

# **Pestivirus Infections in Norway. Serological Investigations in Cattle, Sheep and Pigs**

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**Løken, T., J. Krogsrud and I. L. Larsen: Pestivirus infections in Norway. Serological investigations in cattle, sheep and pigs. Acta vet. scand. 1991, 32, 27-34.** – Serum samples from 1133 dairy cows (187 herds), 3712 ewes (103 flocks) and 1317 adult pigs (877 herds), were tested for neutralizing antibodies against the NADL strain of bovine virus diarrhoea virus. The prevalence rate of seropositive animals was 18.5 % in cattle, 4.5 % in sheep and 2.2 % in pigs, such seroreactors being found in 28 % of the cattle herds and 18 % of the sheep flocks. In all three species the rate showed considerable herd and geographical variation. In cattle the seroreactor rate was similar in herds with normal reproduction and in 62 herds with problems of repeat breeding.

Of 31 pig sera containing antibodies against the NADL strain, 27 were also positive in a neutralization test for antibodies against swine fever virus (Baker strain). However, all sera showed a higher titre of antibodies against the bovine strain than against the swine fever virus. It was concluded that the immune response of the pigs had been induced by ruminant pestivirus, and not by swine fever virus.

bovine virus diarrhoea; border disease; swine fever; neutralizing antibodies.

## **Introduction**

Members of the genus *Pestivirus* in the family *Togaviridae* infect several species of domestic and wild ruminants, and also pigs (Baker *et al.* 1954, Acland *et al.* 1972, Doyle & Heuschele 1983, Harkness 1985). Diseases caused by pestiviruses encompass the bovine virus diarrhoea/mucosal disease (BVD/MD) syndrome in cattle (Baker 1987), border disease (BD) in sheep and goats (Terpstra 1985), and swine fever (SF) (Harkness 1985). As regards goats, signs and lesions of BD have been reported on only one occasion, in a single kid in Norway (Løken *et al.* 1982a), however, serological investigations in some countries have indicated widespread infection with pestivirus antibodies in this species (Løken 1990). Infec-

tion with pestivirus in cattle have a worldwide distribution, prevalence of antibodies ranging mostly between 50 % and 90 % (Baker 1987). Pestivirus antibodies in sheep have also been recognised in several countries, but usually the prevalence has been shown to be much lower than in cattle (Barlow & Patterson 1982, Terpstra 1985). Outbreaks of SF occur worldwide (Harkness 1985), but the Nordic countries are currently free of this disease.

Bovine and ovine isolates of pestivirus have been shown to be closely antigenically related (French & Snowdon 1964), and should probably be regarded as strains of the same virus (Done *et al.* 1980). They may be pathogenic not only in cattle and sheep, but

also in pigs (Terpstra & Wensvoort 1988). Though to a lesser degree, ruminant strains are antigenically related also to SF virus (Mengeling et al. 1963, Plant et al. 1973), which appears to infect pigs only (Harkness 1985). Neutralizing antibodies against one strain of pestivirus will also show neutralizing activity against the other viruses in this genus (Hafez et al. 1976, Vantsis et al. 1980). In pigs, infections with ruminant pestiviruses induce a distinctly higher antibody titre against such strains than against strains of SF virus, and vice versa (Jensen 1985, Terpstra & Wensvoort 1988).

While BVD/MD in cattle and BD in sheep are endemic in Norway, this has never been the case for SF, the last outbreak occurring in 1963 (Animal Health Yearbook 1987).

The purpose of the present study was to estimate the prevalence rate of neutralizing antibodies against pestivirus in populations of cattle, sheep and pigs in this country.

## Materials and methods

### *Serum samples*

**Cattle:** Blood samples were randomly taken during the period 1984–86, from altogether 1133 dairy cows of various ages over 2 years in 187 herds of Norwegian Red Cattle (Table 1). Herds sampled were selected so as to ensure a reasonably wide geographic representation. Of the 1133 sampled cows, 536 were from 62 herds with reproductive problems of repeat breeding, 495 from 99 herds (5 from each herd) providing bull calves for testing as potential semen donors for artificial insemination, and 102 cows from 25 randomly selected herds in the county of Troms in northern Norway.

**Sheep:** A total of 3712 ewes of different breeds in 103 flocks distributed throughout the country, were sampled during 1980–84 (Table 2). Of these, 2993 samples were from 80 flocks taking part in a maedi/visna con-

trol scheme, 205 from 6 flocks affected by different disease problems not associated with pestivirus, and 514 from 17 flocks in Troms.

