DAIRY VETERINARY NEWSLETTER

May 2025

Protection Against HPAI/H5N1 is Another Reason to Pasteurize Milk for Calves

Another reason to adopt pasteurization of waste milk to be fed to dairy calves has emerged. There is new evidence that infected milk can transmit HPAI/H5N1 virus efficiently to dairy calves. An online publication by K. Sarlo Davila et al. in CABI, February 3, 2025 reports on a study of the transmission of HPAI in unpasteurized milk fed to calves. The entire paper URL is: https://www.cabidigitallibrary.org/doi/epdf/10.31220/agriRxiv.2025.00303

The objective of the study was to "determine [whether HPAI/H5N1] genotype B3.13 strain (the common HPAI strain now isolated from dairy cattle) could be transmitted to calves fed unpasteurized milk from virus positive lactating cows". The study population was 5 Holstein calves at the USDA Agricultural Research Service's National Animal Disease Center in Iowa. Before the study, all 5 calves were test-negative for influenza A virus using reverse transcription quantitative real-time PCR (RT-qPCR) and test-negative for H5 antibody via ELISA.

4 of the 5 Holstein calves from 7 to 11 weeks old were fed one quart of milk twice daily for 4 days from 2 cows that had been infused intramammarily with HPAI B3.13; milk from 2 other cows that had not been infused with HPAI was fed to 1 control calf on the same schedule as well. I found it interesting that the calves were fed at an age such that many calves would either be weaned, or would be weaned at one more week of age, and that there was only one control calf. It would have been more like the inference population of commercial dairy farms if the milk from HPAI infused cows were fed to younger calves.

Clinical evaluation and sample collection from calves

Behavior of calves was evaluated for 6 days, beginning with the first treated (or control) milk feeding. Clinical scoring was adapted from a University of Wisconsin method, which is shown in Table 1 below. Nylon swab samples were collected from eyes, nares, and rectums. Jugular vein blood samples were placed into serum separator tubes. Saliva was collected using an absorbent pad; all samples were taken prior to feeding milk each day.

Euthanasia and necropsy

All calves were euthanized and necropsied. Two HPAI - fed calves and the negative control calf were necropsied at

Nasal swab Performagene

Necropsy Colorado State

6 days post - inoculation (DPI) while the remaining 2 HPAI exposed calves were necropsied at 13 DPI.

Testing milk for HPAI virus

Each day's pool of milk - that was fed to the 4 experimental calves from cows that had been infused with HPAI - was tested using an influenza A virus RT-qPCR. It was interesting that the control cows' milk was not tested; it was assumed to be free of the virus.

Table 1. Clinical Scoring of Dairy Calves. (Sarlo Davila et al. adapted their scoring from this table.)

	Points and Description			
Clinical Parameter	**0**	**1**	**2**	**3**
Nasal Discharge	Normal, serous discharge	Unilateral or cloudy discharge	Bilateral, cloudy or excessive mucus	Copious bilateral mucopurulent discharge
Ocular Discharge	Normal	Mild ocular discharge	Moderate bilateral ocular discharge	Heavy ocular discharge
Ear Position	Normal	Ear flicking	Slight unilateral ear drop	Severe head tilt or bilateral ear droop
Cough Score	Normal No cough	Induce single cough	Induce repeated coughs or occasional spontaneous cough	Repeated spontaneous coughing
Rectal Temperature	100-100.9	101-101.9	102-102.9	>= 103
Feces	Normal	Semi-formed pasty	Loose, stays on top of bedding	Watery, sifts through bedding
Navel	Normal	Slightly enlarged, not warm or painful	Slightly enlarged slight pain/moisture	Enlarged, pain, heat malodorous
Joints	Normal	Slight swelling, not warm or painful	Swelling with pain or heat, slight lameness	Swelling with severe pain, heat and lameness
Ultrasound (Lung) Score	0-1	2		3-5
	Normal	Lobular Pneumonia		3=1 lobe 4=2 lobes 5=3 or more lobes Lobar Pneumonia

From the University of Wisconsin Dairy Calf Health Scorer

Testing live animal and necropsy samples for HPAI virus

"Clinical ante-mortem (ocular, nasal, and rectal swabs, serum, and saliva) and necropsy samples were tested using an - - RT-qPCR kit . - - viral RNA was extracted from swabs, serum, and saliva using the MagMAX™-96 Viral RNA Isolation Kit." Extraction of RNA from tissues used the MagMAX™-96 for Microarrays Total RNA Isolation Kit. The RT-qPCR on extracted product used the VetMAX™-Gold SIV Detection kit. Cycle threshold (Ct) values less

than 35 (cycles) were defined as positive, Ct values 35-38 were defined as suspect, and Ct values greater than 38 were defined as negative.

