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Small Ruminant Research 93 (2010) 157–164

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Occurrence and spatial distribution of Toggenburg Orbivirus in Switzerland

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ARTICLE INFO

Article history:
Received 12 February 2010
Received in revised form 21 May 2010
Accepted 28 May 2010
Available online 1 July 2010

Keywords:
Toggenburg Orbivirus
Epidemiology
Prevalence
Goat
Sheep
Cattle

ABSTRACT

Recently, a new member of the Bluetongue virus (BTV) serogroup named Toggenburg Orbivirus (TOV) in goats from Switzerland has been described. The epidemiology and host range of TOV are currently unknown. Since TOV causes cross-reactions in laboratory tests used for BTV diagnosis, this study was carried out in order to determine the spatial and temporal spread of TOV. Therefore, serum samples from a national survey in goats, collected during winter and spring 2008 in Switzerland, were serologically examined. Additionally, cattle and sheep from holdings with seropositive goats were tested for the presence of viral RNA and antibodies against BTV and TOV. All goat samples analysed within routine diagnostics at the Institute of Virology and Immunoprophylaxis from 2008 to 2009 were also tested for the presence of TOV. Finally, goat sera collected 1998 in the Canton of Ticino (TI) were analysed.

Although the TOV index cases had been identified in flocks north of the Alps, no additional TOV-positive herds were found by serological testing in this region. In contrast, south of the Alps, i.e. in the Canton of Ticino (TI), an apparent seroprevalence of 49% in goats was found at animal and 60% at herd level. In the eastern and western part of the Swiss Alps 15.2% and 10% of tested goats were serologically positive, respectively. A within-herd prevalence of up to 100% was found in some of the positive flocks. The positive flocks in TI were mainly found in three of the five districts, but seropositive animals were identified in each district. Certain selected seropositive flocks were investigated virologically. By RT-qPCR and genome sequencing, the presence of TOV could be confirmed in all investigated seropositive flocks.

By testing the goats within routine diagnostics, TOV genome was detected in one goat showing BT-like clinical symptoms from the central Alps and in three healthy animals imported from Germany.

Although 3.8% of the sheep from flocks with TOV-positive goats or in contact with these animals showed a positive antibody reaction, TOV-specific RNA was not found in any of the tested sheep and also not in cattle from flocks with TOV-positive goats.

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doi:10.1016/j.smallrumres.2010.05.016

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Serological and virological test results from archived Swiss goat samples collected in 1998 indicated the presence of TOV already at that time, prior to any Bluetongue disease outbreak in this part of Europe. The results of this study demonstrate that TOV is widespread in certain parts of Switzerland and suggests that this virus has been present in the goat population for at least a decade, albeit without causing any disease signs.

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1. Introduction

The genus *Orbivirus*, one of the 15 genera of the family Reoviridae, includes different non-enveloped dsRNA viruses, such as Bluetongue virus (BTV). Orbiviruses share common features like a genome divided into 10 segments and the transmission by arthropods. Recently, several new species of Orbiviruses have been discovered, such as Yunnan orbivirus in China (Attoui et al., 2005), Middle Point orbivirus (Cowled et al., 2007) and Stretch Lagoon orbivirus (Cowled et al., 2009) in Australia, and Toggenburg Orbivirus (TOV) (Hofmann et al., 2008a; Chaignat et al., 2009) in Europe. The latter is proposed to represent the 25th serotype of the Bluetongue (BT) serogroup. The BTVs are mainly transmitted by haematophageous midges of the genus *Culicoides* species. Although all ruminants are susceptible to BTV infection, severity of disease varies between species and even between breeds of the same species (Gard, 1984). In particular, certain breeds of sheep and deer are clinically affected; whereas disease is rarely seen in goats and cattle and mostly unapparent in many wild ruminant species (MacLachlan, 2004; Lefevre et al., 2008). However, the BTV serotype 8 (BTV-8) strain currently circulating in Europe, is an exception insofar as it regularly leads to clinical disease in cattle (Saegerman et al., 2008).

Active surveillance had been implemented in Switzerland (CH) already since 2003 due to the presence of BTV in Italy and allowed to demonstrate freedom from BT until the first BTV-8 outbreak in late autumn 2007 (Hofmann et al., 2008b). To this end, a nationwide serological survey was conducted in cattle (Cagienard et al., 2006). Furthermore, sentinel cattle were regularly examined in the Canton of Ticino (TI), the region south of the Alps bordering Italy (Racloz et al., 2006). By 2007 a national programme comprising active clinical surveillance in sheep (Stuber et al., 2009) and bulk milk serology in cattle started (Schwermer et al., 2008; Kluiters et al., 2008).

