USDA Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

Technical Brief: Estimating the Probability of Highly Pathogenic Avian Influenza Detection in Bulk Tank Milk at Various Sampling Times Using Disease Event Surveillance Data

Summary

Early detection of highly pathogenic avian influenza (HPAI) in dairy cattle herds is a key surveillance goal and is critical for an effective disease response. Testing aggregate bulk tank milk (BTM) samples using real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) is proven to be an efficient method to detect HPAI; however, as disease progresses within a dairy herd, HPAI virus concentration changes in BTM. This causes HPAI detection probability (test sensitivity) to vary over time. Thus, time-dependent modeling of test sensitivity is critical for surveillance design.

Analysts from the Animal and Plant Health Inspection Service's (APHIS) Center for Epidemiology and Animal Health (CEAH) fit regression models to California and Colorado BTM surveillance data to estimate the probability of detecting HPAI in a dairy herd at various sampling times relative to the onset of clinical signs. This report presents these regression models and their use in evaluating BTM-based HPAI surveillance schemes. CEAH produced these results and modeling approaches to help inform the National Milk Testing Strategy (NMTS) and provide BTM sampling recommendations to industry practitioners, State Animal Health Officials, and other stakeholders.

The results are as follows:

- Based on the California-fitted model for the early phase of within-herd transmission, HPAI is likely detected at a herd level via BTM samples prior to onset of clinical signs, with an average detection probability of 0.69 in the week before the onset of clinical signs.
- For the Colorado-fitted model in the later phase of herd transmission, the average HPAI detection probability via BTM samples declines over time as herds recover, albeit slowly. For example, analysts estimated average detection probabilities of 0.975, 0.94, and 0.86 between 0 to 14, 14 to 28, and 28 to 42 days post-onset of clinical signs, respectively.
- The modeling approaches and regression fits presented here are useful to develop State-level surveillance schemes based on BTM sampling and are beneficial to support further stakeholder technical discussions regarding NMTS with APHIS.
- Based on the available California data, the rRT-PCR cycle times (CT values) in the hospital tanks were in a comparable range to the minimum CT value among non-hospital tanks tested on the same day. These data indicate that sampling hospital tank milk may not increase detection substantially over testing all the non-hospital bulk tanks during the early phase of HPAI infection in a herd.
- The mean duration of BTM HPAI virus positivity was 48 days in a herd based on the Colorado dataset used at the time of this analysis, assuming a CT cutoff of 40; however, there was high variability with 8 out of 62 dairies having positive detections lasting more than 75 days.

Background

Early detection of HPAI in dairy cattle herds is a key component of HPAI surveillance and can lead to a more effective response. Testing individual dairy animals to determine HPAI virus exposure of herds is time and cost inefficient, particularly in the early phases of infection, when few cows are infected. In contrast, testing aggregate BTM samples using rRT-PCR has proven effective for detecting virus in dairy cattle herds. However, the probability of detecting infection in a herd is not able to be calculated using a constant value because test sensitivity in BTM depends on virus concentration, which varies over time due to changes in the number of animals infected and the stage of infection in these animals. Time-dependent modeling is needed to estimate BTM detection probabilities, which are critical for surveillance design. CEAH analysts used the BTM sampling data and regression model fits to estimate the probability of detecting HPAI virus in a dairy herd at various sampling times relative to the onset of clinical signs.

These regression models relied on PCR testing data from surveillance on CT values in BTM from affected herds in California and Colorado. The nature of the two data sets differed based on the different sampling timeframes, which worked well for the modeling. The California surveillance data were best suited and used to inform the detection probabilities in the early phase of herd-level infection when detection probabilities increase over progressive days. The Colorado data were used for regressions to estimate detection probabilities in later infection stages of herds.

The herd-level detection probabilities from these regression models were combined with factors, such as the probability that an infected herd was sampled and the timing of sampling relative to onset of clinical signs, to evaluate various surveillance schemes considered in the development of NMTS.

