

Evaluation of surveillance and sample collection methods to document freedom from infectious bovine rhinotracheitis in cattle populations

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Objectives—To assess the sensitivity of the current surveillance program used in Denmark for detecting outbreaks of infectious bovine rhinotracheitis (IBR) at the herd level and to evaluate the impact of alternative sample collection strategies on the sensitivity of the system in an acceptable time frame.

Sample Population—Data from the Danish Central Husbandry Register on cattle of 24,355 and 25,233 beef herds and on 13,034 and 12,003 dairy herds in the years 2000 and 2001, respectively.

Procedures—Surveillance programs were evaluated under current sample collection conditions and under 3 alternative scenarios by use of simulation modeling. Data from the current detection component of the surveillance system were used as input, taking into consideration the sensitivity and specificity of bulk-tank milk and serologic testing.

Results—The current system identifies infected dairy herds within a 3-month period with desired accuracy largely because of the test characteristics and number of bulk-tank milk samples. The system is less likely to detect infected beef herds in a timely manner because surveillance in beef herds depends solely on serologic testing at the time of slaughter. The efficiency of surveillance in dairy cattle herds was not decreased substantially when the slaughter-surveillance component was omitted.

Conclusions and Clinical Relevance—Geographically targeted sample collection during the high-risk season (winter) was predicted to increase the probability of rapid detection of IBR infection in cattle. This approach can be used for assessing other surveillance systems to determine the best strategies for detection of infected herds. (*Am J Vet Res* 2005;66:2149–2153)

Most international standards for sanitary measures for importation of animals and animal products depend on the disease status of the importing and exporting countries. Countries that wish to use measures that result in a higher degree of protection than would be achieved by measures based on relevant

international standards, guidelines, or recommendations must provide a scientific justification. The documentation of freedom from infection is usually a prerequisite for such measures. Requirements for a country to establish freedom from infection to satisfy the sanitary and phytosanitary agreements are outlined in the World Organization for Animal Health guidelines.¹ Typically, these requirements are based on the information contained in annual surveillance efforts. Definitive proof of the absence of infection or of the agent is virtually impossible to obtain. Therefore, proof is provided in terms of a science-based probability estimate that the infection of concern, if present, is below a specified acceptable prevalence.

There are 2 fundamental objectives for the detection component of a surveillance system for exotic diseases: early detection of newly introduced infection and documentation of freedom from infection.² Surveillance systems need to be based on epidemiologic characteristics of the disease agent, economic implications regarding trade and domestic production, herd demographics (size, breed, and age distribution), prevalence (between and within herds), test characteristics (sensitivity and specificity), and the clinical disease picture, but they must meet the fundamental objectives. For short-incubation contagious diseases, this may mean intensifying surveillance to allow for timely recognition of infection before the problem becomes widespread.

Denmark was declared free of infectious bovine rhinotracheitis (IBR) in 1991. Since 1991, there have been 6 introductions of IBR into Denmark, with the most notable occurring in 1995. In Denmark, a serologic surveillance program is used to screen for IBR.³ The Danish IBR surveillance program was designed 20 years ago, when cattle demographics were different. The number of cattle herds has declined by 60% since 1982 to 23,031 in 2000, and the number of cattle has declined by 30% to 1.8 million in 2000.⁴ The mean dairy herd size is now approximately 70 cows, an increase of 250%.⁴ These demographic changes could substantially alter the probability of detecting an infected herd. For example, detection of infection in beef herds depends solely on slaughter surveillance. Small beef herds may irregularly send cattle to slaughter, making it difficult to detect disease in a timely manner. Larger beef herds might send cattle to slaughter more regularly, which would increase the probability of detecting an infected herd. Despite these demographic changes, evaluation of the national IBR surveillance program has not been performed since implementation in 1991.

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The Danish Central Husbandry Register (CHR)⁵ contains farm-specific information including a unique identification number, type of farm (dairy or beef), mean size of the farm, mean number of adult cows, and mean number of total cattle. Mean values are always being updated as new information is being added to the CHR. Similarly, the register contains information on each individual animal in the farm, such as a unique animal identification number, date of birth, farm of origin identification number, and date of entry to the farm. In addition, date and reason of departure (eg, breeding, slaughter, export, or death) are recorded.

