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Epidemiology of BHV 1 virus infections in dairy herds

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SUMMARY

The epidemiology of bovine herpesvirus 1 (BHV 1) infections was studied in 20 dairy herds. Periodic serological surveillance of these herds during three consecutive years (1980–82) was combined with clinical studies. In 19 herds seropositive cows were found indicating previous exposure to BHV 1.

One herd had its first experience with BHV 1 during the study. No indication of virus circulation for at least three years was found in eight herds. In five herds an interval of 2 years without an indication of virus circulation was followed by infections in yearlings, first- and second-calf cows during the third year. One or two cycles of virus circulation in calves and/or yearlings during the 3 year survey were detected in six herds. Most BHV 1 infections passed unnoticed. Signs of respiratory disease in association with BHV 1 infection were observed in three herds: young animals were most seriously affected. Clinical manifestations of BHV 1 infections were less pronounced than a few years ago when infections in cows caused frank signs and diagnosis was frequently possible on the basis of a typical clinical picture. BHV 1 was the cause of abortions in the herd that experienced its first infections during this survey. A survey of the age-specific BHV 1 neutralizing antibody pattern may be helpful for tracing animals and herds at risk of an outbreak of infectious bovine rhinotracheitis.

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) is a respiratory tract disease characterized by tracheitis, rhinitis and fever that is caused by BHV 1 infection (McKercher *et al.* 1957; Wiseman *et al.* 1980). Moreover, BHV 1 infections play a role among causes of undifferentiated bovine respiratory disease. Outbreaks of BHV 1 infections may cause serious economic losses by abortions and a drop in milk yield (Kirkbride *et al.* 1973; McKercher & Wada, 1964; Owen, Chow & Molello, 1964). A wave of a particularly severe form of IBR appeared to flood the Netherlands in 1972 and the following years (van Bekkum & Straver, 1975) and outbreaks of an acute virulent form of IBR were also reported from other western European countries in the seventies (Anonymous, 1979; Cuthbertson & Wood, 1979; Martel *et al.* 1976; Wellemans, 1975; Wiseman *et al.* 1978). This may have been a consequence of the emergence and spread of a new virulent strain (Nettleton *et al.* 1981) and lack

of immunity in the cattle population. Now the situation seems to have quietened down: serious outbreaks of IBR with frank clinical cases in cows are observed less frequently.

The purpose of this study was (*a*) to gain information on the perpetuation of the virus in dairy herds exposed to the infection in the past, and on the prevalence of clinical manifestations of infection in these herds and (*b*) to develop criteria for a decision about vaccination, based on a better understanding of the ecology of the virus and its aetiologic significance in disease.

MATERIALS AND METHODS

1. *Herd description*

Twenty dairy herds were selected for a 3-year survey of BHV 1 infections. These herds were located in the centre of the Netherlands in the area of the Ambulatory Clinic of the Veterinary School of Utrecht. A mean of 138 animals (range 56–520) were kept in these herds, one-third of the population consisting of calves and yearlings, raised for dairy cattle replacement. Nineteen herds were also included in an investigation of the epidemiology of bovine respiratory syncytial and parainfluenza type 3 virus infections in 1979–80 and 11 in 1980–81. In all herds except five, young stock was raised in a separated house. Replenishments were bought in as follows – 1–8 cows in 10 herds and more than 20 in three herds. No vaccination against BHV 1 infections were done during the course of the study.

2. *Conduction of the serological and clinical survey*

Blood samples were taken from about one-fifth of all animals, from each age group representative for the herd as a whole, in February and March of 1980, before the cows went to pasture. Sampling was repeated in 1981 and 1982, as far as possible from the same animals and, in addition, from yearlings born since the preceding year and, in some cases, from newborn calves.

The stockmen had been alerted for signs of respiratory disease and abortions that might have been caused by a BHV 1 infection and had instructions to warn the clinician in case of illness in their animals.

