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Title: Immunoblotting for the serodiagnosis of alveolar echinococcosis in alive and dead Eurasian beavers (*Castor fiber*)

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1 Immunoblotting for the serodiagnosis of alveolar echinococcosis in alive and dead Eurasian beavers
2 (Castor fiber)
3

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12

13 Abstract

14 A novel species-specific anti-beaver-IgG-alkaline-phosphatase conjugate was synthesized for the
15 development a of a new serological test for echinococcosis in beavers. Two different ELISAs
16 conventionally used for human Echinococcus multilocularis serology (Em18-ELISA and Em2-ELISA)
17 yielded diagnostic sensitivities of 0% and 46%, respectively. In contrast, the subsequently developed
18 immunoblotting assay gave an 85% diagnostic sensitivity (11 out of 13 beavers with alveolar
19 echinococcosis were immunoblotting-positive, i.e. showed reactivity with a specific 21 Mr band), and
20 maximal specificity. In conclusion, this immunoblotting assay should be the method of choice for use in
21 serological studies on E. multilocularis in Eurasian beavers, and the test proved suitable to investigate
22 both animals alive and post-mortem.

23

24 Keywords

25 Echinococcus multilocularis; beaver; Em2-ELISA; Em18-ELISA; immunoblotting; EmVF-antigen

26

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30 Introduction

31 *Echinococcus multilocularis* is one of the most pathogenic parasitic zoonoses prevalent in central
32 Europe. The definitive (final) wildlife hosts in Europe are canids, including predominantly the red fox
33 (*Vulpes vulpes*), but the non-native raccoon dog (*Nyctereutes procyonoides*) and the domestic dog are
34 affected as well (Carmena and Cardona, 2013; Carmena and Cardona, 2014). Adult tapeworms live in
35 the small intestine of the definitive hosts, from which gravid parasite proglottids and eggs are shed with
36 the faeces into the environment. Intermediate hosts are infected when they ingest parasite eggs, which
37 upon release of an oncosphere, develop into the larval stage called metacestode. Metacestodes show a
38 distinct predilection for the liver. At a later stage of infection, metastases develop predominantly in the
39 lungs and brain, though other organs can be affected. The metacestode proliferates continuously and
40 leads to a cancer-like disease in affected intermediate hosts. The sylvatic cycle is completed by
41 carnivore predation of such infected intermediate hosts. In Europe several species of small microtine
42 and arvicolid rodents are the principle intermediate hosts, as well as two larger introduced species, the
43 coypu (*Myocastor coypus*) and the muskrat (*Ondatra zibethicus*) (Mathy et al., 2009). The Eurasian
44 beaver (*Castor fiber*) is another intermediate host. The first cases were reported from Switzerland
45 (Janovsky et al., 2001; Wimmershoff et al., 2012) and Austria (Cronstedt-Fell et al., 2010), and more
46 recent cases were described in the United Kingdom (Barlow et al., 2011) and in Serbia (Cirovic et al.,
47 2012). Humans are paratenic ("accidental") intermediate hosts.

48 A beaver reintroduction to Britain is currently underway as a scientific trial in Scotland, with a large
49 population of free-living beavers now established on the east coast. Further releases have been
50 proposed in Wales, and feasibility investigations are undertaken in England. However, there is now
51 another growing population of free-living beavers, which have either escaped from captivity or been
52 purposely, released in Scotland and England but are not part of government-sanctioned trials. The origin
53 of beavers for importation has been subject to academic debate (Halley 2011, Rosell et al. 2012), the
54 health status of imported animals being a key parameter. Current prevention measures include a six
55 months quarantine period, which is deemed sufficient to prevent the associated entry of rabies.
56 Screening for other infectious organisms is not required during this period, but additional health
57 screening recommendations have been made (Goodman et al. 2012). Among others, the risk of
58 introduction of *E. multilocularis* to non-endemic regions via importation of beavers originating from
59 endemic areas has been assessed following the Office International des Epizooties (OIE) risk assessment
60 framework (Kosmider et al., 2013; Defra, 2012). Subsequently, cases have been detected among beavers
61 meant for reintroductions (Cirovic et al., 2012; Barlow et al., 2011), and it is now recognized that both
62 captive and wild-caught beavers from central Europe represent a risk to import *E. multilocularis* to
63 presently *E. multilocularis*-free areas (Barlow et al. 2011; Campbell-Palmer et al., 2012; Pizzi et al., 2012).