The national surveillance program³ for IBR is based on 4 quarterly samples of bulk-tank milk taken from all dairy herds and blood sample collection from slaughter cattle. Bulk-tank milk samples are collected for the milk quality-control scheme. These samples are identified by the CHR number and used for the surveillance program. The test is a bovine herpesvirus type 1 blocking ELISA with a cutoff value of < 30% for bovine herpesvirus type 1-free status. Nylin et al⁶ estimated herd-level sensitivity (HSe) to be 82% and herd-level specificity to be > 99.9% for bulk-tank milk testing of dairy herds in Denmark. The animal-level sensitivity for the serum samples of cattle at slaughter was assumed to be 99% and the specificity > 99.99% on the basis of previous testing in cattle.⁶

An intensive voluntary testing program is in effect in the region close to the German border (high-risk area for infections), where dairy herds are monitored on a monthly basis by testing bulk-tank milk samples during the winter. If a bulk-tank milk sample result is close to the cutoff value of 30% on the blocking ELISA, the veterinarian will obtain blood samples from 10% of the herd or from at least 10 cattle for clarification of the herd status. Information on individual animal and herd tests for IBR is stored in the CHR register.

Current legislative rules dictate that export-authorized slaughterhouses for cattle must sample blood from every sixth animal that is > 8 months old and domestic slaughterhouses for cattle must sample blood from all slaughtered cattle. If a blood sample result is close to the cutoff value of 30% on the blocking ELISA, the sample will be submitted to the national reference laboratory (Danish Institute for Food and Veterinary Research) for reexamination. The purposes of the study reported here were to assess the sensitivity of the current Danish surveillance system for detecting outbreaks of IBR at the herd level and to evaluate the impact of alternative sample collection strategies on the sensitivity of the system in acceptable time frames.

Materials and Methods

Surveillance assessment and alternatives—Data on 24,355 and 25,233 active beef herds existed in the CHR in 2000 and 2001, respectively. Data on 13,034 and 12,003 active dairy herds also existed in the CHR in 2000 and 2001, respectively. Data from the mandatory CHR⁵ were used in surveillance assessment (ie, current surveillance), and 3 alternative scenarios were developed for surveillance assessment. The 3 alternative scenarios were as follows: 1) slaughter surveillance, assuming current legislative rules are enforced completely (ie, legislative surveillance); 2) slaughter surveillance, assuming current legislative rules for beef

herds but removing the requirement for slaughter surveillance of dairy cattle and relying solely on bulk-tank milk sample collection (ie, beef cattle and bulk-tank milk testing surveillance); and 3) intensification of surveillance in winter of targeted beef cattle designated for slaughter at export-licensed abattoirs in the high-risk zone (near the German border), as determined by the longitude of 10.06° E and latitude of < 55.34° N (ie, risk-based surveillance). This zone is designated as high risk because of historical outbreaks in the region and its proximity to Germany. In the 2 German counties closest to the Danish border, a third of the herds are free of IBR and the remaining have vaccinated all or some of the cattle. These 2 counties detected 28 and 10 infected herds in 2002, respectively. Winter, the high-risk season was defined as the period from the end of October (ie, week 44/52) to the end of March (ie, week 12/52). During winter in the high-risk zone, blood sample collection increased from every sixth animal to either 1 of every 2 slaughtered cattle from small herds (≤ 30 cattle) or 1 of every 3 slaughtered cattle in large herds (> 30 cattle). Blood sample collection during the other weeks of the year (ie, low-risk season) and in the low-risk zone (rest of the country) was performed in 1 of every 10 slaughtered cattle. Cattle slaughtered in domestic abattoirs were all tested regardless of the high-risk season or their zone locations.

Test data for alternative surveillance programs—The IBR slaughter-surveillance testing data expected under each of the programs were created by applying the conditions of each of the 3 scenarios to the original, complete animal-level data set from the CHR. For example, to create the animal-level data for the legislative surveillance program, all cattle > 8 months old were selected from the original data set. Records for cattle slaughtered in the export facilities and those slaughtered in domestic facilities were separated into 2 data sets. Cattle from the domestic facilities' data set were automatically considered to be IBR-tested, which is consistent with current regulations. The surveyselect procedure⁷ was used to select 1 in 6 cattle randomly from each slaughterhouse during each week within the years of interest. The cattle selected by the procedure were considered to be IBR-tested. Once the tested cattle were identified, they were merged back into the original data set with the notation of being tested. The remaining cattle in the original data set that were not identified as tested were placed in the nontested group.

