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Probability of detecting antibodies to bovine herpesvirus 1 in bulk milk after the introduction of a positive animal on to a negative farm

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The purpose of this study was to assess the probability that the introduction of one or more bovine herpesvirus 1 (BHV-1)-seropositive animals would result in the bulk milk of a clean herd becoming BHV-1-positive. Probability calculations (stochastic and deterministic) were based on the distribution of the log(titre) of 828 positive animals and the daily milk production of the herds and of the individual cows. They showed that the probability in average sized herds of 45 dairy cows is only between 10 and 25 per cent and that even in small herds of 25 cows the introduction of a positive animal would go undetected in the majority of cases. It is concluded that if the bulk milk has become BHV-1-positive it is most likely that the infection has spread.

INFECTIOUS bovine rhinotracheitis (IBR) is caused by bovine herpesvirus 1 (BHV-1). In the Netherlands it was first diagnosed in 1971 and at present at least 75 per cent of the 38,000 farms with dairy cattle are affected; the within-herd prevalence may vary from 0 to 100 per cent (van Wuyckhuise and others 1993). Other European countries including Denmark and Switzerland claim to be free of the disease, while in France and Germany several regions have low levels of BHV-1 infections (SGD 1994).

To maintain the current export position for animals and embryos, attempts are being made to eradicate BHV-1 from the Dutch cattle population.

Bulk milk samples were screened for BHV-1 antibodies in autumn 1994. If this test is strongly positive, the herd can be vaccinated with a mutant deletion (marker) vaccine to decrease the number of positive animals. If the bulk milk sample is negative or only slightly positive, the next step is to test all the individual animals. Positive animals can be removed, the herd can be certified free of BHV-1, and bulk milk samples are tested regularly to confirm its negative status (SGD 1994). A similar approach, based on the results of a study in Switzerland, has been recommended by von Forschner and others (1986). However, it is questionable whether such regular bulk milk sampling would detect the reintroduction of BHV-1 (the major route being the purchase of infected animals), owing to the dilution of the infected milk in the bulk. In a German study, only two of 21 bulk milk samples were found positive in herds where less than 10 per cent of the animals were serologically positive (Wizigmann 1987). Because of the intensive national trade in live animals, the introduction of BHV-1 on to an IBR-free farm is likely if no appropriate management measures are taken.

The purpose of this study was to assess the probability that the introduction of one or more seropositive animals would result in a positive bulk milk sample and as a result the loss of the herd's certificate.

Materials and methods

Farms and animals

Single milk samples were available from all 1303 cows in milk in 23 herds whose bulk milk was BHV-1-positive. The samples were taken in April 1994. These 23 herds were from a group of 45 herds which had enrolled voluntarily in a field trial of the efficacy of a marker vaccine and had received a placebo.

Diagnostic test

The samples were tested undiluted with a gB blocking ELISA (Kramps and others 1994). An animal was considered to be positive if the blocking percentage was at least 50 per cent. With blood samples, this 50 per cent cut-off value yields a test with a sensitivity of 99 per cent and a specificity of 96 per cent; with milk samples the sensitivity is 89 per cent and the specificity 91 per cent (J. A. Kramps, personal communication).

Neither the optical density (OD) nor the blocking percentage of undiluted samples is a measure of the relative amount of antibodies in the sample, because an excess of antibody might be washed away in the subsequent step of the test. Serial dilutions indicate to what extent a sample can be diluted before the OD decreases to the cut-off point, and provide a measure of the amount of antibody relative to a standard positive sample. To reduce costs and labour it was decided to use only two dilutions (1:4 and 1:16). The choice of these dilutions was based on the titration of 46 samples taken from three herds which were experiencing an acute outbreak of BHV-1.