During the winter housing, November–March, of 1979–80 and 1980–81 blood samples were collected at monthly intervals from 4 to 12 month-old calves of 19 and 11 herds respectively. These herds were visited every 2 weeks by a clinician, who interviewed the stockmen.

When they are housed in the autumn, calves 4 to 12 months old are usually very susceptible to infectious agents circulating in the herd, especially those of respiratory disease.

About half the number of calves in intensively observed herds were examined clinically and food intake, rectal temperature, nasal discharge, coughing, frequency and type of respiration and abnormal breath sounds on auscultation were recorded. In the case of an outbreak of respiratory disease clinical examinations were repeated on several days. Where BHV 1 infection was suspected as the cause of respiratory disease, nasal swabs were collected from several animals for virus isolation.

3. *Serology*

Sera were tested for neutralizing antibodies to BHV 1 at a 1 in 2 dilution by a plaque reduction test. Sera were incubated for 1 h at 37 °C with an equal volume of BHV 1 suspension, diluted to contain approximately 500 p.f.u./ml. Of each serum–virus mixture 0·2 ml was applied in duplicate to cups of a vinyl Linbro® plate type 96 CV-TC and 1 ml of a suspension of cultured bovine fetal kidney cells in medium was added, giving a confluent monolayer after 24 h of incubation. Plates were incubated for 3 days at 36 °C, cell sheets were then fixed with ethanol and stained with 0·1 % amidoblack and plaques were counted. Sera which reduced the number of plaques counted in the control lacking serum to one-third or less were considered to be positive.

4. *Virology*

Extracts of nasal swabs were inoculated into secondary cultures of bovine fetal kidney cells for virus isolation. Cultures were incubated at 36 °C with maintenance medium and examined microscopically three times a week for at least 10 days for cytopathic changes. Cytopathic agents were identified by electron microscopy. Recognition of characteristic morphological structures of a herpesvirus in combination with a seroconversion for BHV 1 antibodies was considered proof of an infection. Pieces of liver, kidney, spleen and lung of aborted fetuses and cotyledons were ground with sand in a mortar. Tissue extract in Hanks' balanced salt solution with antibiotics was inoculated into secondary cultures of bovine fetal kidney cells. Inspection of cultures for cytopathic effect and identification of BHV 1 virus was as above. Cryostat sections of liver, kidney, spleen and lung tissue of aborted fetuses and cotyledons were tested for the presence of BVD- and BHV 1-virus antigen by a direct fluorescent antibody technique (Terpstra, 1979). Tissues of aborted fetuses and cotyledons were fixed in 10 % formol saline and processed and sectioned by standard procedures. Tissue sections were stained by Giménez' method to identify chlamydia (Nabli & Tarizzo, 1967).

5. *Compilation of data*

Sera were tested for neutralizing antibodies to BHV 1. If yearlings were positive at the end of the winter housing period, the samples collected at monthly intervals were tested to detect the time of seroconversion. If yearlings were found positive on returning from pasture for winter housing, virus circulation was considered to have occurred during the grazing season. Results of serological surveys of 3 years were arranged according to age group. Animals born from July until the end of June of the successive year were assigned to one age group. Calves born before July will be considered to be yearlings from July until the end of June of the successive year for practical reasons, although this does not fulfil the official definition of yearlings (calves from 12 to 18 months old).

Herds were allocated to four groups, according to results of the serological survey: group I, no seroconversion occurred during the last 3 years; group II, no seroconversion occurred for 2 years and virus circulation was confirmed for the last year; group III, one or two cycles of virus circulation were detected during the

Table 1. *Survey for BHV 1 antibodies during 3 consecutive years of two herds in which no indication of virus circulation had been found for 5 and 3 years respectively*

	Herd A tested in			Herd B tested in		
Animals born in	1980	1981	1982	1980	1981	1982
1968–76	5/7*	7/7	5/5	15/15	15/15	8/8
1976–7	0/4	0/4	0/2	3/3	3/3	3/3
1977–8	0/5	0/5	0/4	2/2	2/2	1/1
1978–9	0/11	0/11	0/8	0/12	0/12	0/7
1979–80	NT	0/8	0/5	NT	0/8	2/7
1980–1	—	NT	0/5	—	NT	0/5
1981–2	—	—	NT	—	—	0/2

* Number of animals seropositive for BHV 1 antibody/number of animals tested in each age group.
NT, not tested.

survey and group IV, infection was introduced for the first time in the course of the survey.

