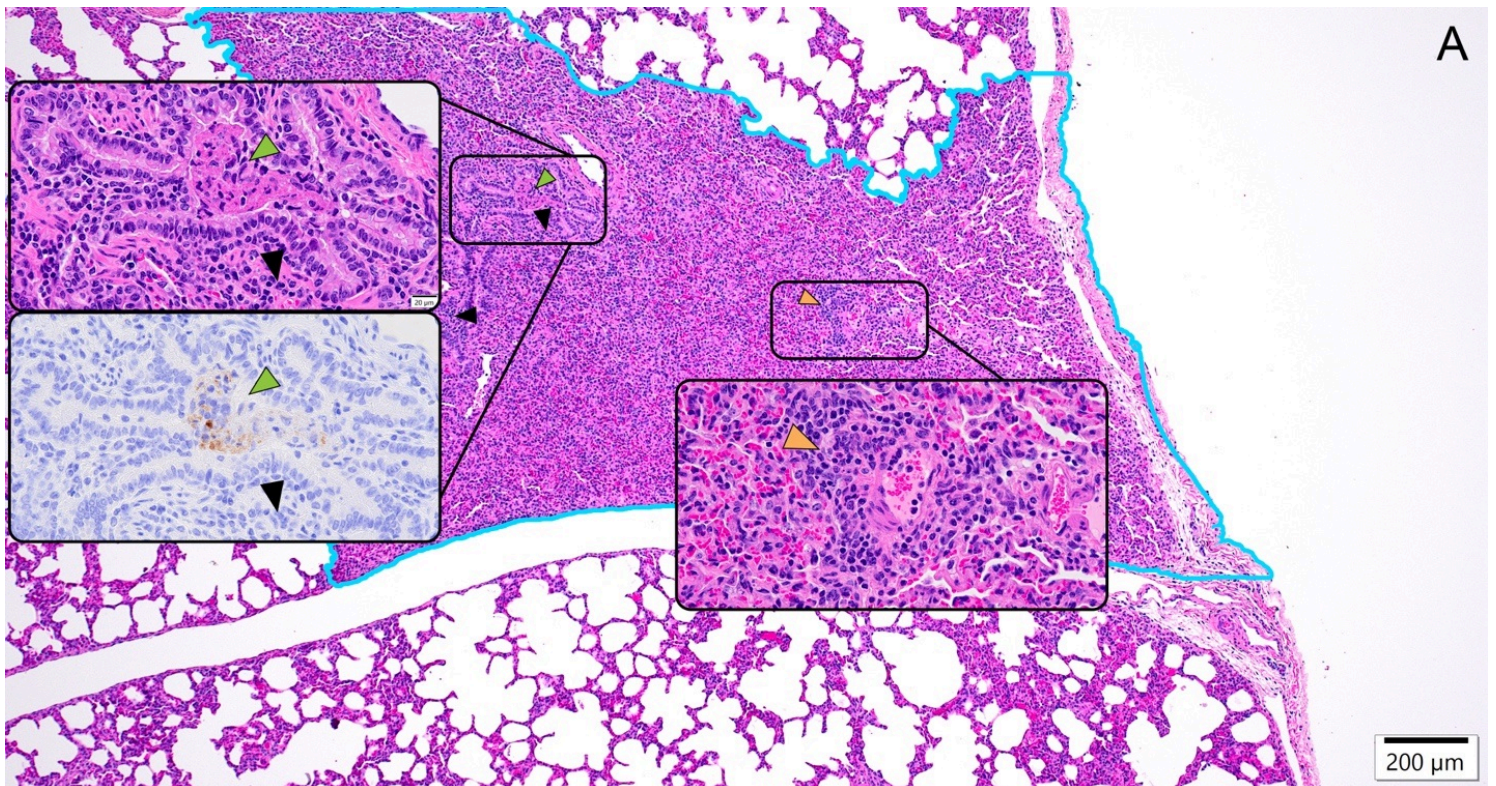


Figure 4

Figure 5. Bronchiolitis and pulmonary atelectasis in Group One Calf 2414 . Accumulation of cellular debris (green arrowhead) in a bronchiole, peribronchiolar (black arrowhead) and perivascular (orange arrowhead) lymphocytic infiltrates, and influenza A virus nucleoprotein antigen detection (brown) by immunohistochemistry (bottom inset) in the luminal debris of the affected airway. Area of obstructive atelectasis outlined in blue.

