

1 New World camelids and BVDV (Bovine Virus Diarrhea Virus) infection in Switzerland:
2 a retrospective study

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11
12 Abstract

13 The aim of this study was the search for persistently infected (PI) New World camelids
14 (NWC) as a possible source of Bovine Virus Diarrhea Virus (BVDV) infection for other
15 NWC or cattle in Switzerland, where an eradication program for BVDV has been
16 implemented. Different organs from 166 animals and 101 sera from different parts of
17 Switzerland were tested for BVDV antigen by means of immunohistochemistry and
18 HerdChek*BVDV Ag/Serum Plus. None of the organs and sera was found to be
19 positive for antigen.

20 Up until now, PI NWC are infected mainly by BVDV genotype 1b. In Switzerland most
21 PI cattle harbor the subgroup BVDV-1e, followed by 1h, 1k and 1b. The subgroup
22 BVDV-1b is found in less than 10% of the cattle cases. Therefore, assuming that
23 NWC are more prone for a persistent infection with the subgroup 1b, it could be
24 hypothesized that the infection rate of NWC is lower than in other countries because
25 of a lower circulating level of this type in ruminants in Switzerland. We conclude that
26 NWC are a negligible threat to the eradication efforts for BVD in Switzerland.

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32 Key Words: Llama, Alpaca, Bovine virus diarrhea virus, ELISA, necropsy,
33 immunohistochemistry

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36 Introduction

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38 New World camelids (NWC) are becoming more and more popular as pets and for
39 leisure activities like trekking. In 2009 2'652 llamas and 2'094 alpacas were registered
40 in Swiss farms (Bundesamt für Statistik, Berne CH). A wide range of viral infections
41 are known in NWC, like West Nile Virus, contagious ecthyma and Bovine virus
42 diarrhea virus. Persistent BVDV (Bovine Virus Diarrhea Virus) infections have been
43 reported in recent years in alpacas (Goyal et al., 2002; Mattson et al., 2006; Foster et
44 al., 2005 and 2007; Carman et al., 2005; Barnett et al., 2008; Byers et al., 2009; Kim
45 et al., 2009) and in llamas (Belknap et al., 2000; Wentz et al., 2003).

46 BVDV belongs to the genus pestivirus within the flaviviridae family. The genus
47 consists of the two genetic species BVDV-1 and -2, which are again divided into
48 numerous subgroups. Among BVD viruses, cytopathogenic (cp) and non-
49 cytopathogenic (ncp) types are distinguished due to their properties when cultured on
50 cells. Only the non-cytopathogenic type causes persistent infection in cattle when
51 affecting fetuses during the immunotolerant phase of gestation (Bachofen et al., 2009;
52 Van Amstel and Kennedy, 2010). In alpacas an ncp BVDV genotype 1b was isolated
53 (Goyal et al., 2002; Carman et al., 2005; Byers et al., 2009; Kim et al., 2009) and in
54 llamas also a BVD type 1b was recognized (Wentz et al., 2003). A serological survey
55 among 63 alpaca herds all over the USA found 16 (25.4%) of them to harboring
56 seropositive crias and 4 (6.3%) with persistent infected (PI) crias (Topliff et al., 2009).
57 A similar survey in Switzerland including 53 alpaca and llama herds with 109 sera
58 examined detected a seroprevalence of 4.6% (Danuser et al, 2009). A newer
59 serological survey of 596 serum samples showed that the prevalence of BVDV
60 carriers was 0% (Mudry et al., 2010).

61 Attempts were made to provoke the birth of PI crias by experimental infection of 4
62 pregnant llamas between days 65 and 105 of gestation. But neither were clinical signs
63 observed in the mothers nor were PI crias born (Wentz et al., 2003). Because the

64 gestation period in NWC is longer than in cattle (alpacas: range 335 to 356 days;
65 NWC mean 345 days), the period of susceptibility of the camelid fetus for a persistent
66 infection has not been determined with certainty (Carman et al., 2005; Mattson et al.
67 2006; Byers et al., 2009; Kapil et al., 2009; Van Amstel and Kennedy, 2010).
68 Supposed that the ontogenesis of the immune system is similar to bovids, Mattson et
69 al. (2006) postulate the development of a PI cria until approximately 145 days of
70 gestation to occur/be possible. Other authors (Byers et al., 2009) propose that the
71 gestational exposure time for BVDV immunotolerance in alpacas may be only the first
72 trimester. In a newer study, transplacental infection during early gestation of alpacas
73 naturally exposed to BVDV type 1b was confirmed in 7 out 10 live-born crias
74 (Benedice et al, 2011).