Methods

Both California and Colorado BTM data included bulk tank and hospital tank rRT-PCR CT values (influenza A matrix gene) on different sampling days and the corresponding dates of onset of clinical signs. These data included sampling dates both prior to and following the onset of clinical signs for several premises. At the time of this analysis, the dataset from Colorado primarily covered the sampling timeframe post-onset of clinical signs, while the dataset from California was used for sampling prior to the onset of clinical signs. The California dataset included a total of 95 dairies that tested positive at least one time and a total of 630 tests across 291 unique farm-days between 26 August 2024 and 20 November 2024. Here, a farm-day refers to a dataset row with a unique combination of the dairy farm and sampling day when at least one of the non-hospital bulk tanks was tested. The Colorado dataset had a total of 62 dairies that tested positive at least one time and a total of 1,280 tests across 859 unique farm-days between 23 April 2024 and 17 October 2024.

Bulk Tank Milk Regression Models

Logistic regression models were fit to each dataset with the time since onset of clinical signs as the independent variable and detection in at least one of the non-hospital bulk tanks as the dependent variable. The model was fit to farm-by-day sampling, with all BTM non-detections equaling 0, and at least one BTM detection equaling 1. In the application of the models for surveillance evaluation, the early phase California regression fit was used for days 0 to 14 prior to symptom onset (assuming that a herd is infected through the duration of this interval) and the Colorado regression model from the day of symptom onset onward. For the California regression, it was assumed that for the period prior to the onset of clinical signs, negative tests on all non-hospital bulk tanks represented a failed detection. For the Colorado regression, it was assumed that any negative tests preceding

the final positive test (i.e., a negative between two positives) was a failed detection and that infection was cleared by the date that all future samples were negative.

The logistic regression estimates for the early herd infection phase based on California data are shown in Table 1, and a plot of model predictions relative to onset of clinical signs is shown in Figure 1. Regression estimates for the later phase based on Colorado data are shown in Table 2 and Figure 2. The McFadden's pseudo R^2 for the California model is 0.54, indicating a strong fit. The pseudo R^2 for the Colorado model was lower at 0.21, indicating less variance explained (the raw data show moderately high variation across time among farms). However, despite much variation in the data, model predictions (Figure 2) do track well with the central tendency of the data. Further, given the focus on detection in the early phases of dairy-level infection, the California model is relied upon somewhat more than the Colorado regression.

For the California-fitted model, the average detection probability was 0.16, 0.69, and 0.97 between 14 to 7 days prior to clinical signs onset, 7 to 0 days prior to clinical signs onset, and the week following clinical signs onset, respectively. For the Colorado-fitted model, the average detection probability was 0.975, 0.94, and 0.86 between 0 to 14, 14 to 28, and 28 to 42 days post-onset of clinical signs, respectively.