Testing for antibody vs. HPAI/H5N1

A blocking ELISA detected antibodies to the nucleoprotein Influenza A (Influenza A Ab™) in serum. The cut-off sample to negative optical density ratio was ≤ 0.6. "Serum samples were also tested using a - - multi-species ELISA to detect antibodies against the hemagglutinin H5 (IDScreen® Influenza H5 Antibody Competition 3.0 Multi-Species)." The paper also described an H5 specific hemagglutination inhibition (HI) assay.

Statistical Analysis

There was a description of statistical analysis in the full paper. To me, with only 5 calves fed milk for 4 days, the major outcome of interest in this preliminary study was whether HPAI was detected in calves fed milk from infused cows, including whether that milk as well as the calves tested positive, and whether the control calf indeed remained negative for the virus. (Nonparametric biologically significant analysis.)

Results

Control calf consuming milk from non-inoculated cows: "- - did not develop any clinical signs or fever during the study - - All samples tested from the negative control calf were negative for [HPAI] by RT-qPCR." This lends some support to the authors' apparent assumption that the cattle at the National Animal Disease Center were free of HPAI except for those infused with it into the mammary glands.

4 calves consuming unpasteurized milk from experimentally HPAI inoculated cows: " - - mild lethargy and slightly increased respiratory effort were observed in all [4] beginning 1 DPI. By 2 DPI, loose stool and nasal discharge were observed in 3 of the 4 calves." Mild lethargy, loose stool, increased respiratory effort and nasal discharge were observed intermittently from 3 to 7 DPI; 2 of the calves were normal at 6 DPI. Rectal temperatures between 102.4° F and 105.1° F were observed from 2 to 6 DPI in all 4 calves; their mean temperature of 102.2° F was within the normal range.

Pooled [HPAI] "infected" milk fed to calves: Ct of 37.0 (suspect) on day 0 and 18.5, 24.7, and 29.0 (all strongly HPAI - positive) on 1, 2, and 3 DPI, respectively. There was no mention of the 4th day; apparently the milk of the cows infused intramammarily no longer tested positive on that final day when fed.

Nasal swab HPAI test results: 4/4 exposed calves pos at 2 and 3 DPI; 3/4 pos at 4 DPI; 2/4 pos at 5 DPI. **Ocular swabs:** All 4 suspect from 2 to 4 DPI, except 1 calf was pos at 3 DPI. **Saliva samples:** 1/4 pos at 1 DPI, 1/4 pos (a different calf) at 3 DPI. **Fecal and serum samples:** All remained HPAI-negative throughout.

The paper includes a lot of **necropsy findings**, including supplemental data provided. Major results were: both HPAI-exposed calves necropsied at 6 DPI had obstructive atelectasis in multiple lung lobes, lymphadenopathy of retropharyngeal and mandibular lymph nodes, and 1 calf had hemorrhage in a mesenteric lymph node. **Histological lesions:** bronchiolitis, luminal cellular debris in bronchioles, atelectasis, perivascular lymphocytic infiltrates, erosion of ciliated epithelium of pharyngeal tonsil in both calves. **HPAI antigen:** Detected by IHC in retropharyngeal, mandibular, parotid, and tracheobronchial lymph nodes of both calves euthanized at 6 DPI, and in a single bronchiolar lumen in 1 calf. The 2 remaining exposed calves euthanized at 13 DPI included 1 with multifocal atelectasis.

Serology: All 5 calves were seronegative for antibody through 6 DPI. The 2 remaining exposed calves were HPAI antibody seropositive by all serological test methods on both antibody testing days, 10 DPI and 13 DPI.

5600 Old Main Hill
Logan UT 84322-5600

Discussion/Conclusion

The authors stated, “This manuscript describes the first model of experimental infection of calves fed raw milk from cows inoculated with [HPAI/H5N1] genotype B3.13 strain. Seroconversion confirms infection from this route of transmission. Calves showed clinical signs including nasal discharge, mild fever, mild lethargy, loose stool and slightly increased respiratory effort for 5-6 days. - - The clinical signs observed in calves including nasal discharge and loose stool are common in pre-weaned dairy calves and could easily be [misdiagnosed]. - - Milk diverted from the human food supply in H5N1 positive dairy herds or from suspect cows should not be fed to calves without pasteurization.”

Save the Date - August 19, 2025 - Dairy Extension Conference, Logan, UT

There will be a dairy extension conference on **Tuesday August 19, 2025** at the Extension Building at the Cache County Fairgrounds in Logan, UT (there is no charge to attend, including for the lunch). More details will be forthcoming, but the subjects covered will include observations from numerous dairy herds with HPAI in California, new dairy technology, and cutting edge information regarding dairy cattle nutrition. The conference speakers include multiple new USU Extension faculty and at least one speaker from industry. These speakers are bringing in some exciting new information from their own research and expertise. We plan to have RACE credits for veterinarians; this should be confirmed soon along with the complete program details. We hope to see you there; save the date.

Please let us know your comments and suggestions for future topics. I can be reached at (435) 760-3731 (Cell), or David.Wilson@usu.edu.

David Wilson, DVM, Dairy Extension Veterinarian