RESULTS

Serological survey

In 1980 cows with neutralizing antibodies to BHV 1 were found in all herds except herd T. Where seroconversion occurred in 1981 and 1982, it involved nearly all animals of one or more age groups; occasionally only a single animal became seropositive. All yearlings of a group appeared to become seropositive in the same month indicating exposure of all yearlings to virus. Herds A and B are typical of group I (Table 1). In the 1980 survey of herd A five of seven cows born before or in 1975–6 were found serologically positive and younger animals were negative. All positive animals remained positive in 1981 and 1982 and no further seroconversions took place. The youngest seropositive animals in this herd were 6 years old (fourth-calf cows) in 1982. In the 1980 survey of herd B all cows born before or in 1977/8 were positive. Younger animals were negative. First- and second-calf cows remained seronegative until 1982, except for 2 first-calf cows. Similar results were obtained in six other herds: no animals had seroconverted during the last 3 years, indicating no spread of BHV 1 to seronegative animals for at least 3 years. In 1982 the youngest seropositive age groups in herds of group I were 7 (herd C), 6 (herd A and D), 5 (herd E) and 4 (herd F, B, G, H) years old. In the other 12 herds (I–T), assigned to groups II–IV, at least one cycle of virus circulation was confirmed during the last 3 years: 16 cycles in total (Fig. 1). Virus circulation was confirmed by seroconversion of yearlings during their winter housing, or by some cohorts having become seropositive in 1981 or 1982. Moreover, the finding of seropositive yearlings at the time of housing in 1979 was an indication of BHV 1 circulation during the previous grazing period.

Results of herds M and K are presented as examples of group II (herds I–M) (Table 2). In 1980 in both herds first-calf cows and older were seropositive. Only one yearling (1978–9) in herd K was positive. In 1981 one further animal born in 1978–9 (herd M) was positive, as were five out of six calves, but these may be

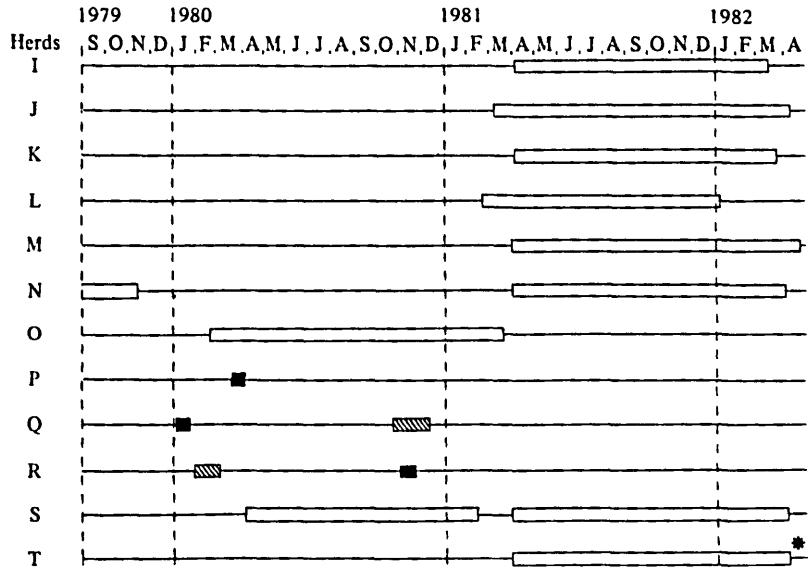

Fig. 1. BHV 1 circulation in 12 dairy herds during a survey of 3 years. □, Period during which BHV 1 circulation occurred in one or more cohorts, confirmed by seroconversion in yearly surveys. ■, Clinical signs of BHV 1 infection in yearlings, confirmed by seroconversion and virus isolation. ▧, Circulation of BHV 1 confirmed by seroconversion in monthly blood samples from calves and yearlings. * Abortions in association with BHV 1 infections.