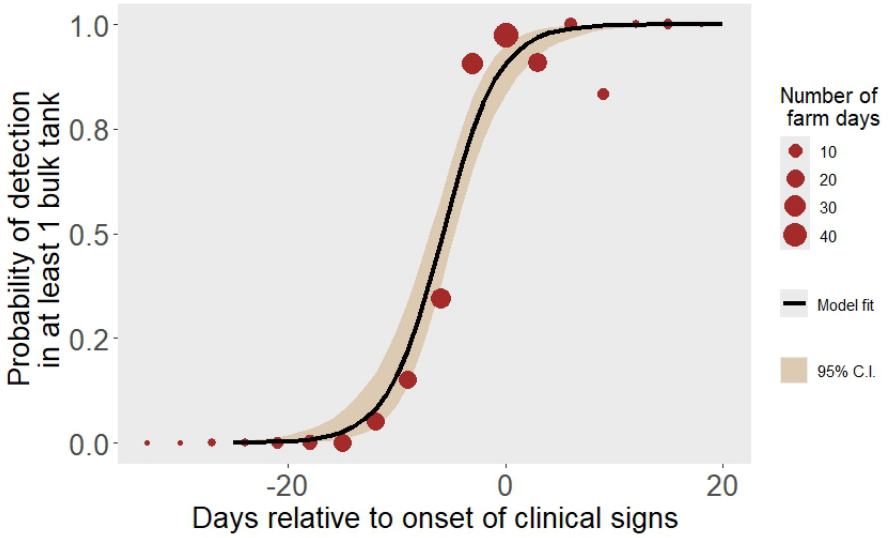

Figure 1. Logistic regression fit and data for early phase of herd HPAI infection based on the California event. Points show the proportion of all unique farm-by-day samples (the total of which is represented by sample size) that were positive over 3-day time periods. Data is binned here for visual clarity. The sample size represents the number of unique farm days sampled and lines and envelopes show logistic regression model fits (mean and 95% confidence interval [CI]).

Table 1. Logistic regression fit details for early herd infection phase based on California data.

	Estimated	Standard error	P-value
Intercept	2.27	0.34	<0.001
Days post-onset of clinical signs	0.39	0.05	<0.001

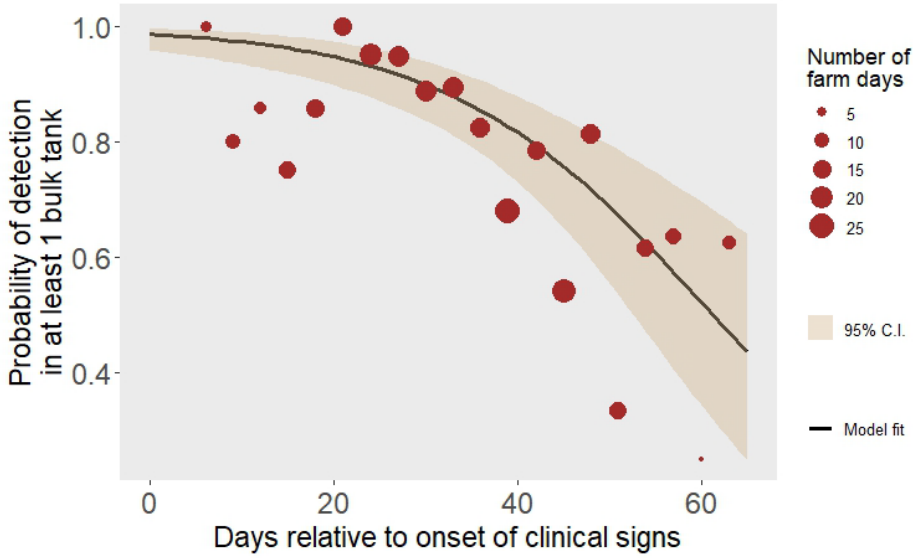

Figure 2. Logistic regression fit and data for later phase of herd HPAI infection based on Colorado BTM data. Points show summarized raw data (binned into 3-day time periods) and lines and envelopes show logistic regression model fits (mean and 95% CI).

Table 2. Logistic regression fit details for later herd infection phase based on Colorado data.

	Estimated	Standard error	P-value
Intercept	4.29	0.31	<0.001
Days post-onset of clinical signs	-0.07	0.008	<0.001

Modeling HPAI Detection at Potential Additional Dilutions of Bulk Tank Milk

An rRT-PCR standard curve provided by the Agricultural Research Service's Southeast Poultry Research Laboratory (ARS–SEPRL; Figure 3) was used in dilution calculations to predict CT values at additional dilutions of BTM (e.g., tankers or silos). The standard curve was based on experimental CT value data from milk spiked with a poultry origin A/tk/IN/3707/2022 clade 2.3.4.4b virus at various dilutions.