75 In PI crias clinical symptoms like ill thrift, anorexia, decreased weight gain, chronic
76 recurrent debilitation and infections as well as diarrhea can be found and some show
77 congenital defects. Stillbirths and abortions are seen too in affected herds (Evermann,
78 2006; Foster et al., 2007; Byers et al., 2009; Passler and Walz, 2009; Topliff et al.,
79 2009; Van Amstel and Kennedy, 2010; Bedenice et al., 2011).

80 For diagnosis of BVDV in NWC the same diagnostic test designed and used for the
81 identification of BVDV infection in cattle can be used, as immunohistochemistry,
82 antigen detection ELISA, PCR or virus isolation (Carman et al., 2005; Kapil et al.,
83 2009). By using immunohistochemistry large amounts of antigen can be found in PI
84 crias in several organs (Byers et al., 2009). As source of infection in NWC movement
85 of animals is suspected, which means, e.g. female alpacas with crias dislocating
86 between premises for mating. Another possibility would be the contact to infected
87 cattle, mixed animal husbandry and communal pastures (Evermann, 2006; Foster et
88 al., 2007; Barnett et al., 2008; Danuser et al., 2009; Passler and Wetz, 2009; Topliff et
89 al., 2009; Van Amstel and Kennedy, 2010).

90 Because an eradication of BVDV is in progress in Switzerland, the aim of this study
91 was to exclude NWC as a possible source of reinfection of bovines by identifying
92 BVDV infected animals. In this study we therefore used retrospective paraffin-
93 embedded material available at the institute of veterinary pathology from 1996 to 2009
94 and serum samples from NWC collected in 2007 to identify possible infection with
95 pestiviruses, mainly BVDV.

96

97 Material and methods

98

99 Paraffin-embedded material from, 94 alpacas, 59 llamas, 5 guanacos and 8 vicuñas
100 referred for necropsy to the Institute of Veterinary Pathology, between 1996 and 2009
101 was available.

102 The llamas send in for necropsy were from neonatal to adult animals, one abortion
103 was examined and the oldest animal was 16 years old, the female to male ratio was
104 50:50. 74 % of alpacas in the necropsy were females. The age of the incoming
105 animals was from neonatal to 27 years, among them 6 abortions were investigated.
106 From the 5 guanacos 3 were females, 2 out of them neonatal and one 12 years old,
107 one animal was a male of 3 years and one animal an abortion. Five vicuñas were
108 female from neonatal to 23 years, one male animal was 4 days of age. Furthermore,
109 one stillbirth and one abortion were examined. Most animals were from the German
110 part of Switzerland (mostly from the canton Zurich, see table 1 and figure 1).

111 In addition to this retrospective data, 99 serum samples from sound animals collected
112 during a study for the survey of infectious diseases (Kaufmann et al., 2010; Zanolari et
113 al., 2010) were analyzed by ELISA for BVD antigen. Serum samples originated from
114 77 alpacas and 24 llamas. Age and gender distribution of this sample is presented in
115 table 2.

116

117 Immunohistology

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119 Tissue sections mostly of brain but also skin, spleen, kidney, liver or gastrointestinal
120 tract were deparaffinized and rehydrated, watered in tap water for 5 min. and
121 counterstained for 2 min. in hemalaun. Endogenous peroxidase was inactivated by
122 treatment with 3% H₂O₂ (3% H₂O₂ with 0,2% NaN₃ (sodium azide) in water) for 10 min.
123 at room temperature (RT). Afterwards the slides were digested with proteinase k for
124 10 min. at room temperature (Dako REAL Proteinase K (40x), S2019). For 1 h at 37°
125 C the slides were incubated with the following two antibodies: C42 (dilution 1:400;
126 Prof. V. Moennig, Institute of Virology, Hannover, Germany) and 15c5 (dilution 1:
127 1000; Dr. E. Dubovi, Cornell University, USA). Afterwards the EnVision-method

128 (DAKO, K 4001 EnVision, peroxidase mouse, Zug, Switzerland) was applied as
129 described by the manufacturer. As chromogen AEC (Aminoethyl Carbazole Substrate
130 Kit, 00-2007, Invitrogen) was used. As a positive control the analogous stained brain
131 section from a PI calf was used.