The TOV was first detected in December 2007 in Swiss goats tested for the presence of BTV-specific genome and antibodies (Ab) prior to the transport outside of the protection zone established because of the first BTV-8 cases. The animals were found to be positive for BTV genome in quantitative RT-PCR (RT-qPCR) and Ab in commercial BTV-serogroup specific ELISA. Partial genome sequencing demonstrated that these results were due to TOV and not to any other known BTV serotype (Hofmann et al., 2008a). No clinical signs were observed in TOV-affected adult goats in the field or in experimentally infected goats (Chaignat et al., 2009).

It is not possible to differentiate TOV from other BTV serotypes, either serologically with the currently available ELISAs, or by RT-qPCR used for BTV serogroup genome detection at our Institute (RT-qPCR-panBTV), based on segment 10 (S10) (Hofmann et al., 2008b). Hence, we developed a TOV-specific RT-qPCR (RT-qPCR-TOV), based on segment 2 (S2) (Hofmann et al., 2010) to allow differentiation of TOV from all other BTV serotypes.

Since (i) goats do not show clinical signs when infected with TOV, (ii) in the two TOV index cases a large proportion of these flocks was positive for TOV and (iii) these two flocks had no epidemiological link and were located >100 km apart, we hypothesised that TOV could be widespread within CH. Therefore, a serological cross-sectional national survey for TOV in the goat population was performed in the winter 2007/2008. Samples from seropositive herds were additionally examined for TOV-specific RNA. Screening for Ab was suitable as at that time only few BTV-infected holdings had been identified in a small area in the northern part of the country, and no BTV vaccination was performed before June 2008.

Additionally, there were concerns about possible affection of cattle and sheep by TOV. These animals are economically more relevant than goats, and cattle are often exported from CH to BT-negative countries.

To distinguish whether TOV had been newly introduced into CH or had been present in the country for several years, goat serum samples from 1998 were also examined for TOV.

Depending on the methods used for Ab and RNA detection, i.e. the used RT-qPCR, TOV cannot be detected in all the cases. Sometimes TOV-infected goats could be serologically positive for BTV but negative for viral genome by BTV RT-qPCR targeting segment 1 or 5 instead of S10 (Hofmann et al., 2008a). To shed more light on the prevalence of TOV in Europe, testing of imported goats for TOV was implemented, and collaboration programmes were started with neighbouring countries (Italy, France).

In this paper we present results about the investigations on spatial distribution, susceptibility of sheep and cattle to TOV in the field, and the temporal presence of TOV in CH.

2. Materials and methods

2.1. Goat sera from a CAE cross-sectional study in 2008

The sampling procedure is described in Fig. 1. The 2033 samples from the cross-sectional survey of Caprine Arthritis Encephalitis (CAE) were collected between February and March 2008 (CAE08), prior to any vaccination against BTV-8, and were used for serological screening.

In a first phase, 1123 randomly selected samples (839 from north of the Alps, 113 from Canton of Grisons [GR], 66 from Canton of Valais [VS] and 105 from TI), 5 samples per herd and representative for the Swiss goat population, were examined for the presence of BTV Ab. Due to the overrepresentation of TI for seropositive animals, all serum samples (910) of CAE08 from this region were examined in a second step.

2.2. Goat sera collected in 1998 from TI

For the retrospective look into the TI goat population, 323 serum samples from 29 holdings, collected in 1998 and kept in a serum bank (Institute