Using this PCR standard curve, BTM CT values can be adjusted to estimate what a given BTM CT value would be if that BTM were diluted into a tanker or silo. By applying this adjustment to California BTM values, logistic regressions can be used to estimate detection probability over time assuming a given dilution factor. Here, a CT value above 40 was assumed to be negative. The logistic regression model fits for HPAI detection with dilution factors of 50 and 400 relative to BTM are shown in Figure 4 and Figure 5 for California and Colorado data, respectively. Analysts chose 50-fold and 400-fold dilutions to reflect a realistic range of possible BTM-to-silo milk dilutions. For example, for a 50-fold dilution, an 800-gallon bulk tank into a 40,000-gallon silo, and for a 400-fold dilution, a 2,000-gallon bulk tank into an 800,000-gallon silo.

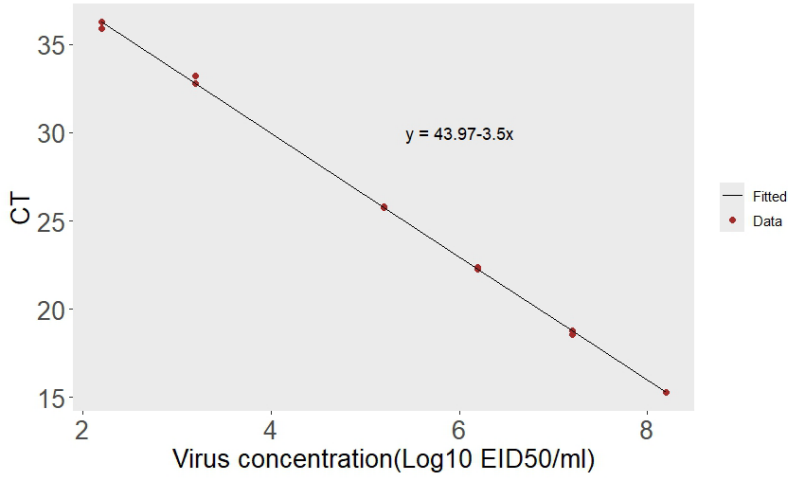

Figure 3. rRT-PCR standard curve for (poultry-origin HPAI clade 2.3.4.4b virus RNA from egg yolk) spiked in milk samples provided by USDA–ARS–SEPRL.

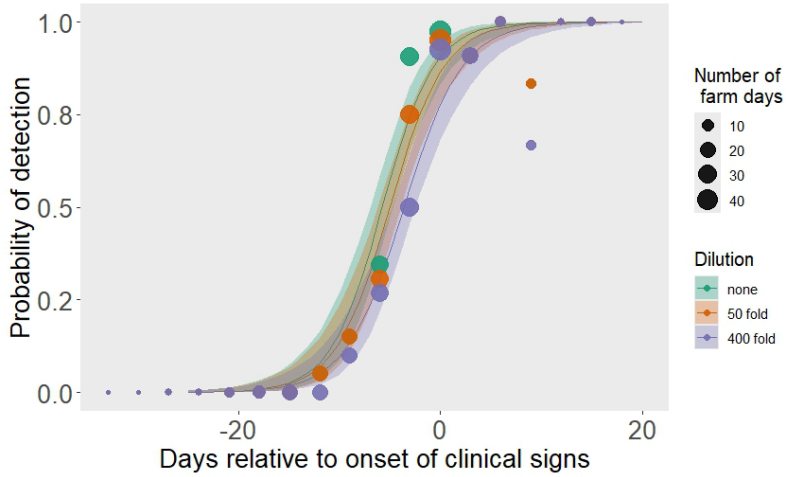

Figure 4. Regression fit to California BTM data at three different dilutions. Points show summarized raw data (binned into 3-day time periods) and lines and envelopes show logistic regression model fits (mean and 95% CI).