132

133 HerdChek*BVDV Ag/Serum Plus

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135 This ELISA detects BVDV antigens in serum, plasma, whole blood and ear notch
136 tissue samples. Specific monoclonal antibodies (Er^{ns}/gp44-48) are coated on the
137 microtiter plates, so that captured BVD-antigens can be detected (HerdChek* BVDV
138 Antigen ELISA Ear-Notch/Serum Test Kit; Idexx Laboratories). This ELISA is not
139 established in NWC; however, it was used in previous studies or mentioned in reviews
140 (Foster et al., 2005; Kapil et al., 2009; Van Amstel and Kennedy, 2010).

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142 Results

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144 Necropsy

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146 In 18 out of 59 llamas sent in for necropsy the main problem was a moderate to
147 severe infestation with *Dicrocoelium dendriticum* and subsequent liver necrosis and
148 fibrosis (cirrhosis). 7 had a severe infestation with intestinal parasites like *Eimeria* sp.
149 or *Nematodirus* sp., 5 had a septicemia, 3 had each a lymphoma and two a carcinoma
150 in diverse organs, 2 had a *Clostridium perfringens* enterotoxaemia (type D) and the
151 rest of the animals had diverse problems like arthritis, cardiovascular problems, birth
152 trauma, lung problems or no diagnosis. No abortion cause could be determined in the
153 only abort examined.

154 An infestation with *Dicrocoelium dendriticum* was seen at the autopsy in 12 out of 93
155 94 alpacas, 1 animal was infested with *Fasciola hepatica*, 11 animals showed
156 cachexia mostly because of dental problems (hooks and waves) but also because of
157 parasitic infestation, 6 animals had intestinal parasites like trichostrongylides, from the
158 6 abortions two had a septicemia, one a pneumonia and the placenta a suppurative

159 placentitis suggesting a bacterial infection, one showed a intrauterine asphyxia and
160 two had no diagnosis. Two neonatal alpacas died because of inanition, two because
161 of poorly unfolded lung and two adult alpacas had a generalized mycobacterial
162 infection, 7 out of 93 had a pneumonia or bronchopneumonia, 4 had a endocarditis
163 valvularis, birth deformities like ventricular septum defect, choanal atresia, spina bifida
164 occulta, hydrocephalus internus were found in single juvenile animals and the oldest
165 animal (27 years) had bilateral ocular cataract. Other problems like septicemia (3
166 animals), tubulonephrosis and urolithiasis (one animal each) were also found.

167 The abortion and one adult animal in the guanaco group (n=5) had no diagnosis, one
168 animal died from a cerebrocortical necrosis, one showed degeneration of muscle
169 fibers due to vitamin E/selenium deficiency and one died because of trauma induced
170 by a herdmate.

171 Lymphoma was found in an adult vicuña, one neonatal animal showed septicemia,
172 another uremia due to a glomerulonephritis and tubulonephrosis, a 4 days old animal
173 showed a suppurative meningitis, the stillbirth and the aborted animal had no
174 diagnosis, one animal had arthrosis and one stomatitis papulosa.

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176 Immunohistology

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178 Tissues of 166 animals were examined for BVDV antigen by means of
179 immunohistochemistry as described above. In no animal, BVDV antigen could be
180 detected by this test (Figure 2).

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182 HerdChek*BVDV Ag/Serum Plus

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184 Two out of the 101 sera examined were repeatedly positive. A skin biopsy of these
185 cases was requested. For immunohistochemistry, however, only one case could be
186 examined and revealed to be negative in IHC.

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188 Discussion

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190 Organs from 166 animals and 101 sera were tested for BVDV antigen. Assuming that
191 the population number of NWC in Switzerland was around 4'000 at the time of
192 sampling (Bundesamt für Statistik, Berne, Switzerland), approximately 6.7% of this
193 population was involved in our investigation. Danuser et al. (2009) found a
194 seroprevalence of 4.9%; however, as a population of mainly sick animals in a referral
195 centre was analysed there is a potential bias in this study. Mudry et al. (2010) found
196 an overall pestivirus seroprevalence of 5.75% and a seroprevalence of 0% for BVDV
197 in the year 2008 in NWC in Switzerland, showing that the infection rate is low and that
198 the exposure of NWC with BVDV is a rare event. Both exposition and susceptibility
199 can be regarded as low. Still movement of animals for mating or contact to infected
200 cattle and mixed animal husbandry and communal pastures has to be regarded as a
201 source of infection in NWC (Foster et al., 2007; Barnett et al., 2008; Topliff et al.,
202 2009; Danuser et al., 2009). The review of the necropsy reports demonstrates that
203 infestation with *Dicrocoelium dendriticum*, seldom with *Fasciola hepatica*, but with
204 intestinal parasites too has been (Wenker et al., 1998) and still is one of the bigger
205 problems in NWC farming.