The calculation of the log(titre) of a sample was based on the log-logit method described by Ritchie and others (1981). The log(titre) of a sample is defined as the Log_2 of the dilution at which the OD is exactly 50 per cent of the highest OD measured on

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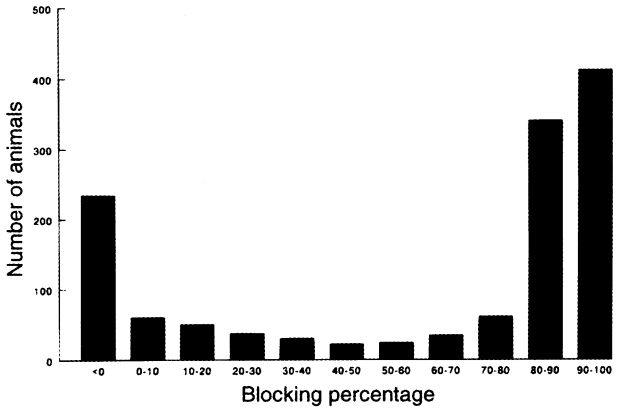

FIG 1: Distribution of blocking percentage of 1303 milk samples

the positive standard (OD). A regression line was calculated from the transformed ODs of the serial dilution of a positive standard. Subsequently, a sample regression line was calculated using the OD of the sample dilution with an OD closest to 50 per cent of OD and assuming that it would be parallel to the line based on the positive standard. The sample's log(titre) was determined from this sample regression line.

Probability calculation

On the basis of the frequency distribution of the log(titre), the probability that a positive animal would convert the bulk milk status from negative to positive can be assessed. For this, the daily milk production of the whole herd and of the positive animal must be specified. The probability of conversion can be calculated in a deterministic or stochastic way; the latter gives information about the variability of the probability.

Using a deterministic model, the probability of conversion is calculated from the dilution factor (D) of the infected milk in the total bulk. The log(titre) of the positive milk should then be at least equal to the Log_2 of this dilution, LogD. The probability of conversion equals the proportion of the animals that have a log(titre) ≥LogD.

The stochastic approach consisted basically of 100 random selections of a positive animal. Subsequently, it was determined whether the log(titre) of the selected animal exceeded LogD. By making 100 selections the probability of conversion was calculated as the number of conversions divided by 100. To assess the random variability this process was repeated 100 times, resulting in 100 probabilities.

Results

Blocking percentage

Of the 1303 milk samples 870 (67 per cent) tested positive in the undiluted blocking ELISA. The distribution of the blocking percentages of the 1303 samples is shown in Fig 1. The mean blocking percentage of the 870 positive samples was 87 per cent, the median being 85·8 per cent. The distribution indicated that most of the positive samples (those with a blocking percentage greater than 50 per cent) needed to be diluted in order to quantify the relative amount of antibody in the sample.

Titres

A log(titre) of zero indicates a positive animal because the blocking percentage in the undiluted sample is in that case exactly 50 per cent. The log(titre) frequency distribution of 828 positive milk samples is shown in Fig 2. For 42 of the samples, the log(titre) calculation was considered invalid, in most cases because

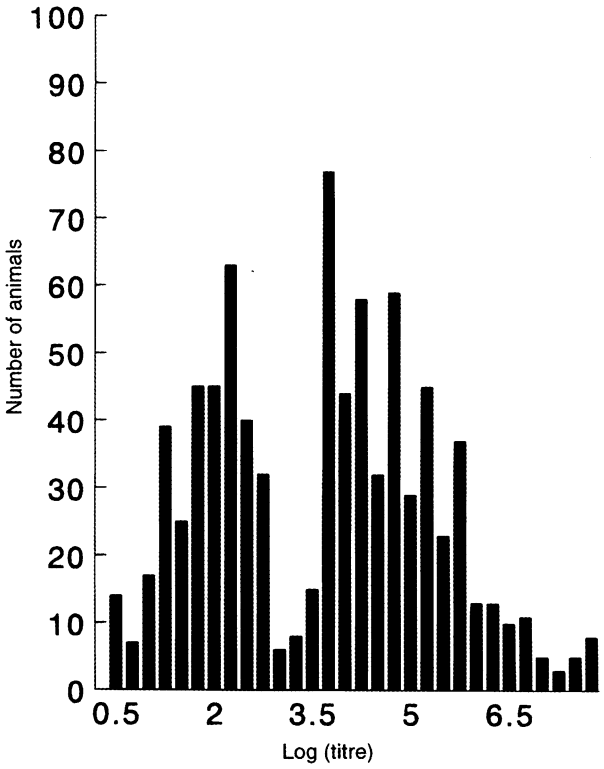

FIG 2: Distribution of log(titres) of 828 positive milk samples

the blocking percentage at a dilution of 1:16 was higher than at a dilution of 1:4. The mean log(titre) was 3·50, and the median was 3·65. Fig 2 shows that the log(titre) distribution was bimodal.