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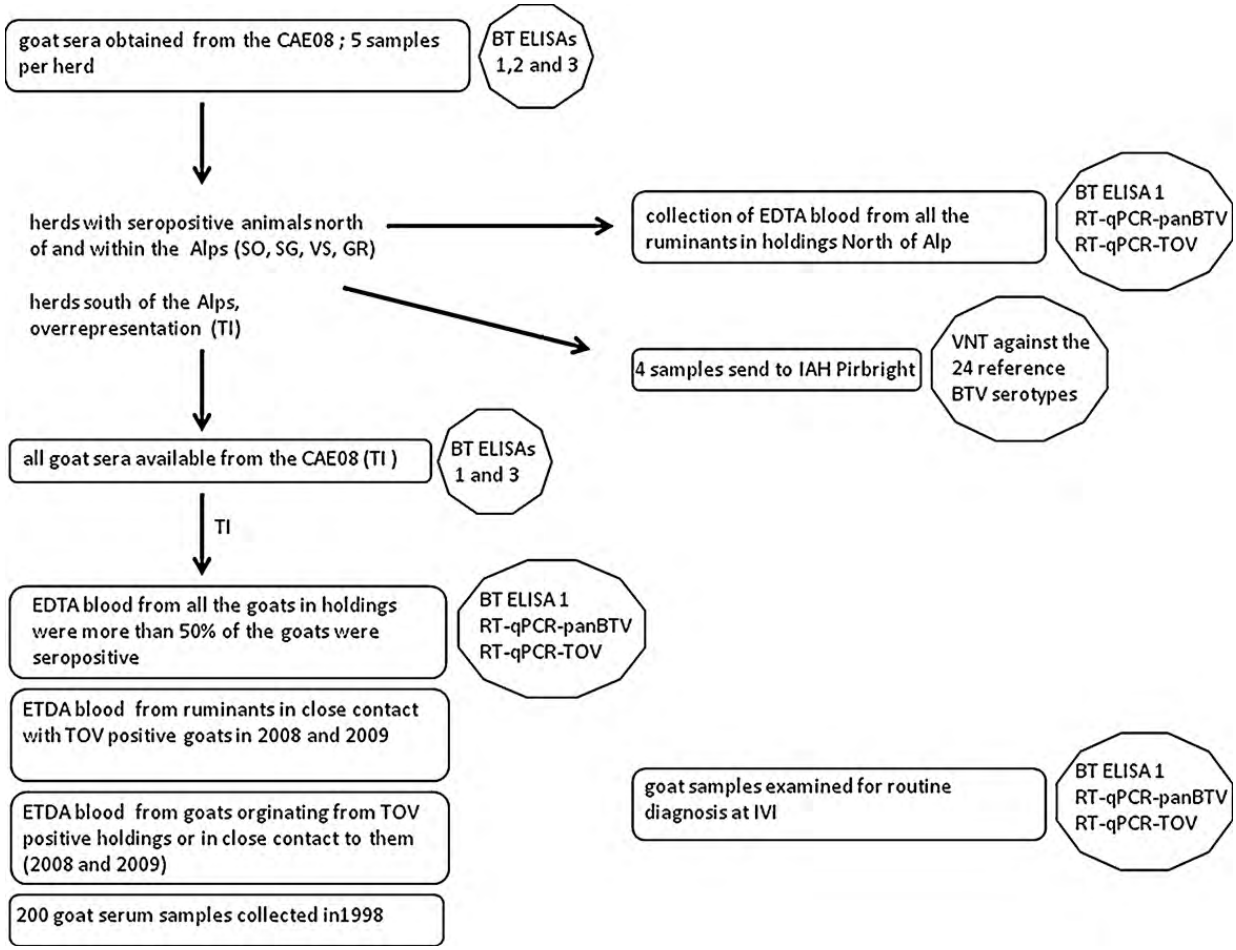


Fig. 1. Workflow of sampling and testing procedure. Serum samples from the cross-sectional survey of Caprine Arthritis Encephalitis, collected between February and March 2008 (CAE08) were examined using the following BT ELISAs: BT ELISA VMRD (BT ELISA 1), BT ELISA IDVET (BT ELISA 2), BT ELISA INGEZIM (BT ELISA 3). Herds in the Cantons of Sankt-Gallen (SG), Valais (VS), Ticino (TI) and Solothurn (SO) were examined more closely by new sampling.

of Virology, Vetsuisse Faculty, Zürich, CH) at −20 °C, were tested for BT. At first, 200 samples were analysed. Then 3–10 more samples from each holding with seropositive animals were examined, altogether 123 further samples. Wherever positive or doubtful ELISA results were obtained, BTV and TOV genome detection in serum was carried out (see below).

2.3. Cattle and goat EDTA blood samples from seropositive holdings outside TI

From 2 holdings north of the Alps (Cantons of Solothurn [SO] and St. Gallen [SG]), another one from the western Swiss Alps (VS) and from 2 out of 3 in the GR, in the eastern Swiss Alps, where seropositive animals were found, EDTA blood was collected from all cattle ($n = 23$) and goats ($n = 48$) on the farm and tested for BTV Ab and TOV genome. From the third holding (GR), no newly collected samples were available for confirmatory testing.

2.4. Additional samples from TI collected in 2008–2009

It was decided to collect EDTA blood in the autumn of 2008 in TI, from flocks with over 50% seropositive goats in CAE08 and not vaccinated against BTV-8 during the summer. First, EDTA blood was collected in autumn 2008 from all goats. Secondly, also cattle and sheep were tested for TOV. Some contact animals from other holdings were also tested. Overall 267 goats, 87 cattle, 77 sheep and 19 animals identified only as small ruminants which had been in contact with TOV-positive animals, were sampled in 2008. From known TOV-infected holdings, 425 offsprings or not previously investigated animals (281 goats, 63 cattle and 81 sheep) were additionally sampled in 2009. All these samples were examined for the presence of BTV Ab and if seropositive, RT-qPCR-panBTV and RT-qPCR-TOV were performed.

2.5. Goat samples from routine diagnostics

With each of the 5 RT-qPCR-panBTV-positive goat samples from the routine diagnostics at the IVI, tested between August 2008 and March 2009, RT-qPCR-TOV was performed.