**Pigs:** Serum were obtained at random from 1317 adult breeders in 877 Norwegian landrace or Yorkshire herds in 1984 or 1986. The samples were collected at slaughter at 8 abattoirs in different regions of Norway (Table 3).

### *Laboratory examinations*

All sera, stored at  $-20^{\circ}\text{C}$ , were tested in two-fold dilutions for neutralizing antibodies against the cytopathic NADL strain of BVD/MD virus as described by Løken et al. (1982b). The lowest serum dilution was 1:4. One half of the sheep sera which were positive for NADL-antibodies (titres  $\geq 4$ ), were similarly tested for neutralizing antibodies against 3 additional cytopathic strains of pestivirus (Løken 1990): the oregon strain (Gillespie et al. 1960), a 20-passage Norwegian MD isolate, and a low passage English ovine BD isolate (obtained from Dr. P. Roeder, Central Veterinary Laboratory, Weybridge, UK).

Pig sera which proved to contain NADL-antibodies, were similarly tested for antibodies against 100 median tissue culture infectious doses (TCID<sub>50</sub>) of the non-cytopathic Baker strain of SF virus (obtained from the Central Veterinary Laboratory, Weybridge, UK) by a neutralizing peroxidase-linked antibody assay (NPLA) based on the technique described by Jensen (1981). A swine-anti-SF-virus (Alfort strain) serum (obtained from the State Veterinary Institute for Virus Research, Lindholm, Denmark) was used as primary antibody in the immuno-peroxidase technique. The test sera were all examined in two-fold dilutions in microtitre plates with primary cultures of pig kidney cells. The lowest serum dilution was 1:2.

Neutralization titres are expressed as the reciprocal of the highest serum dilution that prevented virus replication in at least one of the two replicate cultures, recorded as cytopathic effect or as positive immunoenzymatic staining (NPLA).

Three different antisera were used in the control work. One was the Alfort strain antiserum, and the other two were from a pig (D4/85) and a calf (D5/85), both inoculated intranasally and intramuscularly with 17 different bovine and ovine strains of pestivirus. These sera had neutralizing antibodies against the NADL strain in titres of 8, 128 and 2048, and against the Baker strain in the NPLA in titres of 1024, 8 and 16, respectively.

## Results

### Cattle

Neutralizing antibodies against the NADL strain were found in 210 cows (18.5 %) in 53 herds (Table 1), the prevalence rate of seropositive cows in different areas ranging from 4 to 56 %. The highest rate of positive animals found was in the county of Rogaland in western Norway. The overall rate in northern Norway was as low as 6.5 %, being particularly low in the county of Troms (4 % of 155 cows). Samples from herds with in-

creased proportion of repeat breeders were positive for antibodies in 19.6 % of 537 animals investigated. A similar prevalence was found in the 495 cows examined in herds from which potential semen donors were selected. In 21 herds, all animals examined were found to be antibody positive, while in 12 others only a single cow was found to be positive in each herd. When only the positive herds (53) were included, a rate of 56 % seropositive cows was demonstrated, a figure which is more than 3-fold higher than for the material as a whole. The titres showed a normal distribution curve, ranging from 4–2048 (Table 1), a peak number of 47 samples having a titre of 128.

The presence of antibodies seemed to be positively correlated to age, the seropositive rate being 16 % in cows aged between 2 and 4 years, 24 % in 5-year olds, and 26 % in 6-year olds or above. The titre distribution was similar within the different age groups.

### Sheep

A total of 166 ewes were positive for antibodies against the NADL strain (Table 2). The prevalence rate of seropositive sheep ranged between 1 % and 100 % in the positive flocks, the overall rate being 4.5 %. Considerable regional differences were

Table 1. Prevalence and titre distribution of neutralizing serum antibodies against bovine virus diarrhoea virus (NADL strain) in dairy cows.

Region of Norway	Number of positive		Individual prevalence rate (%)	Distribution of titres		
	herds	cows		4–16	32–256	512–2048
South-eastern	26 (N= 80)	126 (N= 520)	24.2	8	81	37
Western*	17 (N= 54)	66 (N= 337)	19.6	4	53	9
Northern**	10 (N= 53)	18 (N= 276)	6.5	4	13	1
Totals	53 (N=187)	210 (N=1133)	18.5	16	147	47

N = Total number of herds/cows.

\* Included animals in the county of Rogaland (N=19/N=100).

\*\* Composed of animals in the counties of Sør-/Nord-Trøndelag, Nordland, Troms, Finmark.

Table 2. Prevalence and titre distribution of neutralizing serum antibodies against bovine virus diarrhoea virus (NADL strain) in ewes.