206 Immunohistochemistry is described in the literature as a strong tool for identifying PI in
207 NWC (Carman et al., 2005; Byers et al., 2009). With the HerdChek*BVDV Ag/Serum
208 Plus we had 2 positive results out of 101 sera. One of the positive sera was confirmed
209 to be false positive by IHC. Kapil et al. (2009) described this phenomenon and they
210 postulate that commercial antigen-capture ELISA can cause false positive results
211 because of high background. The antigen-ELISA has not been validated for camelids
212 (Van Amstel and Kennedy, 2010). However, the second positive serum sample could
213 not be rechecked by IHC testing because the owner of the animal was not willing to
214 carry out further examinations.

215 The PI NWC described in the literature up until now were infected mostly by the
216 genotype 1b. In Switzerland the viral genetics of 169 Swiss isolates from bovines
217 confirmed the presence of the BVDV-1 subgroups b, e, h and k. No BVDV type 2 was
218 detected in this study (Bachofen et al., 2008). In another study in PI cattle, most
219 animals harbored the subgroup BVDV-1e, followed by 1h, 1k and 1b. The subgroup
220 BVDV-1b was found in less than 10% of the cases (Bachofen et al., 2009). Therefore,
221 assuming that NWC are more prone for a persistent infection with the subgroup 1b it

222 can be hypothesized that the infection rate of NWC is lower than in other countries
223 because of a lower circulating level of this type in ruminants in Switzerland.
224 We conclude that in Switzerland there is a low risk of an infection of NWC with the
225 subgroup BVDV-1, mostly with the genotype 1b and that the PI in NWC crias can be
226 regarded as a rare event. Furthermore, NWC are a negligible thread to the eradication
227 efforts for BVD in Switzerland.

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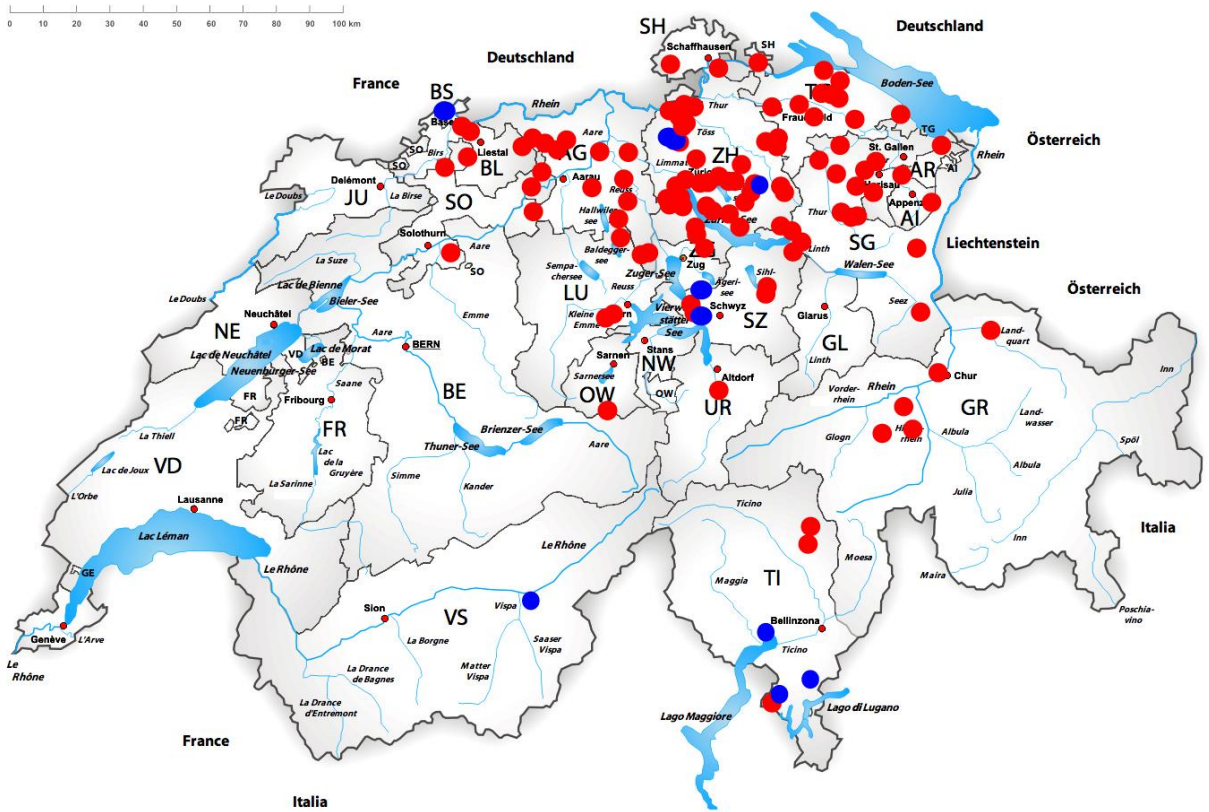
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1 Figure 1: Nachbarländer Bezeichnung in Englisch (Italia -> Italy)