2.6. Antibody detection

Commercial BTV Ab ELISAs cross-react with TOV Ab (Chaignat et al., 2009). Therefore, for screening purposes, samples were analysed using Bluetongue Virus Antibody Test Kit (VMRD, Pullman, WA, USA) (BT ELISA VMRD). Positive results were confirmed with the ID.Screen Blue Tongue Competition ELISA Kit (ID.VET, Montpellier, France) (BT ELISA IDVET) and the double recognition ELISA INGEZIM BTV DR1 (INGENASA, Madrid, Spain) (BT ELISA INGEZIM). In cases where questionable results were obtained, Blue Tongue Competitive ELISA Kit (B.D.S.L., Irvine, UK) (BT ELISA BDSL) was additionally used. They were used according to the manufacturer's instructions.

In 2008, 4 ELISA-positive samples from TI were sent for serotype determination to the Institute for Animal Health (Pirbright, UK) (IAH Pribright), the OIE reference laboratory for BT, where virus neutralisation test (VNT) against the 24 reference BTV serotypes was performed. The test was performed according to the method of Haig et al. (1956), using South African BT reference viruses as described in a previous study (Chaignat et al., 2009).

2.7. Viral RNA detection

Viral RNA was detected using RT-qPCR-panBTV and a first version of RT-qPCR-TOV. The latter was slightly adapted when sequences of newly detected TOV were available (Hofmann et al., 2010). Total RNA

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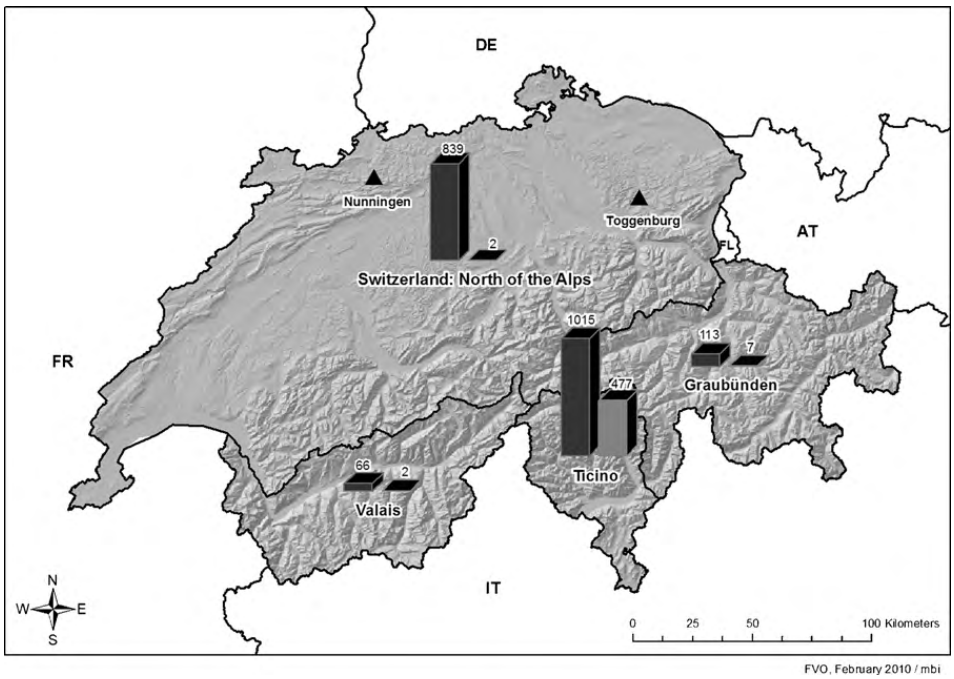


Fig. 2. Overall prevalence of TOV in Switzerland. Localisation of index cases (▲) and spatial distribution of the seropositive goats as observed in the CAE08 (number of analysed samples [dark column] and number of seropositive [light gray column]).

was extracted manually from EDTA blood, blood coagula or serum by Trizol® (Invitrogen, Paisley, Scotland) or semi-automatically from EDTA blood and blood coagula using NucleoSpin Multi-96 Virus extraction kit (Macherey-Nagel, Düren, Germany) on a Genesis Freedom EVO robot (TECAN, Männedorf, CH) according to the manufacturer's instructions. All samples were tested in triplicates. Any cycle threshold (Ct) value <50 was interpreted as positive.

2.8. Nucleotide sequencing

To confirm the RT-qPCR-TOV-positive results and to adapt these primers and probe to the newest sequence information, partial sequencing of S2 was performed according to Hofmann et al. (2010).

2.9. Statistical methods

Data were prepared in Microsoft Access 2007 and descriptive statistics were calculated with Microsoft Excel 2007. Two by two tables were analysed in NCSS 2007 (Kaysville, USA) and statistical evidence was established by Fisher's exact test with α of 5%. The 95% binomial confidence intervals (CI) for proportions were also calculated with NCSS. Differences between means of groups were also tested for statistical significance with T-test in NCSS. A simulation model was run to determine the true in-herd prevalence with the beta-function in @risk (Palisade Decision Tools [Ithaca, USA]).

Maps showing the geographical distribution of the results from examined flocks were produced with ArcGIS 9.2 (Esri Redlands, USA) and visually analysed. Raw prevalence estimates for herds and goats aggregated by district were also visually analysed.