Region of Norway	Number of positive		Individual prevalence rate (%)	Distribution of titres		
	flocks	sheep		4-16	32-256	512-1024
South-eastern	7 (N= 56)	13 (N=2216)	0.6	5	6	2
Western*	12 (N= 30)	153 (N= 982)	12.6	66	70	17
Northern**	0 (N= 17)	0 (N= 514)				
Totals	19 (N=103)	166 (N=3712)	4.5	71	76	19

N = Total number of flocks/ewes.

\* Included animals in the county of Rogaland (N=17/N=591).

\*\* Composed of animals in the county of Troms only.

found, the seropositive rate being high in western Norway (12.6 %, with 17.1 % in the county of Rogaland), low in the south-eastern region (0.6 %), and zero in northern Norway. Considering the positive flocks alone, i.e. flocks in which at least 1 seropositive animal was detected (19 flocks, 845 examined sheep), the prevalence rate of seropositives was 19.6 %, a figure which was about 4-fold higher than in the sheep material as a whole. Antibody titres in sheep also showed a normal distribution curve, ranging from 4 to 1024, samples peaking numerically at a titre of 16. Sera with titres ranging from 8-32 comprised 79 (48 %) of the 166 positives.

All but 2 of the 79 sera which were titrated against 4 different virus strains, showed a maximum 4-fold variation in titres for the different strains. The geometrical mean titre (GMT) of these sera with respect to the Norwegian MD isolate was 177, and ranged between 101 and 111 with respect to the 3 other strains.

#### Pigs

Neutralizing antibodies against the bovine NADL strain were demonstrated in 31 pigs in 30 herds (Table 3). As in cattle and sheep, the rate of NADL-antibody positive animals showed considerable regional variation: 6.4 % in the county of Rogaland on the one

Table 3. Prevalence and titre distribution of neutralizing serum antibodies against bovine virus diarrhoea virus (NADL strain) in pigs.

Region of Norway	Number of positive		Individual prevalence rate (%)	Distribution of titres		
	herds	pigs		4-16	32-128	256-512
South-eastern	18 (N=670)	18 (N=1026)	1.8	13	3	2
Western*	12 (N=159)	13 (N= 204)	6.4	8	5	
Northern**	0 (N= 48)	0 (N= 87)				
Totals	30 (N=877)	31 (N=1317)	2.2	21	8	2

N = Total number of herds/pigs.

\* Composed of animals in the county of Rogaland only.

\*\* Composed of animals in the counties of Nord-Trøndelag, Nordland, Troms, Finmark.

hand, and zero in northern Norway on the other. Though antibody titres ranged from 4 to 512, 21 (70 %) of the sera had titres as low as from 4–16.

In the NPLA for SF-antibodies, 4 sera with BVD/MD-antibodies were negative (titre < 2). A titre of 2 was found in 14 sera, and titres of 4 or 8 in 12 sera. One serum, which had NADL-antibodies in a titre of 512, showed a titre of 32. In all 27 samples positive for SF-antibodies, the titre of antibodies against the bovine virus was higher by two-fold (7), four-fold (9) or more (11) than that against the SF virus.

### Discussion

Neutralizing antibodies against the NADL strain of BVD/MD virus were found in cattle, sheep and pigs. The prevalence rate of 18.5 % seropositive cows detected in Norwegian dairy herds is low compared to the situation in several other countries as reviewed by Baker (1987). In Finland, however, Alenius (pers. com.) found antibody-positive cows in only 3 % of 200 dairy herds. The rate of 4.5 % seropositive sheep is in accordance with findings in many other countries (Barlow & Patterson 1982). Pigs positive to antibodies against a bovine strain of pestivirus at a rate of 2.2 % is in the lower range reported from other European countries (Jensen 1985, Terpstra & Wensvoort 1988).

Transplacental infection with BVD/MD virus in cattle may cause a range of different reproductive failures (Done *et al.* 1980, Duffell & Harkness 1985). Also outbreaks of early foetal death causing repeat breeding have been found to be a facet of the BVD/MD syndrome (Zemjanis & Hurtgen 1975, Løken *et al.* 1989). The prevalence of pestivirus antibodies in the herds with problems of repeat breeding in this study, however, was found similar to that in the herds

with normal pregnancy rates. These findings accord with other studies (Whitmore *et al.* 1981, Grahn *et al.* 1984), indicating that infection with BVD/MD virus is not a major cause of repeat breeding in cattle.

