Age Distribution of Animals Persistently Infected with Bovine Virus Diarrhea Virus in Twenty-two Danish Dairy Herds

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ABSTRACT

The objectives of this study were to compare the age distribution of animals persistently infected (PI) with bovine virus diarrhea virus (BVDV) in 12 herds with clinical BVD compared to ten herds without clinical BVD and to examine the incidence of PI calves born after the oldest PI animal. Blood samples from all animals were tested for bovine virus diarrhea virus and antibodies. In five herds, blood samples were obtained from calves born after the whole herd had been tested. All calves born by PI dams were also blood tested.

In herds with clinical BVD the median age of PI animals was 248 days and in herds without clinical BVD the median age was 144 days. There was no significant difference between the age of PI animals in herds with clinical BVD compared to herds without clinical BVD ($p = 0.48$) suggesting similar epidemiology of the occurrences of PI animals in the two herd categories.

Thereafter, all herds were used to study the incidence of PI animals. A total of 129 PI animals were found. In ten herds with 72 PI animals the age range of PI animals was more than six months. In these herds 26.3% of the PI animals were born within the first two months after birth of the oldest PI animal, no PI animals were born 2-<6 months, 52.7% were born 6-<14 months, 6.9% were born 14-<22 months and 13.9% (all born by PI dams) were born later than 22 months after the oldest PI animal. The incidence of PI animals was significantly different from non-PI animals ($p < 0.001$). It is suggested that the infection is introduced initially by a few animals and is then reinforced by birth of PI animals. Therefore, PI animals are often clustered in two separate age groups.

RÉSUMÉ

Le but de cette étude était de comparer les variations d'âge chez des animaux infectés de façon permanente (IP) avec le virus de la diarrhée à virus des bovins (V-BVD) dans 12 troupeaux présentant des signe clinique de BVD, comparativement à 10 troupeaux sans signe clinique de BVD, et d'examiner l'incidence de veaux IP nés après l'arrivée du plus vieil animal IP. On a analysé le sang de tous les animaux pour détecter la présence du V-BVD et d'anticorps contre ce virus. Chez cinq troupeaux, on a obtenu du sang de veaux nés après que le troupeau entier ait été testé. Tous les veaux issus de mère IP ont aussi été testés.

Dans les troupeaux où il y avait une forme clinique de BVD, l'âge moyen des animaux IP était de 248 jours et dans les troupeaux sans forme clinique de BVD, l'âge moyen était de 144 jours. Aucune différence significative entre l'âge des animaux IP dans les troupeaux ayant eu la forme clinique du BVD et dans les troupeaux sans forme clinique de BVD n'a été observée ($p = 0,48$) suggérant une épidémiologie similaire dans le fait de trouver des animaux IP dans les deux catégories de troupeaux.

Tous les troupeaux furent donc utilisés pour étudier l'incidence d'animaux IP. Au total 129 animaux IP ont été détectés. Dans 10 troupeaux comptant 72 animaux IP, l'écart d'âge des animaux IP était de plus de six mois. Dans ces troupeaux, 26,3 % des animaux IP étaient nés moins de deux mois après la naissance du plus vieil animal IP; aucun animal IP n'était né entre 2-<6 mois, 52,7 % des animaux IP étaient nés 6-<14 mois, 6,9 % étaient nés 14-<22 mois et 13,9 % (tous issus de mères IP) étaient nés plus de 22 mois après la naissance du plus vieil animal IP. L'incidence d'animaux IP était significativement différente de celle des animaux non-IP ($p < 0,001$). On pense que l'infection est introduite d'abord par quelques animaux, puis elle prend de l'ampleur par la naissance d'animaux IP. C'est pourquoi les animaux IP sont souvent regroupés en deux groupes d'âge différents. (*Traduit par Dr Pierre Lamothe*)

INTRODUCTION

Bovine virus diarrhea virus (BVDV) has a world wide distribution. The prevalence of infection in different areas of the world has been reviewed recently (1). In Denmark the annual incidence risk of postnatal infection, i.e. the risk of infection within one year has been calculated to be 34% (2). Postnatal infection with BVDV is most often subclinical although transient diarrhea, fever and drop in milk production may be seen. The most serious consequence of infection occurs after *in utero* infection of the fetus. Experimental infection of pregnant seronegative heifers caused fetal death and abortion, congenital malformations and growth retardation (3). Fetal infection between 42–125 days of fetal life induced specific immunotolerance against BVDV (4). These calves will be persistently infected (PI) for the rest of their life. Persistently infected animals are considered the most important transmitters of infection. Persistently infected calves may be born weak and undersized, but many of them appear normal at birth (4). Later they may develop unthriftiness or mucosal disease. Any control program for BVDV should include identification and removal of PI animals (5).