3. Results

The results are presented in Table 1.

3.1. TOV seroprevalence in CH

The overall prevalence of TOV, detected in CAE08 samples, was 2.4%. The distribution is presented in Fig. 2.

3.1.1. North of the Alps

Out of 839 samples tested, 2 (0.2%) were found to be BTV Ab-positive. These samples originated from SO and SG, from holdings without any disease anamnesis. Samples from all the ruminants from these farms (in SO 16 cattle and 4 goats, in SG 2 cows and 5 goats) were all negative for BTV Ab.

3.1.2. Within the Swiss Alps

In GR, 7 out of 113 samples were BTV Ab-positive or questionable by screening. These positive samples originated from three different flocks. This represents an apparent seroprevalence of 6.1%. All 17 supplementary samples from CAE08 originated from one of these flocks were tested positive for BTV Ab. This means that in this flock all goats were seropositive. From the second seropositive sampled flock, the animals were vaccinated in the meantime and, therefore BT Ab-detection was not useful anymore.

In 1 out of 10 tested holdings from VS, 2 seropositive (4%) animals were found in CAE08. An additional sampling on this farm (5 cattle and 18 goats) was performed in autumn. All cattle were seronegative, 14 goats seropositive (including two vaccinated ones), 2 questionable and 2 were negative. The observed in-herd seroprevalence for the non-vaccinated goats was 75%.

Overall, the apparent seroprevalence in goats in the Swiss Alps outside of TI was 6.3% (95% confidence interval (CI) 5–7.8%).

3.1.3. TI

A total of 1015 samples from CAE08 from TI were examined and 477 of them were positive in one or more ELISAs. The apparent seroprevalence was estimated at animal level to be 49% (CI 44.7–50.9%) in goats. The CAE08 samples originated from 95 farms and in 57 of them at least one positive

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Table 1
Results overview.

Region/sample origin		Species	# samples	# BTV Ab-positive	Sero-prevalence	# positive in RT-qPCT-panBTV	# positive in RT-qPCR-TOV
North of the Alps	CAE08	Goat	839	2	0.2%	0	0
	SO[a]	Cattle	16	0		0	0
		Goat	4	0			
	SG[a]	Cattle	2	0		0	0
		Goat	5	0			
Within the Alps	CAE08 (GR)	Goat	113	7	6.1%		
	GR 1[a]	Goat	30	30	100%	17	5
	GR 2[a]	Goat	4	ND		1	1
	CAE08 (VS)	Goat	66	2			
	VS[a]	Cattle	5	0		0	0
		Goat	18	14	75%	14	2
South of the Alps	CAE08 (TI)	Goat	1015	477	49%	10	6
	2008–2009 (TI)[a]	Cattle	150	0			
		Goat	548	132		48	35
		Sheep	158	6	3.8%	0	0
	1998 (TI)	Goat	200	20	20%	1	0

CAE08: Samples from the cross-sectional survey of Caprine Arthritis Encephalitis collected between February and March 2008. ND: not performed.
[a] Additional samples from Canton of Solothurn (SO), of St. Gallen (SG), of Valais (VS), of Grisons (GR), of Ticino (TI).

sample was found; therefore the herd prevalence was 60%. The mean estimated in-herd prevalence was 33.5% with an observed maximum of 100%. Based on 79 farms with a known number of goats and only sampled in either 2008 or 2009, the simulated in-herd prevalence was 44.4%. The affected herds were distributed over the whole canton, but with a concentration of cases in the districts of Blenio, Vallemaggia and Riviera (Fig. 3). For the sampling rounds in autumn 2008 and in 2009, 132 and 36 sera respectively, were positive or doubtful in at least one ELISA, and the distribution on the district level is only slightly different; however; this should be interpreted cautiously, as these samples are not representative.

3.1.4. Serum bank 1998 from TI

From the samples collected in 1998, 40 out of 200 sera were positive in both BT ELISA VMRD and BT ELISA VMRD BDSL. These samples originated from 13 holdings. They represented a seroprevalence on animal level of 20%. The apparent in-herd prevalence ranged from 14% to 75% and the herd level prevalence was 41%. As only small sample volumes were available, no further serological analyses like VNT could be performed.

3.2. Serological characterisation

Four samples from different seropositive holdings from TI taken in 2008 sent to IAH Pirbright did not react with any of the 24 BTV reference serotypes when tested by VNT.

3.3. Viral RNA detection in goat samples

3.3.1. North of the Alps

None of the tested ruminant samples scored positive in BTV serogroup-specific RT-qPCR-panBTV.

3.3.2. Within the Swiss Alps

By testing 30 goats from the herd in GR with an in-herd seroprevalence of 100%, 17 goats were positive in BTV serogroup-specific RT-qPCR-panBTV. Among these, 5 were positive and 9 negative in RT-qPCR-TOV. From the second herd, 1 out of 4 goats was positive in RT-qPCR-panBTV and also in RT-qPCR-TOV. Partial S2 sequencing confirmed these results (Hofmann et al., 2010).