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64 So far, cases of echinococcosis in beavers have mainly been diagnosed by post-mortem investigation,
65 principally based on methods that have been widely used to identify the larval stage of *E. multilocularis*
66 in other rodents and in human patients. Beside conventional histopathology, a molecular analysis by
67 PCR and/or direct immunofluorescence yields a reliable species-specific identification of the parasite.
68 This is particularly helpful in the rare cases where histology findings are inconclusive (Diebold-Berger et
69 al., 1997).

70 In contrast, diagnosing alveolar echinococcosis in live beavers is challenging. Imaging procedures may
71 provide a certain degree of information, as it has been demonstrated in other rarely infected
72 intermediate hosts, e.g. dogs (Scharf et al., 2004), rats (Asanuma et al., 2005) and non-human primates
73 (Kishimoto et al., 2009) but it is not reliable enough to rule out an infection. Currently, investigation of
74 live beavers prior translocation includes a time-consuming combination of clinical examination and
75 diagnostic imaging, such as detailed abdominal ultrasonography combined with endoscopic surgical
76 visual examination of the liver and other abdominal organs in anaesthetized animals (Pizzi et al., 2012).
77 A serological test could be used as a rapid diagnostic tool that could considerably reduce such
78 investigations in beavers. Besides its application to prevent the entry of the parasite via imported
79 individuals, serology could also be useful to assess exposure in captive or free-living populations
80 considered potential sources for translocation projects as well as to estimate prevalence in infected
81 populations. To our knowledge, serological diagnosis of parasitic infections in beavers has not yet been
82 reported. The goal of this study was to elaborate and evaluate serological tests regarding their suitability
83 to diagnose an *E. multilocularis* infection in the Eurasian beaver.

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84 Materials and Methods

85 Study design, animals and samples

86 We comparatively evaluated several conventional antigens presently used to detect anti-*E.*
87 *multilocularis* antibodies in intermediate hosts such as humans and small rodents (crude vesicle fluid,
88 EmVF-antigen [Müller et al., 2007], Em2-antigen [Gottstein et al., 1991], Em18-antigen [Sako et al.,
89 2002).

90 In a first step we developed a new anti-beaver-IgG-specific secondary antibody, because we had found
91 out in preliminary experiments that heterologous conjugates (anti-mouse IgG; protein A, protein G)
92 yielded unsatisfactory results with beaver samples. This host-specific antibody, at an affinity-purified
93 status, was coupled to alkaline phosphatase by using a conventional procedure provided by the
94 manufacturer (Sigma-Aldrich, http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-
95 explorer/analytical-enzymes/alkaline-phosphatase/conjugation.html).

96 In a second step, we compared two ELISAs and immunoblotting as previously evaluated for human
97 alveolar echinococcosis serology (Müller et al., 2007). Test evaluation was done using the samples from
98 three different groups of beavers: (1) a "positive" group of 13 beavers confirmed to be infected with *E.*
99 *multilocularis* by post-mortem investigations including histopathology and PCR (nine animals of Swiss
100 origin and four animals of Austrian origin); (2) a "negative" group consisting of 27 beavers originating
101 from a region known to be non-endemic for *E. multilocularis* (Scotland), i.e., animals expected to have
102 not been exposed to the parasite; (3) another "negative" group including 29 beavers from areas
103 endemic for *E. multilocularis* (25 dead animals from Switzerland and four from Austria), i.e., with
104 possible previous exposure. All negative beavers (groups 2 and 3) were necropsied and did not present
105 lesions consistent with the presence *E. multilocularis* infection. For statistical determination of a
106 negative-positive-threshold value in ELISA, only negative animals from group (2) were used.