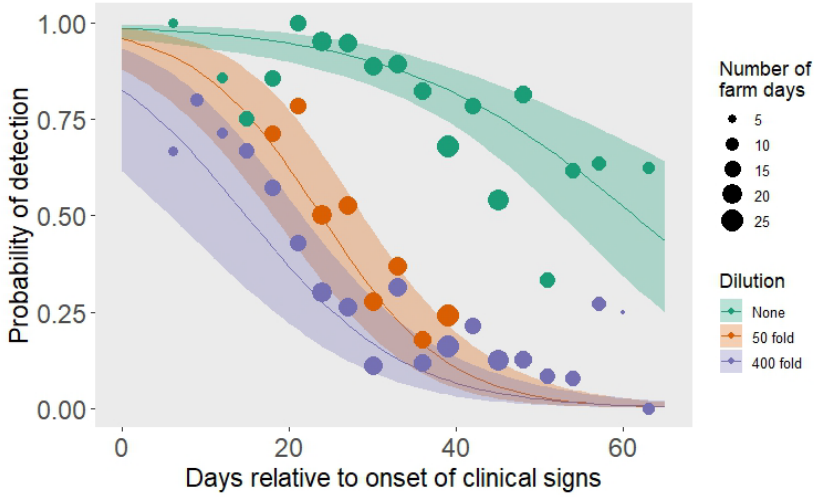

Figure 5. Regression fit to Colorado BTM data at two different dilutions. Points show summarized raw data (binned into 3-day time periods) and lines and envelopes show logistic regression model fits (mean and 95% CI).

Comparison of the CT Values in Hospital verses Non-Hospital Bulk Tanks

Available data from California farms, including hospital and bulk tanks, were tested to compare detection across these tank types. The difference between the hospital tank CT value and the minimum BTM CT value from the same sampling day on a farm was not significant (mean 0.29; 95 percent probability interval from -5.4 to 7.4) as shown in Figure 6. In addition, in 90 percent of cases where the hospital tank was positive, HPAI was also detected in one of the regular bulk tanks on the same sampling day. The small percentage of cases (10 percent) where a hospital tank was positive and other bulk tanks were negative all occurred in later herd infection stages more than 20 days post-exposure. Overall, these California data indicate that the inclusion of hospital tanks may not increase detection substantially over testing all the non-hospital bulk tanks during the early phase of HPAI infection in a herd.

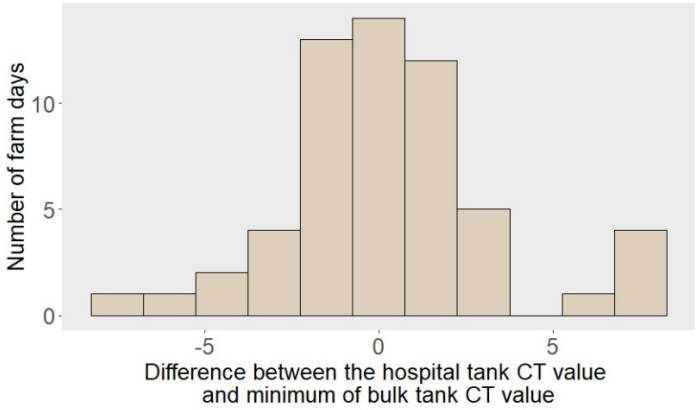

Figure 6. Difference between hospital tank and minimum of BTM CT values on days when the hospital tank tested positive, based on California event data.

Estimate of the Duration of HPAI Detection BTM on an Infected Premises Based on Colorado Data

Colorado data were used to estimate the duration of BTM HPAI virus positivity from the date of clinical signs onset. The mean duration of BTM HPAI virus positivity in Colorado was 48 days, assuming a CT cutoff of 40 (Figure 7). However, variation among dairies was high, with a standard deviation of 20 days and 8 out of 62 dairies having positive detections lasting greater than 75 days at the time of this analysis.