Genome detection by RT-qPCR-panBTV was performed for all the ruminants ($n = 23$) from the holding in VS. Fourteen goats were found to be weakly positive in this BTV serogroup-specific test (albeit with high Ct values) and 1 of them was positive for TOV; the other caprine samples were negative in RT-qPCR-TOV. None of the cows was positive in any RT-qPCR.

3.3.3. TI

Genome detection in serum was performed on 65 out of 477 samples from BTV-seropositive herds from CAE08 and 10 sera, all from the same holding, scored positive in RT-qPCR-panBTV, 6 of them were also positive in RT-qPCR-TOV.

From the samples collected in the second phase (autumn 2008 and spring 2009), 121 out of 132 BT ELISA-positive samples were analysed by RT-qPCR-panBTV. Forty-eight were positive in this assay and 35 of them, originating from 6 holdings, were also positive in RT-qPCR-TOV. This observation was supported by partial sequencing of S2 and S10 (Hofmann et al., 2010).

3.3.4. 1998 serum bank samples

From the first 200 samples, 1 was weak positive in RT-qPCR-panBTV. From the second series of 123 samples, 3 were positive and 5 were doubtful (only one of three triplicate was positive) in RT-qPCR-panBTV. The samples scored negative in RT-qPCR-TOV.

3.3.5. Viral RNA detection in samples from routine diagnostics

Three goats imported from southern Germany (Baden-Württemberg) in 2009 were examined for TOV after they were positive in RT-qPCR-panBTV. All 3 goats were also

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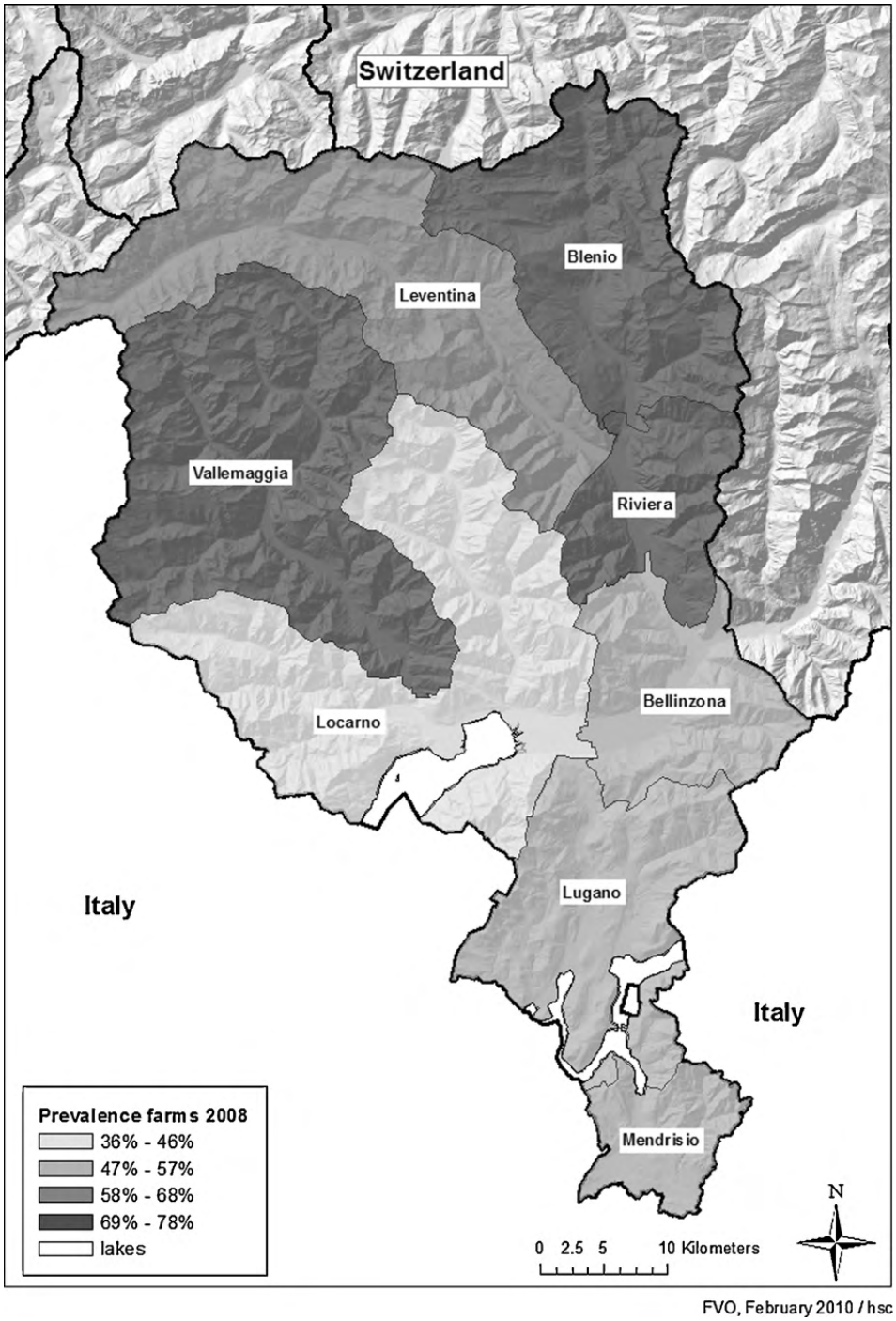