107 For all beavers, blood samples consisted in sero-sanguinous fluid collected post-mortem, either
108 obtained from clotted heart-blood, or (if not available) muscle juice obtained as described elsewhere
109 (Berger-Schoch et al., 2011). Samples were kept frozen at -20°C until further analysis.

110 Anti-beaver IgG-conjugate

111 One ml of frozen beaver blood was used to purify IgG with the ammonium sulfate precipitation
112 technique as described by Page and Thorpe (2002), by applying a three-cycle-precipitation at 40%, 40%
113 and 50% saturation conditions. Purified beaver IgG was sent to Gallus Immunotech Inc. (Ontario,
114 Canada) for the production of affinity-purified chicken anti-beaver-IgG, based on IgY isolation from eggs
115 derived from hens immunized with 100µg beaver-IgG emulsified in Freund's adjuvants (indications refer

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116 to one immunization shot; hens received 1 primary immunization and 3 boosters every 10 days). Egg
117 yolk IgY was subsequently immuno-affinity purified on a solid-phase beaver-IgG column as previously
118 described (Felleisen and Gottstein, 1993). All subsequent steps to prepare the final anti-beaver-IgG-
119 alkaline phosphatase-conjugate were carried out as described elsewhere (Baumann and Gottstein,
120 1987).

121 For a primary validation of the newly synthesized anti-beaver-IgG-conjugate, we electrophoretically
122 separated the purified beaver-IgG by SDS-PAGE and applied a Coomassie-blue staining, followed by
123 immunoblotting.

124 ELISA

125 All blood samples were examined for antibodies directed against the Em2- and recEm18-antigen from *E.*
126 *multilocularis* by ELISA as previously published for foxes (Gottstein et al., 1991) but using the beaver-
127 IgG-specific alkaline phosphatase conjugate described above. Beaver samples were considered sero-
128 positive when the ELISA A405nm-values exceeded the average negative control value plus 4 standard
129 errors (S.E.). The actual threshold value discrimination between negative and positive reactions was
130 based on a 99.9% range exhibited by the 26 “negative” beaver samples from non-endemic areas, this
131 was performed for each antigen separately. We used the mean plus 4 S.E. to calculate the threshold
132 values for the Em2- and the recEm18-antigen, respectively. All values above these cut-offs were
133 regarded as positive, all others below as negative. A ROC based approach was not feasible due to the
134 low number of ”positive” cases.

135 Immunoblotting

136 Immunoblotting was performed as previously described for *E. granulosus* hydatid fluid (EgHF) antigen
137 (Poretti et al. 1999), with the exception that EmVF (7 µg per cm slot) was used instead of EgHF (Müller
138 et al., 2007). The conjugate was identical to that used for the ELISAs described above.

139 Statistical analyses

140 Data were analyzed using the computer program SPSS 17.0. One-way ANOVA and Student’s t-test were
141 used to determine threshold values and to compare differences between groups. $P<0.05$ was considered
142 as indicating statistical significance.

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143 Results

144 Anti-beaver IgG-conjugate

145 Electrophoresis (Figure 1A) and Coomassie blue staining revealed the presence of two predominating
146 bands, the upper one corresponding by relative molecular mass (Mr) to the heavy chain of the
147 antibody, and the lower one to the light chain. Immunoblotting analysis of these two bands upon use of
148 the anti-beaver-IgG-alkaline-phosphatase conjugate revealed the binding capacity of the conjugate to
149 both antibody chains, with a stronger activity to the heavy chain (Figure 1B).

150 ELISA

151 Table 1 shows the results obtained with the two ELISAs for the beaver samples from the "positive"
152 group. With the Em18-ELISA, all samples from this group yielded negative findings. With the Em2-ELISA,
153 six out of 13 "AE-positive" beavers showed a serological reaction. Thus, the diagnostic sensitivities of
154 both tests were very low (0% and 46%, respectively).