Probability calculation

Table 1 shows the results of deterministic calculations for various levels of milk production of the herd and of the positive animal. In larger herds with a high daily output of milk, the probability of conversion would be very low (less than 10 per cent) and hardly varies with the milk yield of the positive animal. The introduction of more than one animal increases this probability. Suppose that the milk of two cows, producing 20 and 30 kg, is added to a bulk of 1000 kg. Then, the probability of conversion is almost equal to one minus the probability of adding two of these animals that both do not convert the bulk milk status: 1-[(1-0·095) x (1-0·175)] = 0·25 (data from Table 1).

TABLE 1: Probability of conversion of bulk milk status by the addition of different quantities of BHV-1-positive milk to different quantities of bulk milk, calculated by deterministic and stochastic principles

Kg in bulk (herd size)	Kg added	Deterministic Probability	Stochastic Probability	(range)
500 (23)	20	0·244	0·242	(0·14 – 0·37)
	30	0·383	0·383	(0·26 – 0·51)
	40	0·477	0·473	(0·35 – 0·57)
1000 (46)	20	0·095	0·091	(0·03 – 0·17)
	30	0·175	0·174	(0·09 – 0·26)
	40	0·244	0·245	(0·14 – 0·36)
1500 (69)	20	0·051	0·052	(0·01 – 0·11)
	30	0·095	0·092	(0·03 – 0·18)
	40	0·155	0·153	(0·08 – 0·22)
2000 (92)	20	0·029	0·028	(0·00 – 0·07)
	30	0·056	0·056	(0·01 – 0·12)
	40	0·095	0·093	(0·03 – 0·17)
2500 (115)	20	0·019	0·021	(0·00 – 0·05)
	30	0·047	0·049	(0·01 – 0·11)
	40	0·066	0·066	(0·02 – 0·13)

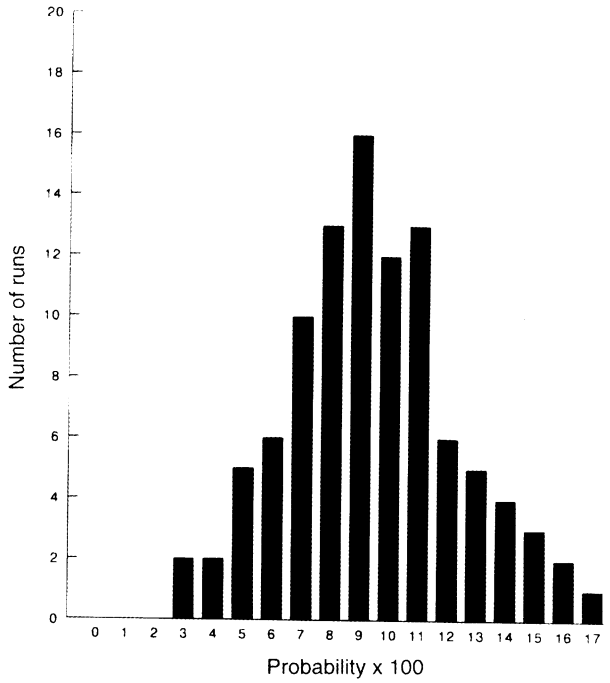

FIG 3: Distribution of probabilities to detect a conversion in a bulk of 1000 kg to which 20 kg of a randomly chosen positive cow are added (the random procedure was repeated 100 times)

The results of the stochastic calculation are shown in Table 1. The averages of the stochastic probabilities approached the deterministic probabilities. The distribution of the calculated probabilities for a specific case in which 20 kg of positive milk was added to 1000 kg of bulk milk are shown in Fig 3. The stochastic probabilities in this specific case ranged from 0·03 to 0·17, indicating the worst and the best case, respectively.