Fig. 3. Spatial distribution of seropositive goats and flocks aggregated on the district level in TI from the CAE survey 2008.

clearly positive for TOV. A goat clinically suspicious for BT from the Central Swiss Alps (Canton of Uri) was found to be positive for TOV in 2008. The fifth goat from CH was infected with BTV-8 and no TOV genome was detected in this animal.

3.4. Affected species

All the ruminants (158 sheep and 150 cattle) kept on the same farms as TOV-positive goats were examined by ELISA and RT-qPCR. Thirty-seven sheep were tested in BT ELISA, this represented a seroprevalence of 23%. All the animals were negative for BTV genome.

4. Discussion

TOV is a recently discovered Orbivirus being closely related to the BTV serogroup. The virus has been found in the Swiss goat population in different regions of the country, as well as in goats from southern Germany and in the north of Italy (Sozzi et al., 2009). The overall prevalence in Switzerland was 2.4% but, by looking more closely at the

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regional distribution, clear geographical differences were seen. In the present study no infected animals could be found north of the Alps. As it was not possible to confirm the seropositivity of the animals north of the Alps, they were determined as false positive. The specificity of the used ELISAs varies between 99% and 100% (data from manufacturer and own validation not shown), this could explain this result in the screening test. Within the Alps, there were regions such as GR with up to 6.1% or VS with 3% seropositive animals. In contrast to these areas, in TI almost 50% of the goats were seropositive. The TOV genome-positive goats originated from 7 flocks, distributed throughout the canton. This national spatial distribution leads to the assumption that TOV had spread from the southern part of CH, which is the warmest area, to the north. However, as the transmission mode of TOV is currently not known, all assumptions about the cause for the observed distribution are only speculations that might lead to the formulation of further research hypotheses.

One TOV-positive goat was found in Canton of Uri. This animal was presented for a BT suspicion (lameness and stomatitis) and a TOV infection was diagnosed. However, as none of the experimentally or the naturally infected adult goats (Chaignat et al., 2009; own observation) exhibited any obvious clinical signs, we assume that the clinical signs of this goat were not related to this TOV affection.

Since TOV still cannot be propagated outside its natural host (Chaignat et al., 2009; personal observation), no TOV-specific VNT could be performed. However, several samples reacting strongly positive in the BT ELISAs did not neutralise any of the 24 reference BTV serotypes in VNT. Therefore, we assume that Ab against TOV were present in these samples.

A further diagnostic challenge was that some weakly positive RT-qPCR-panBTV samples could not be confirmed to be TOV by RT-qPCR-TOV. Validation data with TOV-positive samples from experimentally infected animals have revealed a lower sensitivity for RT-qPCR-TOV than for RT-qPCR-panBTV, especially for weakly positive samples (data not shown). One reason for the weakly positive results obtained with many samples could be the suboptimal sample material; i.e. sera, some stored for more than 10 years, instead of EDTA blood. A difference of up to 12 Ct values could be observed between both sample materials, as derived from our validation data (data not shown). Furthermore, TOV strains demonstrated a higher degree of genetic variability in their S2 sequences, where the RT-qPCR-TOV primers and probe bind, than in the S10 where the RT-qPCR-panBTV primers and probe are located. The RT-qPCR-TOV was designed based on the S2 sequence of recent TOV samples, therefore primers and probe might not optimally fit to older TOV strains. Hence, viral genome detection might have shown false negative results. However, even in cases where not all the seropositive results were confirmed by the detection of TOV RNA, it can be assumed that TOV was involved, because (i) there were no notified BTV-8 cases in TI and GR and (ii) there was no sample testing positive for BTV-8 VNT (data not shown). Furthermore, no BTV cases in other species (cattle and sheep) were observed in the context of the active surveillance in effect since 2003 in TI and since 2007 in GR. Considering the fact that the time point of sampling in 2009 was presumably not ideal – samples collected in spring containing probably only traces of viral RNA – a relatively high number of goats were still found positive by RT-qPCR-TOV.