155 All 29 beaver samples of the "negative" group from endemic areas were serologically negative, i.e., the
156 obtained values were all in the same range as the 27 "negative" samples from non-endemic areas used
157 to determine the cut-off point. Statistically, there was no difference between the median value of the
158 negative sera from non-endemic areas and those from endemic areas.

159 Immunoblotting

160 Immunoblot profiles, as shown for two samples from the "positive" group (beavers B1 and B2, Figure 2),
161 demonstrated antibody reactivity with one major immunoreactive band and two minor side bands of
162 approximately Mr 21 (major band), and Mr 19 and Mr 40 (minor bands). The localization of these three
163 bands corresponded to the localization of bands obtained with a positive control of human origin (H1).
164 This human serum banding pattern also matched the one described earlier in a large human serological
165 study (Müller et al. 2007). Based on the detection of an anti-21Mr-banding activity, the immunoblotting
166 approach yielded an 85% diagnostic sensitivity, as 11 out of 13 beavers from the "positive" group were
167 seropositive, (Table 1). All "negative" and "true negative" beaver samples were clearly seronegative,
168 considering the absence of any band (exemplified by samples B3 – B5 in Figure 2).

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169 Discussion

170 The purpose of this study was to develop and evaluate the suitability of serological tests for the
171 detection of *E. multilocularis* infection in the Eurasian beaver. Such a rapid diagnostic tool is urgently
172 needed to facilitate the procedures aiming at minimizing the risk of introducing the parasite via
173 translocated animals, which requires both the testing of translocated individuals in vivo prior to release,
174 and screenings of potential source populations.

175 A serological test would be applicable on blood samples from both live and dead beavers, making it
176 suitable for in-vivo testing of animals prior translocation and for serological surveys using samples
177 collected post-mortem. The beaver-specific conjugate developed in this study operates methodically
178 very well in ELISAs and also in immunoblotting assays, but the diagnostic performances turned out very
179 different between the two test systems.

180 None of the beavers from the “positive” group showed a seropositive reaction in the Em18-ELISA,
181 indicating that beavers do not develop a humoral immunity against this antigen. Results obtained with
182 the Em2-ELISA were very unsatisfying a well. Thus, the evaluation of these two tests revealed that they
183 are not suitable for diagnosis of *E. multilocularis* infections in beavers. One possible explanation for this
184 phenomenon may not be related to the antigen itself, but may be due to the quality of the beaver
185 “blood” used for serology. Post-mortem decay and degradation may decrease the serological quality of
186 the fluids recovered from the dead animals with regard to application in ELISAs that use highly purified
187 antigens, but may be not with regards to immunoblotting that uses the complex mixture of a crude
188 metabolic antigen. We plan to investigate this aspect upon direct comparison of the Em2-ELISA and
189 EmVF-IB with sera obtained from beavers captivated for translocation. However, such a study may be
190 very lengthy in time, as we would need to investigate their livers post-mortem to get a conclusive
191 diagnosis regarding the presence or absence of AE-lesions and *E. multilocularis* infection, respectively.

192 In contrast, a diagnostic sensitivity of 85% was observed with immunoblotting, which, in terms of
193 serodiagnosis, reaches an acceptable level, especially as specificity reached 100% in our study. Overall,
194 while a seropositive result in immunoblotting unambiguously indicates an infection (high positive
195 predictive value), a negative serological result has to be considered with caution.

