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BORDER DISEASE IN NORWAY

SEROLOGICAL EXAMINATION OF AFFECTED SHEEP FLOCKS

By

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LØKEN, T., B. HYLLSETH and H. J. LARSEN: *Border disease in Norway: Serological examination of affected flocks.* Acta vet. scand. 1982, *23*, 46—52. — Serological examination of 4 Border disease affected flocks of sheep using the neutralization test showed antibody prevalences between 14 and 96 %. Prevalence in yearlings in 3 of the 4 flocks was 37 %, it increased with age to 72 % in 5-year-old sheep. Possible reason for low prevalence (2 %) in yearlings in one of the flocks is discussed.

pestivirus; Border disease; bovine virus diarrhoea; sheep; serology; neutralizing antibodies.

Border disease (BD) in sheep was first described by *Hughes et al.* (1959) and has since been reported in several countries (see *Terlecki* 1977). In Norway BD was first diagnosed in 2 flocks of sheep in 1979 (*Løken & Barlow* 1981). The virus causing BD is a pestivirus in family Togaviridae (*Matthews* 1979), it is antigenically related to bovine virus diarrhoea (BVD) and swine fever viruses (for references see *Horzinek* 1981). BVD virus has frequently been referred to as mucosal disease virus or BVD-mucosal disease virus. Neutralizing antibodies to BD virus may be detected using a bovine strain of BVD virus (*French & Snowdon* 1964, *St. George* 1971, *Harkness et al.* 1978, *Terpstra* 1978). The purpose of the present work was to obtain serological information on the prevalence of antibodies to BD virus within selected flocks where BD had been diagnosed on clinical and pathological grounds.

MATERIALS AND METHODS

Blood samples were collected after the lambing season in May 1980 from all 460 yearlings and older sheep in 4 flocks in south-western Norway, 3 of which had shown BD affected lambs.

Sera were stored at —20°C until examined in the neutralization test (NT). The cytopathogenic NADL strain of BVD virus was propagated in primary calf kidney monolayer cell cultures in bottles at 37°C. Microtiter plates (Culturette®) were used for end-point assay and NT. The plates were incubated at 37°C in an atmosphere of 5 % CO_2 in air. Cell culture growth medium consisted of Eagle's minimum essential medium (MEM) with Hanks' salts (Gibco), 10 % heat inactivated (56°C for 30 min) newborn calf serum, sodium bicarbonate (0.4 mg/ml), penicillin (100 IU/ml), streptocillin (100 µg/ml) and amphotericin B (Fungizone®) (2.5 IU/ml). Maintenance medium was MEM with Earle's salts, 3 % heat inactivated horse serum, sodium bicarbonate (1.5 mg/ml) and the above mentioned antibiotics. Maintenance medium was used as diluent for sera and virus. Heat inactivated test sera were first tested in dilution 1:2. All positive sera were further tested in two-fold dilutions 1:4—1:512 (cf. Table 1). Equal volumes of serum dilution and virus dilution (containing 100 $TCID_{50}$/25 µl) were mixed and incubated at 37°C for 1 h. Monolayer cell cultures were washed twice (PBS, pH 7.5), 3 wells were then inoculated with each serum-virus mixture (50 µl/well) and incubated at 37°C for 1 h before adding maintenance medium (150 µl/well). Positive and negative control sera as well as virus assay were included in each test. Cell cultures were examined microscopically on days 5 and 7 after inoculation. Neutralization titres are expressed as the reciprocal of the highest serum dilution that prevented cytopathogenic effect in at least 2 of the 3 replicate cultures.

RESULTS

Sera from a total of 460 sheep in 4 flocks were examined. Table 1 shows that prevalence of antibodies to BD virus ranged between 14 and 96 %; only 8 out of 216 positive animals had titres ≤ 8. In Table 2 prevalence of seropositive sheep as well as distribution of titres are listed according to age. The prevalence of seropositive sheep was increasing from 37 % for yearlings in 3 of 4 flocks to 72 % for 5-year-old sheep. In animals over 5 years

Table 1. Prevalence and titre distribution of neutralizing antibodies to BVD virus in 4 flocks of sheep in south-western Norway.

Flock No.	Number of animals	Seropositive animals					
		Number	Prevalence %	Distribution of titres[a] 2—8	16—64	128—512	> 512
1	162	87	54	3	18	64	2
2	124	35	28	4	13	17	1
3	84	81	96	0	17	61	3
4	90	13	14	1	4	7	1
Total	460	216	47[b]	8	52	149	7

[a]) Reciprocal of serum dilution
[b]) Average prevalence.

old there was a decline with an average for years 6—8 of 52 %. The prevalence according to age showed similar patterns in the 4 flocks except that in Flock 1 (Table 2) only 1 out of 64 yearlings (2 %) was positive.

Table 2. Age specific prevalence and titre distribution of neutralizing antibodies to BVD virus in 4 flocks of sheep in south-western Norway.

