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

M Hilbe[1], Ch Kaufmann[2], K Zlinszky[1], P Zanolari[3] and F Ehrensperger[1]

[1] Institute for Veterinary Pathology, Vetsuisse Faculty, Winterthurerstrasse 268, 8057 Zürich, [2] Tierpraxis mondo a, Baselstrasse 1a, 4125 Riehen and [3] Clinic for Ruminants, Vetsuisse Faculty, Bremgartenstrasse 109a, 3012 Berne

Abstract

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

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

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

Introduction

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

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

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

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

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

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

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

Material and methods

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

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

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

Immunohistology

Tissue sections mostly of brain but also skin, spleen, kidney, liver or gastrointestinal tract were deparaffinized and rehydrated, watered in tap water for 5 min. and counterstained for 2 min. in hemalaun. Endogenous peroxidase was inactivated by treatment with 3% H_2O_2 (3% H_2O_2 with 0,2% NaN_3 (sodium azide) in water) for 10 min. at room temperature (RT). Afterwards the slides were digested with proteinase k for 10 min. at room temperature (Dako REAL Proteinase K (40x), S2019). For 1 h at 37° C the slides were incubated with the following two antibodies: C42 (dilution 1:400; Prof. V. Moennig, Institute of Virology, Hannover, Germany) and 15c5 (dilution 1: 1000; Dr. E. Dubovi, Cornell University, USA). Afterwards the EnVision-method

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

HerdChek*BVDV Ag/Serum Plus

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

Results

Necropsy

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

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

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

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

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

Immunohistology

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

HerdChek*BVDV Ag/Serum Plus

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

Discussion

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

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

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

can be hypothesized that the infection rate of NWC is lower than in other countries because of a lower circulating level of this type in ruminants in Switzerland.

We conclude that in Switzerland there is a low risk of an infection of NWC with the subgroup BVDV-1, mostly with the genotype 1b and that the PI in NWC crias can be regarded as a rare event. Furthermore, NWC are a negligible thread to the eradication efforts for BVD in Switzerland.

References

Bachofen C., Stalder H.P., Braun U., Hilbe M., Ehrensperger F, Peterhans E.: Co-existence of genetically and antigenetically diverse bovine viral diarhhoea viruses in an endemic situation. Veterinary Microbiology, 2008, 131: 93-102.

Barnett J., Twomey D.F., Millar M.F., Bell S., Bradshaw J., Higgins R.J., Scholes S.F., Errington J., Bromage G.G., Oxenham G.J.: BVDV in British alpacas. Vet. Rec. 2008, 162:795.

Belknap E.B., Collins J.K., Larsen R.S., Conrad K.P.: Bovine viral diarrhea virus in New World camelids. J. Vet. Diagn. Invest. 2000, 12:568-570.

Bedenice D., Dubovi E., Kelling C.L., Henningson J.N., Topliff C.L., Parry N.: Long-term clinicopathological characteristics of alpacas naturally infected with Bovine Viral Diarrhea Virus Type 1b. J Vet Intern Med. 2011 Apr 12. doi: 10.1111/j.1939-1676.2011.0719.x. [Epub ahead of print]

Byers S.R., Snekvik K.R., Righter D.J., Evermann J.F., Bradway D.S., Parish S.M., Barrington G.M.:Disseminated Bovine viral diarrhea virus in a persistently infected alpaca (Vicugna pacos) cria. J. Vet. Diagn. Invest. 2009, 21:145-148.