Table 2. *Survey for BHV 1 antibodies during 3 consecutive years of two herds in which virus circulation occurred after an interval of 2 years. Cohorts in which infections occurred are in italic type*

	Herd K tested in			Herd M tested in		
Animals born in	1980	1981	1982	1980	1981	1982
1971–8	15/17*	15/17	13/13	11/11	24/25	—
1978–9	1/12	1/10	*6/6*	0/6	1/6	*5/5*
1979–80	NT	0/15	*4/5*	0/5	0/15	*3/3*
1980–1	—	NT	*3/5*	—	5/6	*5/5*
1981–2	—	—	1/5	—	—	NT

* Number of animals seropositive for BHV 1 antibody/number of animals tested in each age group.
NT, not tested.

assumed to have had maternal antibodies. In 1982 yearlings (1980–1), first- and second-calf cows were positive, indicating virus circulation during the preceding year.

Similar results were found in another three herds: yearlings were seronegative in 1980, yearlings and first-calf cows were negative in 1981 and yearlings, first- and second-calf cows were positive in 1982. No clinical signs of a BHV 1 infection were noticed in these herds.

Evidence of one or two cycles of virus circulation was found in herds of group III, comprising six herds (N–S). Yearlings of herd Q were seropositive in 1980 and 1981 and so were most of the older animals (Table 3). In 1982 yearlings were found

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Table 3. *Survey for BHV 1 antibodies during 3 consecutive years in two herds in which most yearlings were seropositive in 1980 and 1981, and negative in 1982. Cohorts in which infections occurred are in italic type*

	Herd Q tested in			Herd R tested in		
Animals born in	1980	1981	1982	1980	1981	1982
1977–8 and before	16/16*	12/13	7/8	13/16	13/17	9/11
1978–9	*10/10*	9/10	8/8	*11/12*	8/8	7/8
1979–80	NT	*14/19*	8/8	2/9	*15/15*	4/4
1980–1	—	0/3	0/5	—	NT	1/5
1981–2	—	—	3/4	—	—	3/5

* Number of animals seropositive for BHV 1 antibody/number of animals tested in each group.
NT, not tested.

seronegative. Testing of matched blood samples of yearlings collected monthly during winter housing in 1979–80 and 1980–1 gave evidence of BHV 1 infections in January and November 1980 respectively (Fig. 1). Signs of respiratory disease had been observed in yearlings in the first week of January 1980 and virus was recovered from nasal secretions of affected animals. Infections in yearlings of this herd in November passed unnoticed.

In herd R yearlings were seropositive in 1980 and 1981 and one out of five was positive in 1982 (Table 3). Most animals of older age groups had antibodies. BHV 1 infections of yearlings in February and November of 1980 were confirmed by seroconversions in matched serum samples (Fig. 1). Clinical signs had been observed in yearlings during the second week of November 1980 and infection was confirmed by virus isolation.

In herd N all first-calf cows and older were seropositive in 1980. Moreover, five out of 11 yearlings appeared already positive at housing, indicating a previous BHV 1 infection. No animals had seroconverted in 1981. First- and second-calf cows but no yearlings, had become seropositive in 1982. Infections in this herd passed unnoticed.

All second-calf cows and older animals of herd O were seropositive in 1980, first-calf cows and yearlings were negative. In 1981 second-calf cows only (born in 1977–8) had become seropositive. No signs of a BHV 1 infection had been observed. There were no further seroconversions in 1982.

In herd P most fourth-calf cows and older animals were seropositive in 1980 and most younger animals were negative. BHV 1 circulation was confirmed in March of 1980 by seroconversion in matched serum samples from yearlings. Seven calves had been born at that time and two, having lost maternal antibodies, became infected and developed bronchopneumonia. Animals of all age groups were positive in 1981 and in 1982 yearlings only were found seronegative.