Discussion

This study was motivated by the concern that the introduction of IBR-positive animals on to farms certified free of IBR would go undetected if their negative status was confirmed only by the routine sampling of bulk milk. The major reason for this concern was that the concentration of BHV-1 antibody might be undetectable owing to the large dilution of the positive milk in the bulk milk. Concentrating bulk milk samples, as described by von Forschner and others (1986) and Wizigmann (1987) is too laborious for screening thousands of bulk milk samples (P. Franken, personal communication).

To predict the probability of detecting bulk milk conversions, it is necessary to know the distribution of the amount of antibody excreted in the milk of positive cows. Over 1000 milk samples from individual cows in 23 herds were available, but the corresponding bulk milk samples were not available. It is difficult to say whether these 23 herds (or the positive animals) were a representative selection of the total Dutch population of cows. However, the prevalence of 67 per cent of BHV-1-positive cows was similar to the national prevalence of 75 per cent (van Wuyckhuise and others 1993), and the sizes of the herds, ranging from 19 to 93 cows, were in the normal range. It is concluded that the results should not be biased to a large extent.

Infection with BHV-1 is confirmed by a gB blocking ELISA (Kramps and others 1994) in either undiluted serum or milk, the criterion being a blocking percentage greater than 50 per cent. Using the 1:4 dilution resulted in the loss of some weakly positive samples, because their ODs were out of range. This affected the frequency distribution in such a way that the number of very low log(titre) samples was somewhat underestimated, and as a result there was a slight overestimation of the probability of conversion.

The frequency distribution of the log(titres) appeared to be bimodal. The first part of the bimodal distribution was not due either to positive animals in low prevalence herds or to older animals that had experienced the infection a long time ago, because the herd prevalence and age together explained only 5 per cent of the total variation in log(titre) (multivariate linear regression using StataCorp [1995]). Another possible explanation derives from the type of test used. In a blocking ELISA, epitope-specific antibodies are detected as a result of their competition with a labelled monoclonal antibody, and it is known that the affinity of antibodies to a single epitope has a bimodal distribution (Roitt and others 1993). This implies that some cows produce mainly antibodies with a low affinity while others produce antibodies with a high affinity to this specific epitope. However, if affinity plays a role in this bimodal distribution, the parallel line assumption is violated. It is therefore difficult to conclude from the present data, whether this bimodal distribution has biological significance. Mathematically, the curve did not deviate from a Normal curve; its skewness and kurtosis were 0·16 and –0·70, respectively.

The probability of conversion of the bulk milk status depends heavily on the daily herd milk production, and ranges from 0·2 per cent to almost 40 per cent in the case of a cow producing 30 kg/day. The stochastic calculations show that the variation in the probability is rather large, but that it almost never exceeds 50 per cent. Thus, the majority of introductions of a single positive animal, or the presence of a single reactor, are unlikely to have an immediate effect on the bulk milk status. As a result, if BHV-1 is detected in the bulk milk, there is a high probability that more than one animal is infected and that the infection has spread. This is valuable information for helping to determine the strategy for certification and control programmes.

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Abstract

Hereditary cerebellar degeneration in cats

A PREVIOUSLY undescribed form of cerebellar degeneration in cats, with an autosomal recessive mode of inheritance, which was unassociated with pre- or perinatal infection with feline panleucopenia virus, inherited lysosomal storage diseases (including gangliosidosis and mannosidosis) and feline hereditary neuroaxonal dystrophy, was studied in seven cats. Three cats served as a breeding colony of affected individuals, and four kittens, obtained by backcrossing, developed pure cerebellar dysfunction from six to seven weeks onwards. Magnetic resonance imaging indicated a marked reduction of cerebellar size in diseased cats. The major signs of neurological dysfunction were head tremors, intention tremors, dysmetria and a lack of coordination. Cerebellar cortical degeneration, especially an extensive destruction of Purkinje cells, was observed at post mortem examination.

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