252 Carman S., Carr N., DeLay J., Baxi M., Deregt D., Hazlett M.: Bovine viral diarrhea
253 virus in alpaca: abortion and persistent infection. J. Vet. Diagn. Invest. 2005, 17:589-
254 593.
255
256 Danuser R., Vogt H.R., Kaufmann T., Peterhans E., Zanoni R.: Seroprevalence and
257 characterization of pestivirus infections in small ruminants and new world camelids in
258 Switzerland. Schweiz. Arch. Tierheilk. 2009, 151:109-117.
259
260 Evermann J.F.: Pestiviral infection of llamas and alpacas. Small Ruminant Research
261 2006, 61: 201-206.
262
263 Foster A.P., Houlihan M., Higgins R.J., Errington J., Ibata G., Wakeley P.R.: BVD virus
264 in a British alpaca. Vet. Rec. 2005, 156:718-719.
265
266 Foster A.P., Houlihan M.G., Holmes J.P., Watt E.J., Higgins R.J., Errington J., Ibata
267 G., Wakeley P.R.: Bovine viral diarrhoea virus infection of alpacas (Vicugna pacos) in
268 the UK. Vet. Rec. 2007, 161:94-99.
269
270 Goyal S.M., Bouljihad M., Haugerud S., Ridpath J.F.: Isolation of bovine viral diarrhea
271 virus from an alpaca. J. Vet. Diagn. Invest. 2002, 14:523-525.
272
273 Kapil S., Yeary T., Evermann J.F.: Viral Diseases of New World Camelids. Vet. Clin.
274 North Am. Food Anim. Pract. 2009, 2: 323-337.
275
276 Kaufmann C., Meli M.L., Hofmann- Lehmann R., Zanolari P.: Erster Nachweis von
277 '*Candidatus* Mycoplasma haemolamae' bei Neuweltkameliden in der Schweiz und
278 Schätzung der Prävalenz. Berl. Münch. Tierärztl. Wochenschr. 123, 477-481.
279
280 Kim S.G., Anderson R.R., Yu J.Z., Zylich N.C., Kinde H., Carman S., Bedenice D.,
281 Dubovi E.J.: Genotyping and phylogenetic analysis of bovine viral diarrhea virus
282 isolates from BVDV infected alpacas in North America. Vet. Microbiol. 2009, 136:209-
283 216.

Mattson D.E., Baker R.J., Catania J.E., Imbur S.R., Wellejus K.M., Bell R.B.: Persistent infection with bovine viral diarrhea virus in an alpaca. J. Am. Vet. Med. Assoc. 2006, 8:1762-1765.

Murdy M., Meylan M., Regula G., Steiner R., Zanoni R., Zanolari P.: Epidemiological Study of Pestiviruses in South American Camelids in Switzerland. J. Vet. Intern. Med. 2010, 24: 1218-1223.

Passler T., Walz P.H.: Bovine viral diarrhea virus infections in heterologous species. Anim. Health Res. Rev. 2009, 3:1-15.

Topliff C.L., Smith D.R., Clowser S.L., Steffen D.J., Henningson J.N., Brodersen B.W., Bedenice D., Callan R.J., Reggiardo C., Kurth K.L., Kelling C.L.: Prevalence of bovine viral diarrhea virus infections in alpacas in the United States. J. Am. Vet. Med. Assoc. 2009, 234:519-529.

Van Amstel S. and Kennedy M.: Bovine viral diarrhea infections in new world camelods-A review. Small Ruminant Research 2010, 91:121-126.

Wenker C., Hatt J.M., Hertzberg H., Ossent P., Hänichen T., Brack A., Isenbügel E.: [Dicrocoeliasis in New World camelids]. Tierarztl. Prax. Ausg. Grosstiere Nutztiere 1998, 26:355-361. German.

Wentz P.A., Belknap E.B., Brock K.V., Collins J.K., Pugh D.G.: Evaluation of bovine viral diarrhea virus in New World camelids. J. Am. Vet. Med. Assoc. 2003, 223:223-228.

Zanolari P., Bruckner L., Fricker R., Kaufmann C., Mudry M., Griot C., Meylan M.: Humoral response to 2 inactivated Bluetongue Virus Serotype-8 vaccines in South American Camelids. J Vet Intern Med 2010; 24: 956-959.