196 Interestingly, the tested seropositive beavers showed a very weak banding pattern in immunoblots.
197 While a distinct and rather complex pattern of antigen bands is identified in samples of most human
198 patients with alveolar echinococcosis, beavers exhibit a binding activity with a maximum of three
199 different antigens. This suggests that the *E. multilocularis* metacestode antigens are of very weak
200 antigenicity, which may partially explain why all beavers demonstrated negative results with the
201 conventional Em18-antigen, and why only a very weak diagnostic sensitivity was obtained with the

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202 conventional Em2-antigen. As discussed for the ELISAs above, one of the reasons why a few samples
203 from infected beavers were negative in immunoblotting may have been a decreased quality of the
204 beaver blood due to post-mortem decay. Another reason for the overall weak (methodically and
205 diagnostically) humoral immune response detected in beavers with alveolar echinococcosis may be
206 associated to a high susceptibility to infection of this animal species, i.e., infection and resulting organ
207 lesions may occur in a way that the host cannot mount an appropriate humoral immune response, as
208 compared to other intermediate hosts such as humans who react strongly by the humoral pathway of
209 immunity. Nevertheless, this explanation is unlikely as we know from observations in laboratory rodents
210 that antibody-deficient animals such as the µMT mouse do not show an increased susceptibility (Dai et
211 al., 2004). However, a weak humoral immune response may be associated to a weak cellular immune
212 response, and it is known from murine and human alveolar echinococcosis in immunosuppressed
213 individuals that a weak cellular immune response markedly favours metacestode proliferation (Vuitton
214 and Gottstein, 2010). Referring to beavers, this weak immune response is likely not a particularity of
215 some putatively immunosuppressed individuals but rather a characteristic of the species itself. Indeed, if
216 some beavers would develop a strong antibody reaction (without subsequent lesions), it is probable that
217 part of the beavers from the "negative" group (i.e., animals without lesions but from endemic areas)
218 would have been seropositive. Yet, our sample size was limited and serological investigations of a larger
219 number of beavers originating from endemic areas and submitted to necropsy are necessary to further
220 address this question. Furthermore, as in alveolar echinococcosis susceptibility to disease is usually
221 associated to metacestode fertility, we invite wildlife pathologists to document the frequency of related
222 findings, e.g. protoscolex formation within the parasite tissue, and to carefully record the features of
223 periparasitic inflammatory and immune-mediated processes that may contribute to either accelerated
224 or delayed metacestode proliferation and maturation (Vuitton and Gottstein, 2010).

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226 Conclusions

227 Serodiagnosis of *E. multilocularis* infection in beavers is now possible, and so far the best methodical
228 approach consists in performing immunoblotting based on the detection of anti-Mr21-band-binding
229 activity. Cross- or non-specific reactions did not occur in our study (100% specificity), and the diagnostic
230 sensitivity amounts to 85%. With these diagnostic sensitivity and specificity and a putative prevalence
231 estimated in a future study area, positive and negative predictive values could now be determined. We
232 encourage wildlife health scientists to make use of this tool in order to support further assessment of
233 factors that will help to better interpret serological results.

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241 References

242 Asanuma, T., Matsumoto, Y., Takiguchi, M., Inanami, O., Nakao, M., Nakaya, K., Ito, A., Hashimoto, A.,
243 Kuwabara, M., 2003. Magnetic resonance imaging and immunoblot analyses in rats with
244 experimentally induced cerebral alveolar echinococcosis. Comp. Med. 53, 649-656.

245 Barlow, A.M., Gottstein, B., Mueller, N., 2011. Echinococcus multilocularis in an imported captive
246 European beaver (Castor fiber) in Great Britain. Vet. Rec. doi:10.1136/vr.d4673

247 Baumann, D., Gottstein, B., 1987. A double-antibody sandwich ELISA for the detection of Entamoeba
248 histolytica antigen in stool samples of humans. Trop. Med. Parasit. 38, 81-85.

249 Berger-Schoch, A.E., Bernet, D., Doherr, M.G., Gottstein, B., Frey, C.F., 2011. Toxoplasma gondii in
250 Switzerland: A serosurvey based on meat juice analysis of slaughter pigs, wild boar, sheep and cattle.
251 Zoon. Publ. Hlth. 58, 472-478.

252 Campbell-Palmer, R., Girling, S., Rosell, F., Pulsen, P., Goodman, G., 2012. Echinococcus risk from
253 imported beavers. Vet. Rec. doi:10.1136/vr.e1508

254 Carmena, D., Cardona, G.A., 2013. Canine echinococcosis: global epidemiology and genotypic diversity.
255 Acta Trop. 128, 441-460.