Data for the remaining beef cattle bulk-tank milk and risk-based surveillance programs were created in a similar manner but with modifications to address the conditions for each scenario. For example, in the risk-based surveillance program, the procedure selected 1 of 2 and 1 of 3 slaughtered cattle in small and large herds, respectively, in the high-risk zone during the winter weeks. The procedure was used to select 1 of 10 slaughtered cattle in the high-risk zone in nonwinter weeks and in the rest of the country regardless of season.

Evaluation of surveillance programs—The simulated animal-level data were used as input into the second part of the model in which we simulated the probability of detecting a single infected herd in varying periods. We considered this probability to be the sensitivity of the surveillance system. The program estimated the sensitivity for slaughter and bulk-tank milk testing for each beef or dairy herd over a 4-, 8-, and 12-week period.

The first step in this process was to summarize, for each herd, the total number of cattle slaughtered each week (during the 104 weeks in 2000 and 2001) by slaughterhouse type and determine whether the animal was IBR-tested (on the basis of the random selection procedure from the first por-

tion of the model). The weekly summary was then transposed into a vector of 104 variables representing each of the weeks. The number of tested and nontested cattle in export and domestic slaughter facilities was tracked separately. Bulk-tank milk data were merged with the slaughter data to incorporate the weeks in which bulk-tank milk samples were tested for each dairy herd.

The next step in the second part of the simulation was to summarize the number of IBR slaughter and bulk-tank milk tests taken in specified periods (4, 8, and 12 weeks). A moving window of the number of slaughter and bulk-tank milk tests was created for the relevant period. For example, for the 4-week period, the moving window calculations were continued until the 101st time window, which included the last week of 2001, and were then summarized.

The testing information from each time window was used to compute the sensitivity for each herd as if it were the infected herd. The HSe of the bulk-tank milk testing protocol (ie, HSe_{Dairy}) was calculated for each window by use of the following formula⁸:

$$HSe_{Dairy} = 1 - (1 - 0.82)^t$$

where t is the actual number of bulk-tank milk tests taken during the window and 0.82 is the HSe of the blocking ELISA.

Information from domestic and export slaughter-surveillance records was used to compute the slaughter sensitivity for beef herds. The within-herd prevalence for IBR-infected herds was assumed to be 80% on the basis of previous reinfections detected in Denmark.⁹ The HSe was calculated for each herd that slaughtered at least 1 animal. For each animal tested in a herd, a probability distribution (uniform) was used to decide whether it was infected. If the randomly selected number was ≤ 0.8, the tested animal was considered to be infected. The total number of infected beef cattle that were tested in each herd was calculated and used to estimate the HSe for the time window of interest⁸ (ie, HSe_{Beef}) by use of the following formula:

$$HSe_{Beef} = 1 - (1 - 0.99)^t$$

where t = the number of IBR tests on infected cattle and 0.99 is the animal-level sensitivity of the blocking ELISA.

The sensitivity of each time window for the combination of the testing types was computed for each herd as follows:

$$HSe = 1 - [(1 - HSe_{Beef}) \times (1 - HSe_{Dairy})]$$

The mean sensitivity for each time window was calculated for beef and dairy herds. The total number of herds that contributed to the mean in each time window was deter-

mined. The probability of detecting a single infected herd in each time window, or the survey-level sensitivity, was calculated by multiplying the window sensitivity times the probability of having selected a single infected herd in the respective time window. The likelihood of selecting a single infected herd (reintroduction of IBR) was calculated by use of the hypergeometric distribution as follows¹⁰:

$$\text{Probability} = \frac{\left(\frac{1}{1}\right) \times \left(\frac{M-1}{n-1}\right)}{\left(\frac{M}{n}\right)} = \frac{n}{M}$$

where n is the number of herds sampled and M is the total number of herds.

The probability of detecting a single infected herd in time windows of 8 and 12 weeks was determined in a similar manner. The only change was the number of weeks that was summarized to create the respective windows. Because 8 weeks was used to create a time window, the first 8 weeks of 2000 were in the first window and only 97 windows were created. Similarly, with a 12-week window, only 93 windows were created in the 2-year period.