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

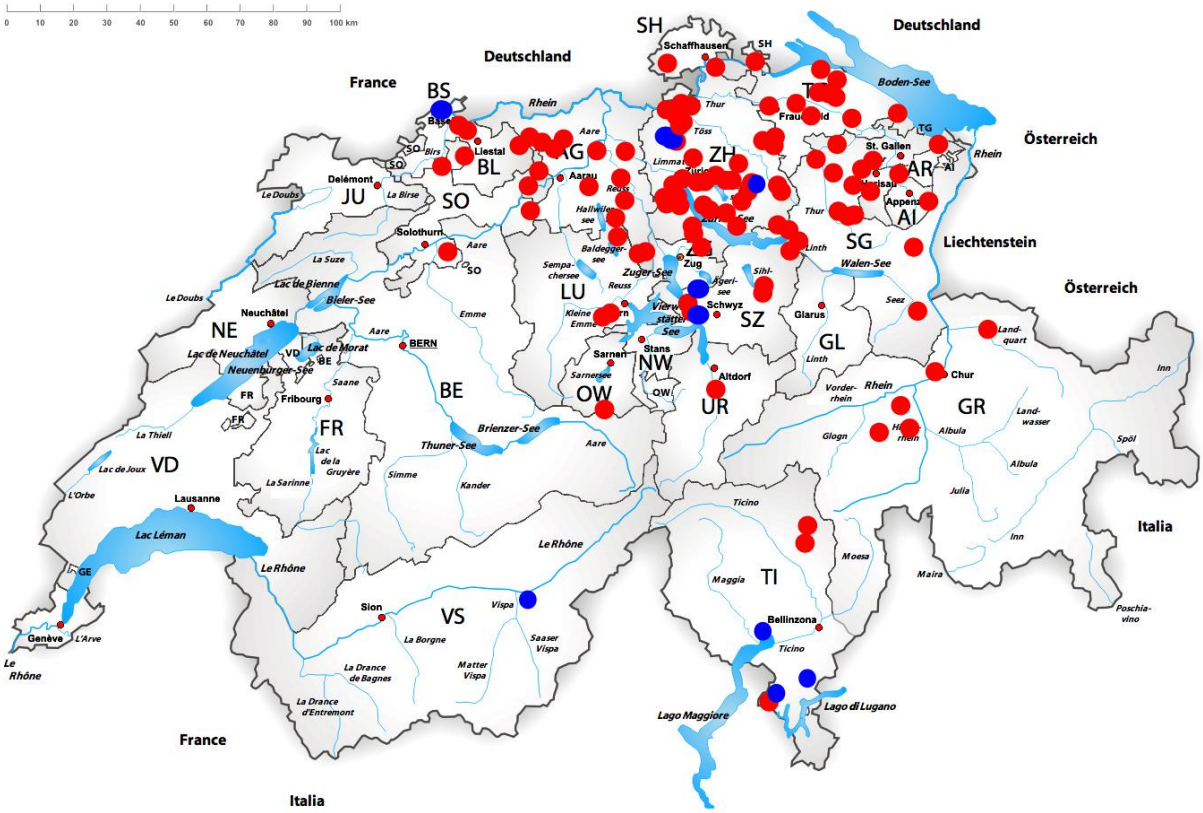

Figure 2:

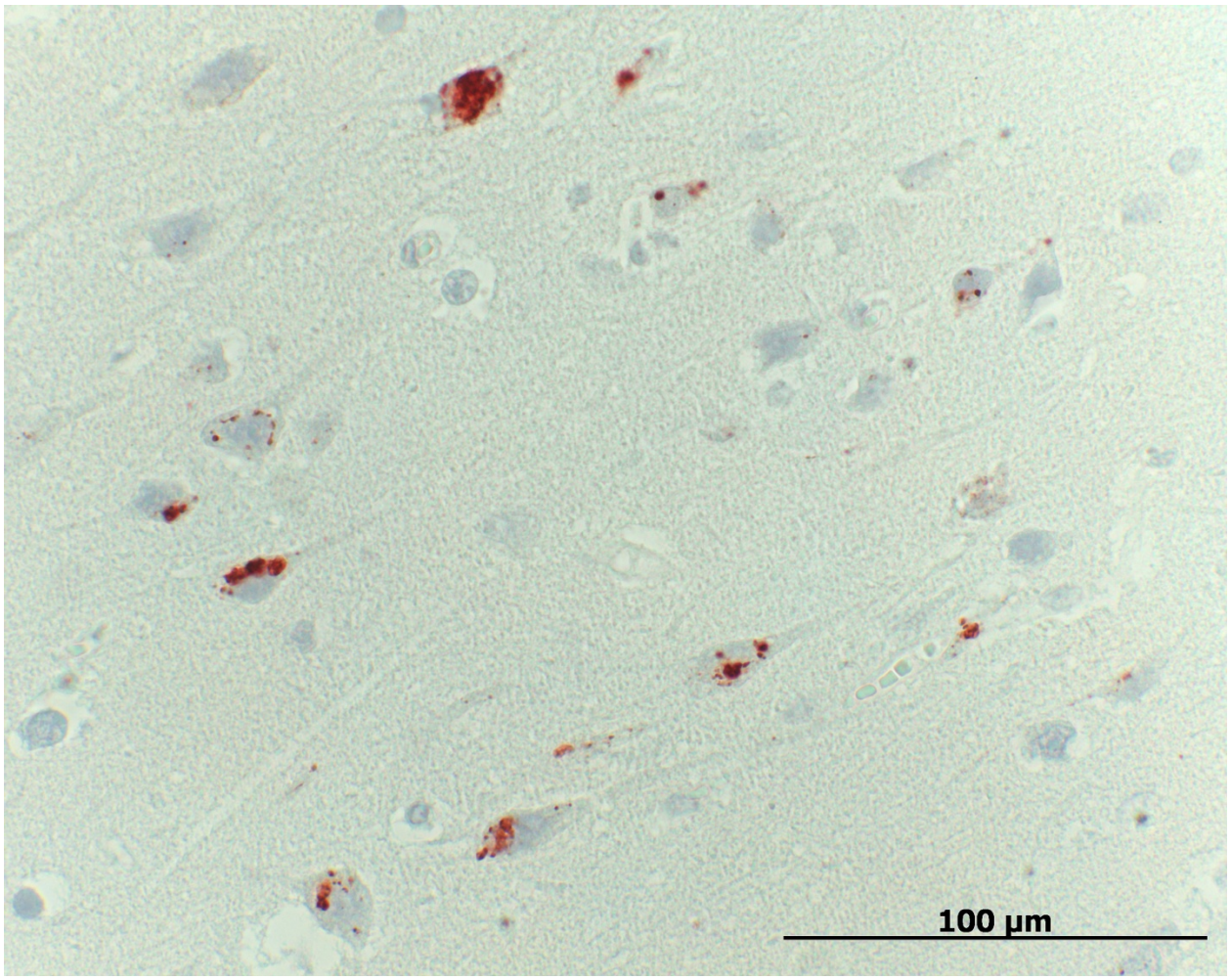

Table 1:

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

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

Table 3:

43 Mycobacterial -> welche Mycobacterien?
44

Species	Number of animals	Necropsy results
Llama	18	Infestation with Dicrocoelium dendriticum
	7	Intestinal parasites, like Eimeria sp. and Nematodirus sp.
	5	Septicemia
	3	Lymphoma
	2	Carcinoma
	2	Clostridium perfringens enterotoxaemia (type D)
	22	Diverse problems, like arthritis, cardiovascular problems, birth trauma, lung problems or no diagnosis
Alpaca	12	Infestation with Dicrocoelium dendriticum
	1	Infestation with Fasciola hepatica
	11	Cachexia due to dental problems or endoparasitosis (e.g. Nematodirus battus)
	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 M. kansasii 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 oculta 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

Legend:

Figure 1: Distribution of the New World camelids 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.

Zanolari Patrik 16.4.11 12:22
Kommentar [1]: 10 blaue Punkte auf der Karte für 59 Lamas und 93/94 Alpacas?

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