In the 1980 survey of herd S all fourth-calf and older cows were seropositive and most of the younger animals were negative. First-, second-, third-, and fourth-calf cows had become seropositive in 1981. In 1982 animals of all age groups, yearlings included, were seropositive, indicating another cycle of virus infections. No signs of respiratory disease had been observed in this herd.

Table 4. *Clinical data of* 12 *yearlings and three calves of Herd Q on day* 0, 1, 2, 3, 4, 6 *and* 10 *of an outbreak of infectious bovine rhinotracheitis*

	12 yearlings		3 calves‡	
Clinical signs	Number of animals*	Frequency†	Number of animals	Frequency
Pyrexia (40 °C)	7	10	1	4
Anorexia	2	4	1	6
Respiratory stridor (snoring)	2	3	0	0
Nasal lesions	7	13	1	3
Ausculation				
Bronchial sounds	0	0	2	7
Bronchovesicular sounds	2	2	2	3
Abdominal breathing	2	2	1	3
Respiratory rate > 50/min.	0	0	2	3
Coughing	11	26	2	8
Nasal discharge	9	24	3	6

* Number of animals showing a certain sign.

† Frequency of observation of a certain sign, based upon a total number of examinations of 12×7 in yearlings and 3×7 in calves.

‡ One calf died on day 8.

Group IV comprised herd T only. Three hundred and twenty cows were kept in this herd and about 100 calves and 100 yearlings were raised. This herd was seronegative in 1980 and 1981. Sixty-seven cows, 11 yearlings and 11 calves were tested in April 1982, and 65, five and two animals respectively were seropositive. No respiratory signs of IBR had been observed during the preceding season and no depression of milk yield was noticed. However, 25 cows aborted in the period from April 1st, until May 13th 1982.

Clinical findings

Outbreaks of BHV 1 infections with signs of respiratory disease, confirmed by virus isolation and seroconversion, were only observed in herds P, Q and R during the 3 years.

In these outbreaks calves and/or yearlings were most seriously affected. Signs of respiratory disease were less characteristic for IBR than those observed a few years before in cows in severe outbreaks (van Bekkum & Straver, 1975). Data from clinical observations of 12 out of 25 yearlings and three calves of herd Q on the day disease was first observed and on days 1, 2, 3, 4, 6 and 10 afterwards are summarized in Table 4.

Signs of upper respiratory tract disease, coughing and mucopurulent nasal discharge were most pronounced. Three calves had been born in this herd at the time disease was observed in yearlings and these were housed together with yearlings. Two calves developed signs of a bronchopneumonia and one of them died. A BHV 1 infection was confirmed by antigen detection by fluorescent antibody staining of sections of pneumonic lung tissue.

Twenty-five out of 320 cows of herd T aborted over a period of 1·5 months. Abortions occurred in cows of all ages between the sixth and ninth months of gestation. No respiratory disease had been observed in this herd. Of nine fetuses

obtained, one was mummified and another showed autolysis. BHV 1 was demonstrated by fluorescent antibody technique in five, BHV 1 being isolated in three. Cotyledons obtained on two occasions were also found positive. No BVD virus was detected. Chlamydia were found in tissue sections of liver and spleen of two fetuses and in cotyledons of another fetus. Two fetuses were positive for both BHV 1 and chlamydia.

DISCUSSION

For practical reasons emphasis in most studies on BHV 1 infections has been on severe signs of disease. In this survey periodic serological surveillance, combined with clinical studies, gave a more complete picture of the epidemiology of BHV 1 by detecting both clinical and inapparent infections.

Once they have experienced an infection, animals maintain BHV 1 antibodies for at least 5 years (Chow, 1972). The date of the most recent cycle of virus circulation in a herd can be fixed by the lowest age at which antibodies are prevalent.