Animals examined		Seropositive animals					
Age (years)	Number	Number	Prevalence %	Distribution of titres[a] 2—8	16—64	128—512	> 512
1[b]	64	1	2			1	
1[c]	81	30	37		2	27	1
2	109	54	50	2	10	37	5
3	76	52	68	3	15	33	1
4	58	34	59	1	12	21	
5	39	28	72	1	8	19	
6	26	15	58	1	5	9	
7	6	1	17			1	
8	1	1				1	
Total	460	216	47[d]	8	52	149	7

[a]) Reciprocal of serum dilution
[b]) Flock 1
[c]) Flocks 2, 3 and 4
[d]) Average prevalence.

DISCUSSION

Serological examination for antibodies against Border disease (BD) virus was carried out using the bovine virus diarrhoea (BVD) virus as test antigen in the neutralization test (NT). In a comparative test on 142 bovine serum pairs the NT has been reported as superior in sensitivity to immunodiffusion test and complement fixation test (*Harkness et al.* 1978). Reliable use of heterologous BVD virus in NT to detect neutralizing antibodies to BD virus requires that these viruses are rather closely related. BD and BVD viruses can cross infect sheep and calves (*Barlow et al.* 1980a and b) and are reported by many workers to be antigenically related (for references see *Horzinek* 1981). The two viruses have even been regarded as strains of the same virus (*Done et al.* 1980) or as pestivirus serotypes (*Horzinek*). Experimental infection with BVD or BD virus in sheep is reported to induce production of cross neutralizing antibodies (*Vantsis et al.* 1979 and 1980a, *Barlow et al.* 1980b) usually showing highest titres to the homologous BD virus (*Barlow et al.* 1980b, *Vantsis et al.* 1980b).

Use of serum dilution as low as 1:2 generally increases the risk of non-specific neutralization of virus. In the present work the proportion of sera with low range titres of 1:2—1:8 represented only about 4 % of the total number of positive sera, and it was not considered a possible error of any consequence. Other workers have even used undiluted ovine serum in NT (*Hamilton & Timoney* 1973).

Diagnosis of BD in Flocks 1 and 2 based on clinical signs, pathology and serology has been reported (*Løken & Barlow* 1981). In Flock 3 BD was diagnosed in twin lambs with characteristic symptoms at the time of serum collection. Flock 4 was originally selected as control since no signs indicative of BD had been observed. However, 1 year after collecting the serum samples that showed a fairly low prevalence of seropositive animals (14 %, Table 1), twin lambs with characteristic signs of BD were borne in this flock and the dam had seroconverted during that year to a titer of > 512.

The prevalence in Flocks 2 and 4 were within the same range as that found in sheep in other countries (*French & Snowdon* 1964, *Hamilton & Timoney*). Flock 3 showed very high prevalence of seropositive animals, and it is remarkable that clinical

cases of BD were not observed prior to serum collection. A possible source of infection in this flock was thought to be a calf which died from a BVD virus infection. Such infections have been recognised for many years in Norway and BVD virus was isolated in 1967 (*Saxegaard et al.* 1971). Ewes inoculated with BVD virus between days 12 and 70 of gestation have been reported to produce signs and lesions in fetuses and lambs characteristic of BD (*Plant et al.* 1976, *Barlow et al.* 1980a).

A positive relationship between increasing age and prevalence of seropositive sheep agrees with earlier reports (*St. George* 1971, *Sands & Harkness* 1978). The increase in prevalence with age shown in the present work (Table 2) indicates presence of a persistent source of virus resulting in a fairly high prevalence in yearlings (37 % in 3 of 4 flocks) and a gradual increase up to 5 year old sheep (72 %). The most likely source of virus is persistently infected lambs from infected ewes as shown experimentally by *Barlow et al.* (1980b) and *Terpstra* (1981). These workers have reported presence of virus in many organs of both apparently normal and clinically affected lambs that were either seronegative (immune tolerant) or had low titres of neutralizing antibodies. Apparently normal but persistently infected sheep can live at least 2½ years and transmit virus to in-contact sheep (*Terpstra* 1981).

In Flock 1 with prevalence of 54 % (Table 1) only 1 of the 64 yearlings was seropositive (Table 2). This indicates a reduced infection pressure during the previous year. One possible reason for this may have been that, since the flock belonged to the State Veterinary Research Station, it was closely watched for BD affected lambs and those with any clinical signs were removed as suggested by *Barlow et al.* (1980b).

The 4 flocks with seropositive sheep had only minor problems with abortions and congenital disease characteristic of BD. Only 10 lambs examined at the State Veterinary Research Station during the last 3 years have been diagnosed as BD, all were from these 4 flocks. Clinical BD is obviously a poor indicator of presence of virus infection. A serosurvey would be necessary to obtain better knowledge of the geographical spread of BD virus infections and of circumstances that influence the spread.

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SAMMENDRAG

Border disease i Norge. Serologisk undersøkelse i berørte sauebesetninger.

Serologiske undersøkelser ved hjelp av nøytralisasjonstest i 4 sauebesetninger med Border disease viste antistoff prevalenser mellom 14 og 96 %. Prevalensen hos årsgamle lam i 3 av besetningene var 37 %, den økte med alder til 72 % hos 5 år gamle sauer. Mulig årsak til lav prevalens (2 %) for årsgamle lam i én av flokkene er diskutert.

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