Since persistent infection is caused by infection during the first three to four months of pregnancy, PI animals are often clustered in certain age

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groups (6). However, spread of infection among adult cows may sometimes be slow and hence PI animals may also appear over an extended period (7). Offspring from PI cows are always PI (8,9).

The purpose of this study was to study the age distribution of PI animals in herds with clinical BVD (mostly fatal mucosal disease) and in herds where clinical signs of infection had not occurred. Further, it was the purpose to examine the incidence of PI calves born after the oldest PI animal. This may provide guidelines for which animals should be blood tested in order to identify PI animals.

TABLE I. Median, range and percentiles of the age of PI animals at the time when the first PI animal was diagnosed. Twelve herds with clinical BVD

Herd number	Herd size No. animals	PI animals				
		Number	Age distribution (days) Median	Range	P5[a]	P95[b]
101	90	—	—	—	—	—
102	115	1	35	0	35	35
103	233	2	39	0	39	39
104	216	1	0	0	0	0
105	176	5	207	197	34	231
106	210	7	87	226	64	290
107	127	9	220	176	136	312
108	117	10	249	97	185	282
109	56	3	327	72	258	330
110	59	7	465	56	450	506
111	68	2	461	244	339	583
112	107	5	416	1072	37	1109
Total	1574	52	248	1109	35	583
			Age distribution for all animals			
			695	6014	47	2033

[a]P5: 5th percentile
[b]P95: 95th percentile

MATERIALS AND METHODS

DEFINITION OF PI ANIMALS

Persistently infected animals were defined according to the following criteria:

a) Animals where the virus was detected in two blood samples taken at least three weeks apart.
b) Animals showing clinical signs of mucosal disease and from which virus was isolated from blood or organs.
c) Asymptomatic animals about the same age as b) and viremic on at least one occasion.
d) Newborn or young calves that were viremic on at least one occasion.

SELECTION OF HERDS FOR THE STUDY

Twenty-two Danish dairy herds were selected for the study. Each herd was visited and blood samples were taken from all animals in the herd. The herds were divided into two groups: Outbreak herds comprised 12 herds (Nos. 101–112) in which there had been clinical BVD 1–19 months prior to the herd blood test (in ten herds the clinical outbreak had occurred less than six months earlier). All outbreaks had been confirmed in the laboratory, either by a rise in serum neutralization (SN) antibody titer (two herds, Nos. 102 and 103), by virus isolation from abortions (five herds, Nos. 101–104 and 107) or by isolation of virus from tissues or blood from animals dying with clinical signs of mucosal disease (eight herds, Nos. 105–112).

Nonoutbreak herds comprised ten herds (Nos. 1–10) where PI animals had been detected in a previous study (2). According to the owners, clinical BVD had not been seen in these herds, except in herd 5 in which the infection had been diagnosed five years prior to the study.

FOLLOW-UP

Outbreak herds — In the two herds (Nos. 102 and 103) in which a rise in SN antibody titer during the clinical outbreak had been demonstrated, blood samples were taken from calves born in the herd over the 12 months following the acute infection, and in one herd (No. 106) blood samples were taken over the 12 months following herd blood test.

Nonoutbreak herds — In two herds (Nos. 4 and 5) blood samples were obtained from calves born during 12 months after the herd blood test. All calves born later by PI dams were also tested.

DATA COLLECTION AND CALCULATIONS

Only animals that were blood tested were included in the calculations. The date of birth was recorded for each animal. The age of PI animals in outbreak herds was calculated as the age at the time when the first PI case was diagnosed. For all other animals the age was calculated as the age at the time of the herd blood test.

Median, 5th and 95th percentiles of the ages were calculated for PI animals and for all animals in the herds. The age range was calculated as the age of the oldest animal minus the age of the youngest animal. The age distribution of PI animals in outbreak herds and nonoutbreak herds was compared by Wilcoxon *t*-test approximation.

In herds in which the age difference between the oldest and youngest PI animals was greater than six months the incidence of PI calves and non-PI calves born after the oldest PI animal was calculated. The incidence of PI animals and non-PI animals born within the first 21 months after the oldest PI animal was compared by Chi-square test. Further, the age differences between each consecutive PI animal in each herd were calculated.

VIROLOGICAL AND SEROLOGICAL EXAMINATION

All blood samples were tested for BVDV infection by virus isolation using serum as inoculum for suspensions of bovine cells in microplates, followed by immunoperoxidase staining. Simultaneously with the virus isolation tests, all samples were tested for neutralizing antibodies using a Danish cytopathogenic BVDV strain as the test strain (10).