The beef cattle and bulk-tank milk testing and risk-based surveillance programs were evaluated by use of the same program as described for the legislative surveillance program. Only the input animal file (created as already described) was changed to reflect the different sample collection strategy. For the risk-based surveillance program, probabilities were separately calculated for the entire country and the high-risk region.

Results

Summarization of data of the current surveillance program revealed that only 10.2% (in 2000) and 13.0% (in 2001) of slaughtered cattle > 8 months of age had been tested, which was below the mandated number (1/6 [16.7%]). The probability of detecting a single infected herd (either a dairy or beef herd) in each window of time was determined (Table 1) to evaluate the impact of the current and alternative surveillance programs.

The probability of detecting a single infected herd if the disease was present for 4-, 8-, and 12-week intervals under the current surveillance program was determined (Figure 1). Bulk-tank milk testing reaches a minimum probability of detection for dairy herds of 0.18 in certain 4-week intervals mainly as a result of summer and winter holidays. The maximum probability of detection of infection for bulk-tank milk testing in dairy herds of 0.92 was observed in a 12-week inter-

Table 1—The probability of (X 100) detecting a single possibly IBR-infected herd in Denmark during different time frames by use of various sample collection strategies in surveillance programs.

Surveillance programs*	Dairy herds									Beef herds								
	4 weeks			8 weeks			12 weeks			4 weeks			8 weeks			12 weeks		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Current	0.43	0.18	0.65	0.70	0.44	0.86	0.86	0.69	0.92	0.07	0.04	0.11	0.13	0.09	0.18	0.18	0.14	0.22
Legislative	0.53	0.32	0.72	0.79	0.61	0.91	0.92	0.82	0.95	0.10	0.06	0.13	0.17	0.14	0.22	0.24	0.20	0.28
Beef cattle and bulk-tank milk testing	0.26	0.00	0.53	0.52	0.21	0.81	0.74	0.50	0.83	0.10	0.06	0.13	0.17	0.14	0.22	0.24	0.20	0.28
Risk-based (nationwide)	0.26	0.00	0.53	0.52	0.21	0.81	0.74	0.50	0.83	0.09	0.05	0.15	0.16	0.10	0.23	0.21	0.15	0.28
Risk-based (high-risk area)	0.34	0.00	0.78	0.62	0.16	0.89	0.81	0.27	0.92	0.14	0.06	0.29	0.25	0.13	0.47	0.33	0.18	0.56

* See Materials and Methods section for description of programs.
Min = Minimum. Max = Maximum.

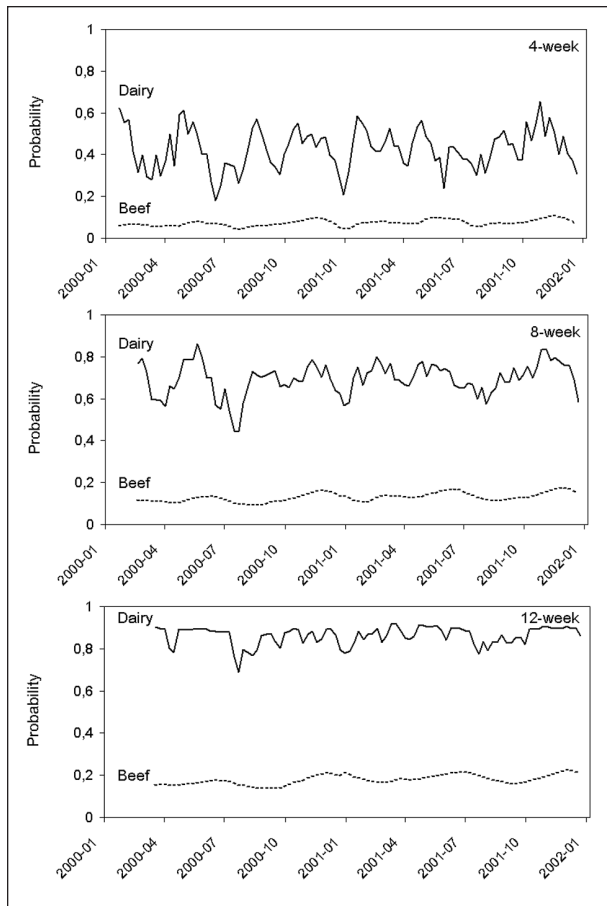


Figure 1—The 4-, 8-, or 12-week probability versus time (year/month) for detection of an IBR-infected herd by use of the current surveillance program.

val. Detection of infection based on testing at the time of slaughter had a minimum probability for detection in beef herds of 0.04 in a 4-week interval and a maximum of 0.22 in a 12-week interval.