When determining the seroprevalence by ELISA it must be considered that the BT ELISAs have a reduced sensitivity for TOV-infected animals. Observations from the first detected TOV cases (Chaignat et al., 2009) demonstrated that although BT ELISA VMRD and BT ELISA BDSL also detect TOV Ab, this detection is not always very efficient, in particular early after infection, often leading to a weak positive or questionable reaction. Upon experimental infection, some animals were found to be seronegative for several weeks after infection, and in field cases not every goat positive in RT-qPCR-TOV showed a positive response in ELISA. Unfortunately, not enough well characterized samples are available to determine the exact sensitivity of BT ELISAs for TOV Ab. To this end, current work in progress in our laboratory is aiming at expressing the TOV VP2 as a recombinant protein to be used as antigen in a TOV-specific Ab ELISA.

Although the southern part of CH was free from BT, as proved by surveys in TI in cattle and sheep since 2003 (Cagienard et al., 2006; Racloz et al., 2006), in 1998 a considerable proportion of goat samples (40/200, 20%) contained BTV Ab, and BTV RNA was detectable in their blood (4/323, 1.2%). At that time, Europe, with the exception of Greece, Cyprus and European Turkey and the Balkans (Mellor et al., 2008), was still BT-free. Moreover, the sequencing of TOV positive samples demonstrated a wide genomic variability (Hofmann et al., 2010). All these observations lead to the hypothesis that TOV was already circulating on a high level in TI 10 years ago. However, our results suggest a slight increase in animal level prevalence, but even more pronounced on farm level. Interestingly, the observed maximal in-herd prevalence was lower in 1998 than in 2008. Unfortunately, it is not possible to allocate this effect unambiguously to the reduced test sensitivity in stored samples or to a biological process.

While in some herds the seroprevalence in goats was as high as 100%, none of the other ruminant species such as South American camelids (in the index cases) (Chaignat et al., 2009) or cattle, kept in the same flocks, was found to be RT-qPCR-panBTV or -TOV positive. Cattle were mainly examined in farms where more than 50% of the goats scored positive in BT ELISA and even in these holdings no TOV-positive cattle was detected. Hence, it could be hypothesized that these animals are not susceptible to TOV. Results obtained after experimental infection of cattle support this hypothesis (Sozzi et al., 2009). In some sheep without anamnesis of disease or BT vaccination, BTV Ab were detected by ELISA. We have shown that sheep can be experimentally infected with TOV but the virus does not replicate efficiently (Chaignat et al., 2009). Thus sheep can be regarded as an accidental host but not as reservoir or main actor of the replication cycle. No TOV genome-positive sheep was observed. The observed difference in susceptibility under field conditions reflects the one seen in animal experiments with sheep and goats. Hence it is likely that TOV susceptibility is restricted, at least in the field, to

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goats. Future studies will aim at determining whether certain wild ruminant species show evidence of TOV infection.

Other Orbivirus also do not cause clinical disease; Middle Point orbivirus and Stretch Lagoon orbivirus which were both identified in several regions of Australia in cattle, goats, donkeys and horses are also apathogenic (Cowled et al., 2007, 2009).

Several commercial RT-qPCR-BTV kits like the one from Adiagene (St-Brieuc, France) (ADIAVET® BTV real-time) and Labor Diagnostik GmbH Leipzig (Leipzig, Germany) (VIROTYPE® BTV real-time RT-PCR Kit) are able to recognise also TOV (data not shown). The RT-qPCRs need to be regularly adapted to the newly detected virus variants. The development of serological tests, such as ELISA, is ongoing. We are working on virus isolation to propagate TOV in cell cultures and establish a VNT.

Considering that no disease has been related to TOV and the virus was only found in goats, the virus itself has, generally, no relevance for international trade. But for diagnostic reasons, it has to be differentiated from the other BTV serotypes. Furthermore, reassortment between TOV and other viruses of the BTV-serogroup cannot be excluded. This novel Orbivirus was also found in goats from Germany and in the north of Italy (Sozzi et al., 2009), i.e. its geographical distribution is wider than previously assumed. Moreover, to better characterize TOV-positive holdings and to determine which are the risk factors for a TOV infection or possible transmission ways, a epidemiological research project was started in spring 2009 in TI.

5. Conclusions

TOV is widespread in CH. High seroprevalence is found mainly in the southern part of the country, in TI and to a lesser extent within the Alps. In the field, only goats seem to be affected by the virus, while other domestic ruminant species do not seem to allow an efficient replication of TOV. The observations made on the serum samples from 1998 sustain the hypothesis that TOV has already been present 10 years prior to its detection. Testing of imported goats confirmed the presence of TOV in goats from Germany.

Acknowledgments

The authors would like to thank J. Feldmann, H. Gobet, M. Horath and Z. Lechissa for their technical support, and the laboratories in charge of CAE diagnosis for submitting the serum samples. Without the cooperation and the kind help of the cantonal and field veterinarians and the farm holders this work would not have been possible. In addition, the authors thank M. Engels, Institute of Virology, Vetsuisse Faculty Zurich, for her support with the 1998 samples. Last but not least, we acknowledge H. Gerber for improving and editing the manuscript in English.

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