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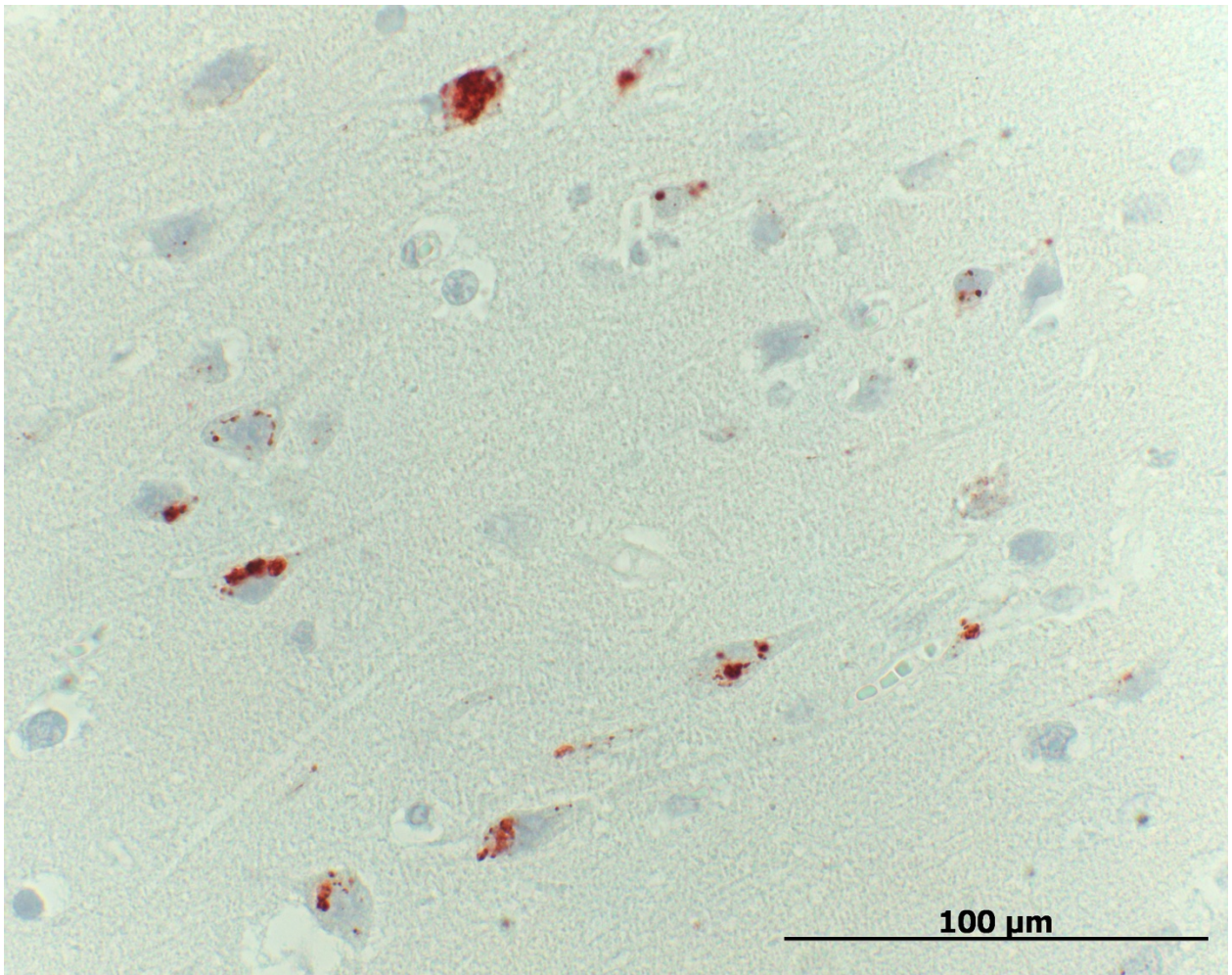
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19 Figure 2:



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34 Table 1:

Canton	Male	Female	Abortion/ unknown
Argovia	4/3/0/0	10/4/0/0	0/0/0/0
Inner Rhodes	0/1/0/0	0/1/0/0	0/0/0/0
Basle-Country	1/1/0/0	1/1/0/0	1/0/0/0
Grisons	0/5/0/0	1/0/0/0	1/1/0/0
Lucerne	2/2/0/0	1/1/0/0	0/0/0/0
Schaffhausen	1/1/0/0	2/2/0/0	0/0/0/0
Solothurn	1/0/0/0	2/0/0/0	0/0/0/0
Schwyz	3/1/0/0	1/1/0/0	0/0/0/0
St. Gall	4/0/0/0	7/2/0/0	2/2/0/0
Ticino	2/0/0/0	2/0/0/0	0/0/0/0
Thurgovia	5/2/0/0	6/2/0/0	0/1/0/0
Uri	1/0/0/0	0/0/0/0	0/0/0/0
Zug	0/0/0/0	2/1/0/0	0/0/0/0
Zurich	12/11/1/1	15/12/3/5	4/1/1/2

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36 Table 2:

Species	Male	Female	Median age	Min. age	Max. age
Alpaca	20	57	3.2	0.1	20
Llama	9	15	3.1	0.1	18.5

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42 Table 3:

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Mycobacterial -> welche Mycobacterien?

Species	Number of animals	Necropsy results
Llama	18	Infestation with <i>Dicrocoelium dendriticum</i>
	7	Intestinal parasites, like <i>Eimeria</i> sp. and <i>Nematodirus</i> sp.
	5	Septicemia
	3	Lymphoma
	2	Carcinoma
	2	<i>Clostridium perfringens</i> enterotoxaemia (type D)
	22	Diverse problems, like arthritis, cardiovascular problems, birth trauma, lung problems or no diagnosis
Alpaca	12	Infestation with <i>Dicrocoelium dendriticum</i>
	1	Infestation with <i>Fasciola hepatica</i>
	11	Cachexia due to dental problems or endoparasitosis (e.g. <i>Nematodirus battus</i>)
	6	Infestation with parasites like trichostrongylides
	7	Pneumonia or bronchopneumonia
	4	Endocarditis valvularis
	4	Neonatal animals: Inanition (n = 2), poorly unfolded lungs (n = 2)
	2	Generalized mycobacterial infection (in one case <i>M. kansasii</i> was isolated and in the other no cultural identification was possible)
	6	Abortion due to septicemia (n = 2), pneumonia and suppurative placentitis (n

		= 1), intrauterine asphyxia (n = 1), no diagnosis (n = 2)
	4	Birth deformities like ventricular septum defect, choanal atresia, spina bifida occulta and hydrocephalus internus
	3	Septicemia
	2	Cataract
	1	Tubulonephrosis
	1	Urolithiasis
Guanaco	1	Cerebrocortical necrosis
	1	Vitamin E/Selen deficiency
	1	Trauma
	2	No diagnosis
Vicuña	1	Lymphoma
	1	Septicemia
	1	Uremia
	1	Suppurative meningitis
	1	Arthrosis
	1	Stomatitis papulosa
	2	Stillbirth and abortion: no diagnosis

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Legend:

Figure 1: Distribution of the New World camelids (NWC) examined in this study. Blue dots are the localization of serum sampling, red dots is the localization of NWC from where animals were received for necropsy.

Figure 2: Immunohistochemistry. BVDV positive control, bovine brain: EnVision-method, 40x. Note the intracytoplasmic red labelling of neurons.

Table 1: Regional and gender distribution of cases analyzed
(Alpaca n=94/Llama n=59/Guanaco n=5/Vicuña n=8)

Table 2: Serum samples for BVD antigen ELISA, age (years) and gender distribution

Table 3: Most common necropsy diagnoses between 1996 and 2009

Zanolari Patrik 16.4.11 12:22

Kommentar [1]: 10 blaue Punkte auf der Karte für 59 Lamas und 93/94 Alpacas?