256 Carmena, D., Cardona, G.A., 2014. Echinococcosis in wild carnivorous species: Epidemiology, genotypic
257 diversity, and implications for veterinary public health. Vet. Parasitol. 202, 69-94.

258 Ćirović, D., Pavlović, I., Kulišić, Z., Ivetić, V., Penezić, A., Ćosić, N., 2012. Echinococcus multilocularis in the
259 European beaver (Castor fibre L.) from Serbia: first report. Vet. Rec. 171, 100.

260 Cronstedt-Fell, A., Stalder, G.L., Kübber-Heiss, A., 2010. Echinococcosis in a European beaver (Castor
261 fiber) in Austria. Poster presentation 36, 9th EWDA conference, Vlieland, September 13-16, 2010.

262 Dai, W.J., Waldvogel, A., Siles-Lucas, M., Gottstein, B., 2004. $\alpha\beta$+CD4+ T cell mediated immune response
263 is crucial for the regulation of parasite 14-3-3 expression and for the control of parasite growth in
264 Echinococcus multilocularis infection. Immunol. 112, 481-488.

265 DEFRA, 2012. What is the risk of introducing Echinococcus multilocularis to the United Kingdom wildlife
266 population by importing European beavers which subsequently escape or are released?
267 http://www.defra.gov.uk/animal-diseases/files/qra-non-nativespecies-echinoccocus-120627.pdf.
268 Accessed July 5, 2012

Accepted Manuscript

ACCEPTED MANUSCRIPT

269 Diebold Berger, S., Khan, H., Gottstein, B., Puget, E., Frossard, J.L., Remadi, S., 1997. Cytologic diagnosis
270 of isolated pancreatic alveolar hydatid disease with immunologic and PCR analyses - A case report.
271 Acta Cytol. 41, 1381-1386.

272 Felleisen, R., Gottstein, B., 1993. Echinococcus multilocularis: Molecular and Immunochemical
273 characterization of diagnostic antigen II/3-10. Parasitol. 107, 335-342.

274 Goodman, G., Girling, S., Pizzi, R., Meredith, A., Rosell, F., Campbell-Palmer, R., 2012. Establishment of a
275 health surveillance program for reintroduction of the Eurasian beaver (Castor fiber) into Scotland. J.
276 Wildl. Dis. 48, 971-978.

277 Gottstein, B., Deplazes, P., Eckert, J., Müller, B., Schott, E., Helle, O., Boujon, P., Wolff, K., Wandeler, A.,
278 Schwiete, U., Moegle, H., 1991. Serological (Em2-ELISA) and parasitological examinations of fox
279 populations for Echinococcus multilocularis infections. J. Vet. Med. B, 38, 161-168.

280 Halley, D.J., 2011. Sourcing Eurasian beaver Castor fiber stock for reintroductions in Great Britain and
281 Western Europe. Mammal Rev. 41, 40-53.

282 Janovsky, M., Bacciarini, L., Sager, H., Grone, A., Gottstein, B., 2002. Echinococcus multilocularis in a
283 European beaver from Switzerland. J. Wildl. Dis. 38, 618-620.

284 Kishimoto, M., Yamada, K., Yamano, K., Kobayashi, N., Fujimoto, S., Shimizu, J., Lee, K.J., Iwasaki, T.,
285 Miyake, Y., 2009. Significance of imaging features of alveolar echinococcosis in studies on nonhuman
286 primates. Am. J. Trop. Med. Hyg. 81, 540-544.

287 Kosmider, R., Paterson, A., Voas, A., Roberts, H., 2013. Echinococcus multilocularis introduction and
288 establishment in wildlife via imported beavers. Vet. Rec. 172, 606.

289 Mathy, A., Hanosset, R., Adant, S., Losson, B., 2009. The carriage of larval Echinococcus multilocularis
290 and other cestodes by the muskrat (Ondatra zibethicus) along the Ourthe river and its tributaries
291 (Belgium). J. Wildl. Dis., 45, 279-287.