Comparison of results from different areas of the country, showed that the prevalence of animals positive for NADL-antibodies was highest in all 3 species in the county of Rogaland. Remarkably, in northern Norway no seropositive sheep or pigs were found at all, while cattle were positive at only a low rate. In a study in Norwegian goats, 3.6 % of the animals were shown to be positive for NADL-antibodies, but again, all animals examined in northern Norway proved to be negative (Løken 1990). The very low prevalence of infection with pestiviruses in northern Norway, and the high prevalence in Rogaland, found in the present study, may be partially explained by the predominance of small, "closed" herds in the former part of the country, with little trade in livestock, and a high intensive system of livestock farming, with high animal density and frequent trade and transfer of animals between herds in the latter.

The apparently positive correlation between age and prevalence of pestivirus antibodies in cattle is in accordance with earlier findings in both cattle and sheep (Sands & Harkness 1978, Løken *et al.* 1982b). The increase in prevalence with age probably reflects accumulation over the years of long-lasting neutralizing antibodies, the production of which has been provoked by pestivirus infections.

Findings of a 3 or 4-fold higher prevalence rate of seropositive animals in the positive cattle herds and sheep flocks as such, compared with that for the material as a whole, probably reflects aspects of the epidemiology of this infection. Transmission of pestivirus from postnatally infected animals is prob-

ably of little significance, whereas introduction of persistently infected animals into susceptible herds is likely to constitute a very efficient, and probably common mode of natural dissemination (Duffell & Harkness 1985). The distribution of NADL-antibodies was similar in cattle and sheep. However, the curves of distribution showed that positive sera in cattle peaked numerically at a titre which was about 8-fold higher than for the corresponding peak in sheep, probably reflecting a closer antigenic relationship between the NADL strain and the field strains in cattle than in sheep.

When comparing titres of antibodies in sheep sera against 4 different pestivirus strains, the highest GMT was found against the Norwegian MD strain, indicating that this bovine isolate is the one antigenically most closely related to the field strains infecting the sheep. Similar serological results have been found in goats (Løken 1990). This apparent antigenic conformity probably reflects a relationship between Norwegian ruminant strains in general, rather than between sheep strains and this particular bovine strain.

In differentiating between antibodies against BVD/MD and SF viruses in pig sera, the neutralization test and the NPLA have been shown to be sensitive and reliable (Jensen 1981, Terpstra et al. 1984). In this study, only 1 pig serum showed a SF titre higher than 8, and all sera showed a lower titre of antibodies against the SF virus than against the BVD/MD virus. The pig and the calf control antisera which had high titres of NADL-antibodies induced by infection with different ruminant strains, had both a low titre of SF-antibodies (8–16), while the SF antiserum showed opposite results. The findings clearly indicated that the immune response in 31 pigs was induced by rumi-

nant strains of pestivirus and not by SF virus. Since persistent infection of ruminant pestiviruses rarely occurs in pigs (Terpstra & Wensvoort 1988), the seropositive animals had probably been infected by incidental virus transmission from ruminants, and not horizontally from other pigs.

Infection with SF virus usually induces a high titre of neutralizing antibodies, which will neutralize also bovine strains at low dilution (Terpstra et al. 1984). However, the choice of test strain may be crucial (Wood et al. 1988). Since SF has not been diagnosed in Norway during the last 25 years, the sera found negative for antibodies against the NADL strain (titre < 4) were not tested for SF antibodies.

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### Samandrag

*Infeksjonar med pestivirus i Noreg.*

*Serologiske undersøkingar hos storfe, sau og gris.*

Serumprøver frå 1133 mjølkekyr, 3712 søyer og 1317 vaksne grisar frå høvesvis 187, 103 og 877 buskapar i ulike område i Noreg vart undersøkte for nøytraliserande antistoff mot bovin virusdiaré-virus (NADL stamme). Prevalens av antistoff-

positive storfe, sau og gris var høvesvis 18,5 %, 4,5 % og 2,2 %. Denne raten hjå storfe var omlag den same i 62 buskapar som hadde problem med mykje omløp. Hjå alle 3 dyreartane varierte raten mykje mellom ulike buskapar og geografiske område. Seropositive dyr vart påvist i 28 % av buskapanane med storfe og i 18 % med sau.

Av 31 svinsera som var positive for antistoff mot NADL stamma, var 27 positive også i ein nøytralisasjonstest for antistoff mot svinepestvirus (Baker stamme). Alle prøvene viste høgre titer av antistoff mot NADL stamma enn mot svinepestviruset. Det vart konkludert med at desse antistoffa skuldast immunsvaret på infeksjon med drøvtyggjarsammer av pestivirus, og ikke svinepestvirus.

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