TABLE II. Median, range and percentiles of the age of PI animals. Ten herds without clinical BVD

Herd number	Herd size No. animals	PI animals Number	Age distribution (days) Median	Range	P5[a]	P95[b]
1	121	1	485	0	485	485
2	133	5	371	627	214	841
3	123	2	176	102	125	227
4	114	1	66	0	66	66
5	124	10	111	29	100	129
6	201	4	168	59	139	198
7	98	6	442	766	60	826
8	96	1	91	0	91	91
9	131	2	370	171	284	455
10	108	2	432	27	418	445
Total	1249	34	144	781	65	830
			Age distribution for all animals			
			650	4706	42	2131

[a]P5: 5th percentile
[b]P95: 95th percentile

TABLE III. Range of the age of PI animals and the maximum age difference between the age of two consecutive PI animals in each herd. Eleven herds (Nos. 102–112) in which there had been clinical BVD and ten herds (Nos. 1–10) without clinical BVD in which PI animals had been found in a prevalence survey

Herd number	Number of PI animals	Range (days)	Maximum age difference (days)
Age range less than six months			
104	8	50	10
107	9	176	42
108	10	97	31
109	3	72	69
110	7	56	35
1	1	0	—
3	2	102	102
5	10	29	15
6	4	59	40
8	1	0	—
9	2	171	171
Age range between 6 and 24 months			
102	2	238	238
103	19	268	155
105	12	385	149
106	9	619	381
111	2	244	244
4	6	389	258
Age range more than two years			
112	5	1072	438
2	7	1123	426
7	7	1073	689
10	3	824	797
Total	129		

RESULTS

The age distribution of 52 PI animals in outbreak herds at the time when the first PI animal was diagnosed is shown in Table I and the age distribution of 34 PI animals in nonoutbreak herds at the time of herd blood test is shown in Table II. In the outbreak herds, the median age of PI animals was 248 days and the age difference between oldest and youngest PI animal (in herds with more than one PI animal) varied between 0 and 1072 days. In the nonoutbreak herds the median age of PI animals was 144 days and the age difference between oldest and youngest PI animal in herds with more than one PI animal varied between 27 and 766 days. There was no significant difference between the age of PI animals in outbreak herds compared to PI animals in nonoutbreak herds ($p = 0.48$, Wilcoxon t-test approximation).

Including the period from clinical BVD until herd blood test and the follow up periods a total of 129 PI animals were found. When all 129 PI animals were considered, the age range of PI animals in each herd was often within a certain interval (Table III). In 11 of the herds all PI animals were born within six months (Herds 104, 107, 108, 109, 110, 1, 3, 5, 6, 8 and 9). In six herds the age range varied between 6 and 24 months (Herds 102, 103, 105, 106, 111 and 4) and in four herds the age range was more than two years (Herds 112, 2, 7 and 10). In all four herds the wide age range was due to vertical BVDV transmission from PI dams to their offspring.

When each herd with more than one PI animal (19 herds with 127 PI animals) was considered separately, 108 age differences between each consecutive PI calf could be established. A total of 88 (81.5%) age differences were less than two months, 14 (13%) were between 2 and 12 months, five (4.6%) were between one and two years and one (0.9%) age difference was between two and three years. Table III shows the maximum age difference between two consecutive PI animals in each herd.

In herds in which the age range of PI animals was more than six months the maximum age difference between individual PI animals was at least 149 days, i.e. five months. Sometimes this age difference divided the PI animals into two age groups where a small cluster of PI animals was followed by a larger cluster of PI animals. The incidence of PI calves and non-PI calves born after the oldest PI animal are shown in Fig. 1. The oldest PI animal from each herd is included in the figure in month 1. Non-PI animals are only shown until 21 months after the oldest PI animal.

In some herds where the oldest PI animal was young, not all animals up to 21 months younger than the oldest PI animal were tested. In herd 102 animals up to 14 months younger than the oldest PI animal were tested, in herd 103 animals to nine months, in herd 4 and herd 10 animals to 15 months and in the remaining herds animals to at