292 Müller, N., Frei, E., Nunez, S., Gottstein, B., 2007. Improved serodiagnosis of alveolar echinococcosis of
293 humans using an in vitro-produced Echinococcus multilocularis antigen. Parasitol. 134, 879-888.

294 Page, M., Thorpe, R., 2002. Purification of IgG by Precipitation with Sodium Sulfate or Ammonium
295 Sulfate. In: JM Walkler (ed.), The Protein Protocols Handbook, Humana Press Inc., Totowa, pp 983-
296 984.

Accepted Manuscript

ACCEPTED MANUSCRIPT

297 Pizzi, R., Cracknell, J., Carter, P., 2012. Echinococcus risk from imported beavers. Vet. Rec.
298 doi:10.1136/vr.e2041

299 Poretti, D., Felleisen, E., Grimm, F., Pfister, M., Teuscher, F., Zürcher, C., Reichen, R. and Gottstein, B.,
300 1999. Differential immunodiagnosis between cystic hydatid disease and other cross-reactive
301 pathologies. Am. J. Trop. Med. Hyg. 60, 193-198.

302 Rosell, F., Campbell-Palmer, R., Parker, H., 2012. More genetic data are needed before populations are
303 mixed: response to 'Sourcing Eurasian beaver Castor fiber stock for reintrodcutions in Great Britain
304 and Western Europe'. Mammal Rev. 42, 319-324.

305 Sako, Y., Nakao, M., Nakaya, K., Yamasaki, H., Gottstein, B., Lightowers, M.W., Schantz, P.M., Ito, A.,
306 2002. Alveolar echinococcosis: Characterization of diagnostic antigen Em18 and serological
307 evaluation of recombinant Em18. J. Clin. Microbiol. 40, 2760-2765.

308 Scharf, G., Deplazes, P., Kaser-Hotz, B., Borer, L., Hasler, A., Haller, M., Flückiger, M., 2004. Radiographic,
309 ultrasonographic, and computed tomographic appearance of alveolar echinococcosis in dogs. Vet.
310 Radiol. Ultrasound. 4, 411-418.

311 Vuitton, D.A., Gottstein, B., 2010. Echinococcus multilocularis and its intermediate host: a model of
312 parasite-host interplay. J. Biomed. Biotechnol. 2010:923193. Epub 2010 Mar 21.

313 Wimmershoff, J., Robert, N., Mavrot, F., Hoby, S., Boujon, P., Frey, C., Weber, M., Café-Marçal, V., Hüssy,
314 D., Mattsson, R., Pilo, P., Nimmervoll, H., Marreros, N., Pospischil, A., Angst, C., Ryser-Degiorgis, M.-P.
315 2012. Causes of mortality and diseases in the reintroduced European beaver population in
316 Switzerland from 1989 to 2009. Proceedings of the joint WDA/EWDA conference, Lyon, July 22-27,
317 2012. P. 37.
318

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318 Table 1: Serological investigation of 13 beavers with hepatic lesions associated with *E. multilocularis*
319 infection, as evidenced by histology and PCR. Abbreviations: CH: Switzerland; A: Austria; WB:
320 immunoblotting.

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diagnosis	origin	Diagn. no.	Em2_ELISA	Em18_ELISA	WB
AE	CH	12S751	neg	neg	neg
AE	CH	12S752	neg	neg	**pos**
AE	CH	W07/0871	**pos**	neg	**pos**
AE	CH	W08/0973	**pos**	neg	**pos**
AE	CH	W07/4586	**pos**	neg	**pos**
AE	CH	W08/973	neg	neg	**pos**
AE	CH	W07/1152	neg	neg	**pos**
AE	CH	12S759	neg	neg	**pos**
AE	CH	W09/1428	**pos**	neg	**pos**
AE	A	12S751	neg	neg	neg
AE	A	12S752	neg	neg	**pos**
AE	A	12D2967	**pos**	neg	**pos**
AE	A	12D2968	**pos**	neg	**pos**
pos/tot			*6/13*	*0/13*	*11/13*
diagn. sensitivity			**46%**	**0%**	**85%**
"negative"*	Scotland	n = 27	n.d.	n.d.	0/27
"negative"	CH	n = 25	n.d.	n.d.	0/25
"negative"	Austria	n = 4	n.d.	n.d.	0/4
neg/tot					0/60
diagn. specificity					**100%**