In the 1982 survey of herd A, cows of 6 years and older were seropositive and no animals born since 1976–7 had become seropositive, indicating no virus circulation over a 5-year period.

Nineteen herds had experienced a BHV 1 infection in the past. Infection had not recurred for 6, 5, 4 and 3 years in one, two, one and four herds respectively. In the remaining 11 herds one or two cycles of virus circulation were detected. The infection was newly introduced into one herd that had been free of the virus.

Most cattle with antibodies against BHV 1 are latently infected and may be considered a source of infection for rearing cattle (Davies & Duncan, 1974; Sheffy & Davies, 1972; Snowdon, 1965). Additions to the herd with a cohort of BHV 1 susceptible calves each year might cause repeated circulation of virus.

An investigation of the epidemiology of bovine respiratory syncytial and parainfluenza type 3 virus infections in the same dairy herds indicated that most calves experienced an infection with these two agents in their first year of life (van Nieuwstadt *et al.* 1982). Epidemiology of BHV 1 differed in that there may be an interval of one or more years before another cycle of BHV 1 occurs. In such a case, animals susceptible to BHV 1 will form a progressively larger proportion of the herd and the risk of serious IBR outbreaks, with economic losses in adult animals, will increase in proportion. The risk would appear greatest in those eight herds where no virus had circulated for 3 or more years. Disappearance of BHV 1 from the herd in the long term might be another outcome.

Where no virus had occurred in six herds for 2 years but yearlings, first- and second-calf cows became infected during the third year, these herds might have been expected to be at risk of abortions due to BHV 1. However, no increase of abortions was reported. The same is true for another four herds in group III, where first-calf cows and older must have been exposed to a BHV 1 infection during gestation.

BHV 1 was the aetiological agent of abortions in herd T, which had its first experience with BHV 1 during the survey. No respiratory signs of IBR had been observed and abortions occurred unexpectedly in April and May. Five cows had

been purchased and introduced in this herd in August, October and December of the preceding year. However, retrospectively it is difficult to decide if they introduced the virus. Again it is not known if the infection had an explosive course or spread gradually in the herd. Chlamydia were demonstrated in aborted fetuses, but the aetiologic significance of chlamydia in abortions is not fully understood.

The pregnant cow may abort following clinical or subclinical infection. The interval between exposure and abortion ranges from 8 days to several months (Kahrs, 1977; Saunders, Olson & Radostits, 1972; Wilson, 1974). Thus fetuses may be expelled while clinical IBR is evident in the herd and as long as 100 days afterwards or even without any indication of a previous BHV 1 infection. Thus, diagnosis of abortions by a BHV 1 infection may easily be missed. Laboratory confirmation of a suspected BHV 1 abortion may be obtained by virus isolation or fluorescent antibody staining of fetal organs from fresh fetuses. However, fetuses are often too decomposed by autolysis. In this study BHV 1 did not appear to be a cause of abortions in herds previously infected with BHV 1.

BHV 1 infection with signs of respiratory disease were detected in three herds and calves and yearlings were most seriously affected. Signs of respiratory disease were generally milder than observed in outbreaks of a few years ago (van Bekkum & Straver, 1975). No respiratory disease was reported from 13 out of 16 serologically confirmed cycles of virus circulation in yearlings or cows, indicating that a considerable proportion of infections must remain unrecognized.

Husbandry practices may affect the epidemiologic pattern of BHV 1. Physical contact between animals favours spread of virus and the practice of rearing young stock separated from the dairy herd in most of these herds probably postponed exposure to the virus.

Virus circulation in one cohort, while other cohorts in a separate house remained free of the infection, was confirmed in herds N, O, Q and R.

This investigation has given no indication of the factors responsible for reactivating virus in dairy herds. The finding that most infections in this investigation were subclinical justifies a re-evaluation of economical losses *versus* costs of vaccination in BHV 1 infections. A survey of the age-specific distribution of antibodies to BHV 1 might be of help in locating herds and animals susceptible to the infection in order to take a decision on vaccination.

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