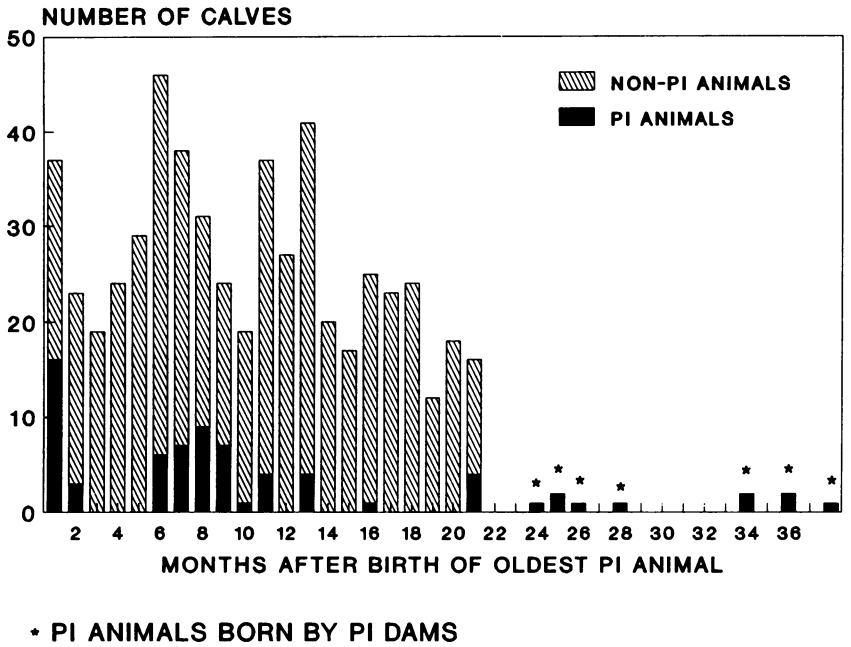

Fig. 1. Incidence of PI animals and non-PI animals born after the oldest PI animal in each of ten herds in which the range of the age of PI animals was more than six months. The oldest PI animal from each herd is included in the figure in month 1.

least 21 months younger than the oldest PI animal were tested.

Out of the 72 PI animals shown in Fig. 1, 19 (26.3%) were born within the first two months after the oldest PI animal, no PI animals were born 2-<6 months, 38 (52.7%) were born 6-<14 months, five (6.9%) were born 14-<22 months and ten (13.9%) were born later than 22 months after the oldest PI animal; these were all borne by PI dams. The incidence of PI animals and non-PI animals within the first 21 months after the oldest PI animal was significantly different (Chi-square test: $p < 0.001$, df. = 20).

DISCUSSION

The present study describes the occurrence of PI animals in 22 herds, 12 of which had experienced clinical BVD, whereas ten herds had no history of recent clinical BVD. The age distribution of PI animals was similar in outbreak herds and nonoutbreak herds suggesting similar epidemiology of these animals whether clinical or not.

The study shows considerable variability in the time span over which PI animals emerge in the herds. Thus, at the time when the first PI animal is born, births of new PI animals can be expected in many herds. In 11 herds all PI animals appeared within six months after the first PI animal, whereas in ten herds the time span between the oldest and youngest PI animal was considerably longer, mainly because PI animals remained undetected and subsequently gave birth to a new generation of PI calves.

Seronegative animals that are pregnant beyond 120 days at the time of exposure to a PI animal will probably give birth to nonviremic calves, whereas seronegative cows exposed between 40 and 120 days will give birth to calves with persistent infection. Thus, a new generation of PI calves will be born into the herd beginning approximately five months after exposure of the dams. A similar pattern has been demonstrated. In a herd with mucosal disease, four virus positive animals were detected, three of which had been born 6-10½ months after the oldest PI animal (11). The characteristic pattern of the incidence of birth of PI animals illustrated in Fig. 1 also shows that in many herds the infection is introduced by means other than by PI animals. Otherwise there would not be the time lag between the birth of PI animals. When PI animals themselves remain undetected and later become pregnant with PI fetuses, the time span where PI animals emerge in the herd is considerably longer, in this study up to three years. Also it appears that the transmission of the infection from PI animals to seronegative stock does not occur over a short time period in all herds. Thus, in some herds of this study, PI calves were borne by non-PI mothers later than nine months after the appearance of the first PI animal in the herd (cf. Fig. 1). This means that the BVDV transmission from PI animals in rare cases may occur over a time period of several months, a finding that has been reported earlier (12).

It may be a drawback that in some herds where the oldest PI animal was young not all animals until 21 months younger than the oldest PI animal were tested. But, except for PI animals borne by PI dams all animals were tested equally. Further, animals sold or slaughtered before blood test would have involved both PI animals and non-PI animals. So, the relationship between PI animals and non-PI animals would remain the same.

Among the 129 PI animals in this study some might have been acutely infected instead of persistently infected because many PI animals were only blood tested on a single occasion. Since 93% of the viremic animals from the prevalence study actually were PI (2), the applied definition of PI animals will be wrong only on rare occasions.

The finding that PI animals were detected in approximately 50% of herds in which BVD outbreak had not been seen (2), emphasizes the importance of BVDV infection in Danish livestock production. Further, it was found that herds with one or more PI animals have a high proportion of seropositive animals (in average 87%), compared to a low proportion in herds without PI animals (in average 43%). This finding may be utilized in the early detection of PI animals. Serological examination of blood samples taken from a limited proportion of the animals in a herd will reveal the presence or absence of PI animals in the herd with high accuracy (13). Together with the age distribution of PI animals it should be possible to find and eliminate PI animals as a part of a control program without heavy expenses.

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