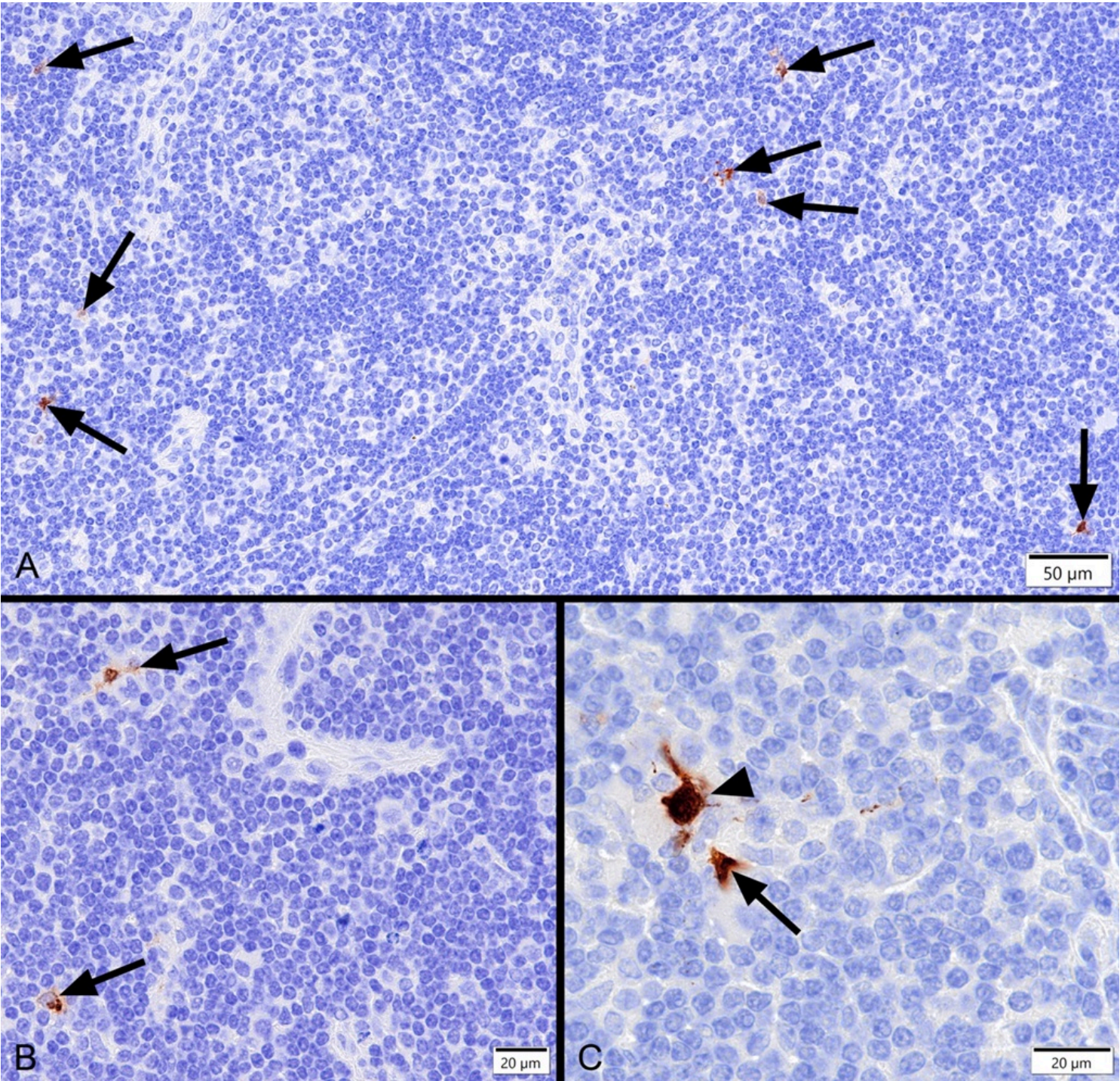


Figure 5

Figure 6. Influenza A virus antigen detection in Group One calf 2410 . Influenza A virus nucleoprotein antigen detection (brown) by immunohistochemistry in the cytoplasm (arrows) and nucleus (arrowhead)

of leukocytes in the parotid lymph node (200X), mandibular lymph node (400X), and tracheobronchial lymph node (600X) of calf 2410 necropsied on DPI 6.

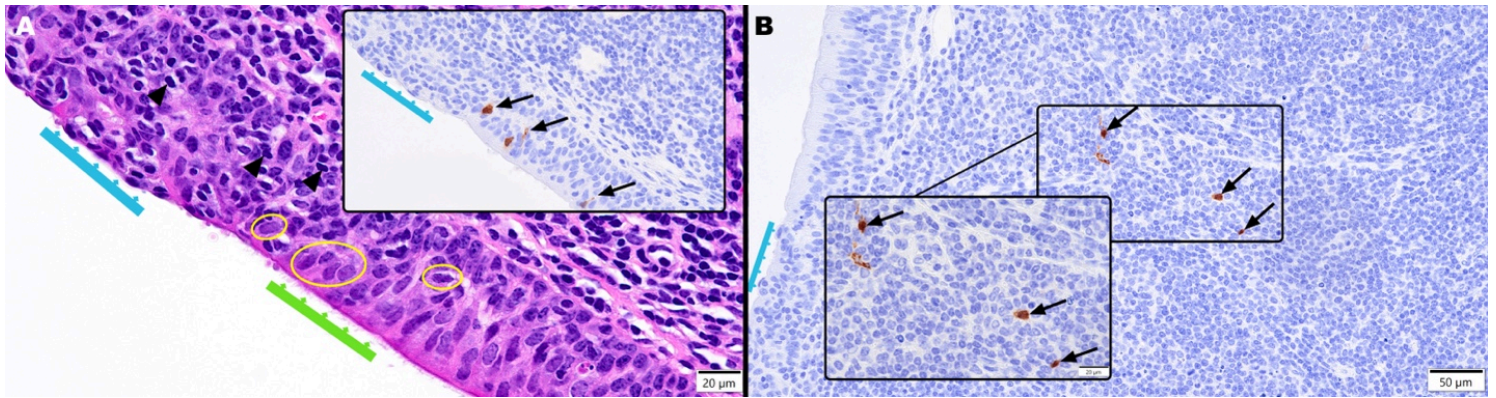


Figure 6

Figure 7. Erosive pharyngitis in Group One Calf 2414 . A) Reticular epithelium (blue bar with arrows) with accumulation of intraepithelial lymphocytes (black arrowhead) adjacent to possible intranuclear viral inclusions (yellow circles) in the area near influenza A virus nucleoprotein antigen detection (arrows) by immunohistochemistry (inset). Intact cilia denoted by the green bar with arrows. B) Reticular epithelium (blue bar with arrows) with subadjacent detection of influenza A virus nucleoprotein antigen (arrows) by immunohistochemistry (inset).

Supplementary Files

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