The probability of detecting a single infected herd if the disease was present for 4-, 8-, and 12-week intervals by use of the legislative surveillance program revealed that bulk-tank milk testing reaches a minimum probability of detection in dairy herds of 0.32 in a 4-week interval. A maximum probability for bulk-tank milk testing of 0.95 was reached in a 12-week interval. Detection of infection based on testing at the time of slaughter had a minimum probability of detection for beef herds of 0.06 in a 4-week interval and a maximum of 0.28 in a 12-week interval.

The probability of detecting an infected herd by use of the beef cattle and bulk-tank milk testing surveillance programs revealed that bulk-tank milk testing reached a minimum probability of detection in dairy herds of 0 in a 4-week interval during the summer holiday in which no laboratory testing was performed. A maximum probability in a 12-week period for bulk-tank milk testing was 0.83. Detection of infection in beef herds based on testing at the time of slaughter had a minimum probability of 0.06 in a 4-week interval and a maximum of 0.28 in a 12-week interval.

The probability of detecting a single infected dairy herd by use of the beef cattle-bulk-tank milk surveil-

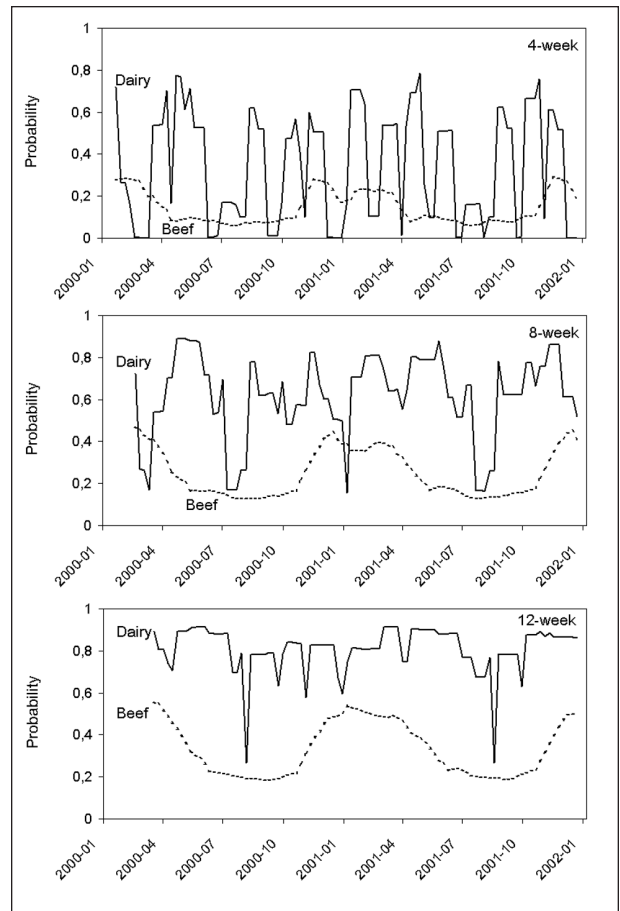


Figure 2—The 4-, 8-, or 12-week probability versus time (year/month) for detection of an IBR-infected herd by use of the targeted risk-based surveillance program in the region close to the German border (high-risk area).

lance program and the risk-based surveillance program (on a nationwide basis) resulted in the same minimum (0) and maximum probabilities (0.83). This similarity occurred despite the removal of the requirement for slaughter surveillance of dairy cattle. Detection of infection in beef herds by use of the risk-based surveillance program (nationwide) had a minimum probability of 0.05 in a 4-week interval and a maximum of 0.28 in a 12-week interval. Although the probabilities for detection of infection based on testing at the time of slaughter are similar to those obtained by use of the legislative surveillance program, these probabilities varied substantially when only the high-risk area was considered (Figure 2). The minimum probability for detection of an infected dairy herd by use of bulk-tank milk testing in the risk-based surveillance program (high-risk region only) was 0 for a 4-week interval, and the maximum probability was 0.92 for a 12-week interval. Detection of infection in beef herds based on testing at the time of slaughter in the high-risk area varied depending on the season. The probability ranges from 0.06 to 0.29 for a 4-week interval and 0.18 to 0.56 for a 12-week interval. The probability of detection in beef herds was determined to be highest in the high-risk area by use of the risk-based surveillance program, compared with other programs including the current surveillance program.