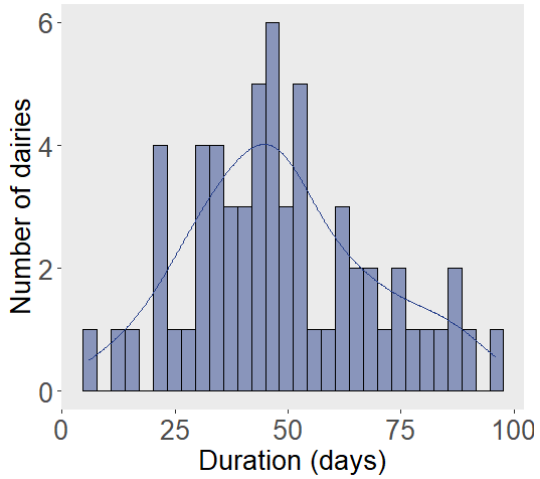

Figure 7. Duration of positivity of all Colorado dairies using a CT value cutoff of 40. Bars for each distribution start at zero (i.e., are not stacked). Density curves show smoothed distributions.

Variation of Bulk Tank Milk HPAI CT Values Over Various Days Relative to Symptom Onset

Overall, there was a substantial variability in the BTM CT values among different farms. Figure 8 shows the raw BTM CT values over various days post-onset of clinical signs from the California data. These data indicate a trend of decreasing CT values prior to onset of clinical signs and gradually increasing CT values from approximately 1-week post-onset of clinical signs onward as the herds recover.

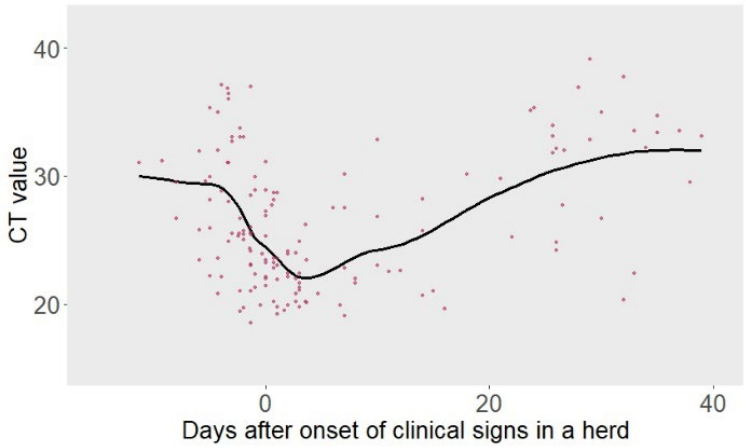

Figure 8. BTM rRT-PCR CT values on various days post-onset of clinical signs based on the California data with a locally estimated scatter plot smoothing (LOESS)-smoothed curve.

Use of Regression Models in Surveillance Design

An important feature of these regressions is that they facilitate calculating the probability that infection would be missed by BTM sampling if HPAI were present in a herd, conditional on sampling design. In particular, the regressions enable consideration of the time dependent sensitivity of BTM sampling and underscore the importance of serial sampling to enhance HPAI detection. As an example, the probability of failing to detect the presence of infection in a State when one or more herds are infected can be estimated for *n* serial samples when *m* dairies are infected and *y*% of all dairies are sampled in each sampling round, conditional on two assumptions: 1) when does sampling begin on infected farms relative to symptom onset, and 2) how long infection lasts. In brief, calculating the probability that no herd-level infections are detected after *n* serial samples involves the multiplication of 1 minus the per-dairy detection probabilities as given by Figure 1 and Figure 2 (i.e., the probability of missing each infection). To address the first assumption, a variety of scenarios can be evaluated and summarized to build an uncertainty interval on detection probability. In scenarios without any disease yet reported, it is realistic (and conservative) to assume the sampling begins one to two weeks in advance of symptom onset. For the second required assumption, infection duration can be sampled from Figure 7. The rRT-PCR standard curve and regression approaches can also be used to predict HPAI detection at additional dilutions of BTM such as a tanker or a silo. The regressions and modeling approaches presented here were used to evaluate the efficacy of alternative surveillance designs to help inform the NMTS and provide BTM sampling recommendations to industry practitioners, State Animal Health Officials, and other stakeholders.