322 *data used to calculate the negative-positive threshold

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324 Legends to Figures

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326 Figure 1: Quality control of the new anti-beaver-IgG-alkaline-phosphate conjugate. (A) Immunoblotting
327 approach with 1 or 5 µg of purified beaver-Ig, SDS-PAGE separated and transferred onto nitrocellulose.
328 Subsequent antibody fragment detection was performed with the new conjugate. (B) Purity status of
329 the purified beaver-IgG used to generate a polyclonal hyperimmune chicken IgY directed against beaver-
330 IgG. The left blue lane shows stained Mr markers and corresponding Mr sizes. The estimated relative
331 molecular mass of the beaver IgG heavy chain is Mr 55 kDa, while that of the light chain appears at
332 approximately Mr 25 kDa.

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335 Figure 1

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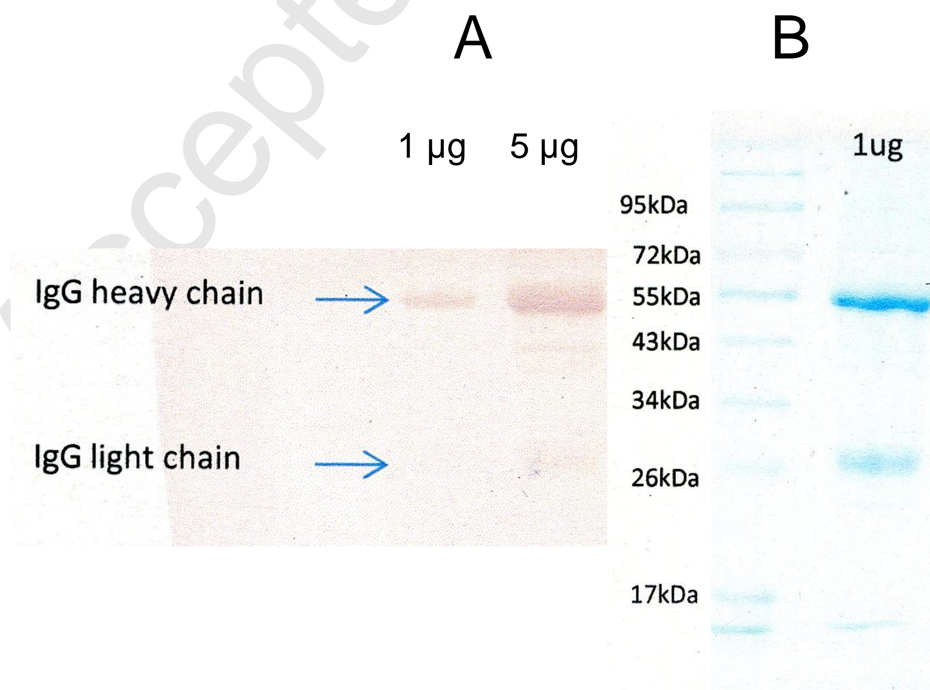

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340 Figure 2: *E. multilocularis* immunoblot analysis with the following sera: H1 – human AE-patient (positive
341 banding pattern control); B1, B2 - samples from beavers with alveolar echinococcosis (infection
342 confirmed by histology and PCR); B3-B5 – samples from three beavers showing no macroscopic evidence
343 for an infection with *E. multiulocularis* (= negative animals). Arrow points at the diagnostic major band
344 at Mr21. Left lane shows stained Mr markers and corresponding Mr sizes.

345

346 Figure 2

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