Discussion

Danish legislation requires that every sixth slaughtered animal that is > 8 months of age must be tested for serum antibodies against IBR if cattle are slaughtered at export-licensed slaughterhouses. However, findings of our study revealed that the proportion of slaughtered cattle being tested was substantially lower. A few herds listed as dairies in the CHR database are not bulk-tank milk tested, and we assume this may be the result of errors in the database caused by herds retaining registration as dairy herds; but in fact, they have either shifted to beef production or closed down milk production. Farmers must report changes in the actual production type to the CHR register on an annual basis, but no official verification process exists.

Our analyses assumed a within-herd prevalence of 80%. Others¹¹ have reported low probabilities for detecting infections in herds with low within-herd prevalences. The outbreak of IBR in Denmark in 1995 had a high within-herd prevalence (> 80%) 4 to 6 weeks after reinfection.⁹ The effect of using the high within-herd prevalence in our simulations is an increase in the HSe. The current surveillance program, even under this within-herd prevalence assumption, is not performing well for national surveillance needs.

The sensitivity of the bulk-tank milk testing is affected by within-herd prevalence and lactation stage.⁶ The sensitivity is decreased when the within-herd prevalence is low (< 10%) or the amount of IgG excreted in milk is low (depending on the lactation stage). However, an intensive and repeated sample collection scheme will, over time, reduce misclassification of false-negative herds resulting from within-herd spread of the disease. In our analyses, sequential bulk-tank milk tests from the same herd were assumed to be independent. Because different cows contribute to the bulk-tank milk and infections can occur in the period between testings, a false-negative herd may not only be a result of poor sensitivity of the test but may also be a result of a true infection that developed in the time interval between sample collections.

Detecting a single infected herd as quickly as possible to reduce the risk for spreading IBR during the transport of cattle requires a much more intensive surveillance program than required for international standards. The current surveillance program can identify dairy herds within an acceptable period primarily as a result of the test characteristics and frequent bulk-tank milk testing. The current surveillance program is less likely to rapidly detect infected beef herds because surveillance in those herds depends solely on serologic testing at the time of slaughter. This decrease in the probability of detection is a result of small beef herds and the low probability of sample collection from at least 1 animal from the herd. The same pattern was demonstrated in Norway.¹²

Modeling revealed that the efficiency of surveillance in dairy herds, which depends on bulk-tank milk testing, would not be substantially decreased if the slaughter surveillance component was discontinued. Surveillance of beef cattle can be improved only by increasing the number of herds tested. Infections of IBR caused by airborne transmission of virus have been recorded only in the southern part of Jutland, close to the German border.¹³ Airborne transmission of viruses has also resulted in the

spread of foot and mouth disease¹⁴ and pseudorabies (Aujeszky's disease).¹⁵

The number of tested cattle slaughtered at the export-licensed slaughterhouses is currently around 70,000. However, by excluding the dairy cattle, this number is reduced to around 25,000 tests (ie, beef cattle only) without substantially lowering the probability of detecting IBR in an 8- or 12-week period in dairy herds. Modeling showed that targeted sample collection during the critical winter season could increase the likelihood of detecting disease in beef herds. Implementation of the targeted risk-based surveillance program requires that approximately 50,000 cattle be tested during winter in the high-risk region of Denmark; this will substantially improve the surveillance of beef herds in the high-risk area and period.

Design of surveillance programs typically is based on a year. However, this time frame may be too extended if an objective of the surveillance is to rapidly detect new infections in the country. Design of surveillance programs with shorter time frames for detection of infection should be based on the test characteristics, modes of surveillance (eg, serum or milk samples), and epidemiologic characteristics of the disease.

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