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1 # Evaluation of Fecal Immunoassays for Canine *Echinococcus* Infection in China

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32 **Running title:** Assessment of echinococcosis tests

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36 **Keywords:** Echinococcosis, *Echinococcus granulosus*, China, Detection, ELISA,
37 sandwich ELISA, Immunopurification

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39 **Abstract**

40

41 Human echinococcosis is present worldwide but it is in China that prevalence is the
42 highest. Western China and in particular the Tibetan plateau is the region where the
43 burden of echinococcosis is the most important. Dogs are a major carrier of
44 echinococcosis and monitoring the presence of *Echinococcus* worms in dogs is
45 therefore essential for efficiently controlling the disease. Detection kits based on three
46 different technologies, i.e. sandwich ELISA, ELISA and gold immunodiffusion are
47 currently marketed and used in China. The objective of this work was to assess the
48 efficacy of these kits, in particular with respect to sensitivity and specificity. Four
49 fecal antigen detection kits for canine echinococcosis covering the three technologies
50 were obtained from companies and tested in parallel on 220 fecal samples. The results
51 indicate that the performance is lower than expected, in particular in terms of
52 sensitivity. The best results were obtained with the sandwich ELISA technology. The
53 gold immunofiltration yielded the poorest results. In all cases, further development is
54 needed to improve the performance of these kits, which represent a key element for
55 the control of echinococcosis.

56

57 **Running title:** *Echinococcus* immunoassays

58

59 **Key words:** Echinococcosis, *Echinococcus granulosus*, dog, fecal immunoassays,
60 sandwich ELISA, ELISA, gold immunofiltration

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62 **Author summary**

63

64 Although present worldwide, human echinococcosis is at its highest prevalence in
65 Western China and particularly on the Tibetan plateau. Controlling echinococcosis is
66 a national priority and routine monitoring must be established. Dogs are the main
67 carriers of echinococcosis and surveying *Echinococcus* worms in dogs is therefore a
68 key issue. Commercial detection kits are currently in use in China for monitoring the
69 presence of Echinococcosis in dogs. These kits are based on three different
70 technologies, i.e. sandwich ELISA with two monoclonal antibodies, ELISA, and gold
71 immunodiffusion. National survey programs are essential for the control of
72 echinococcosis and it is thus very important to assess the efficacy of these kits,
73 planned to be used for the national survey programs. The work was thus undertaken to
74 assess this efficacy, in particular with respect to sensitivity and specificity. Four fecal
75 antigen detection kits for canine echinococcosis covering the three technologies were
76 obtained from companies and tested in parallel on 220 fecal samples. The
77 performance was lower than expected, in particular for their sensitivity, which ranged
78 from 51.5% to 83.9% with only two samples displaying a worm burden lower than
79 100. Three out of four kits showed non-specific cross-reactions with other parasites.
80 The best results were obtained with the sandwich ELISA technology, whereas gold
81 immunofiltration yielded the poorest results. However, in all cases, further
82 development is strongly needed to improve the performance of these kits, which
83 represent a key element for the control of echinococcosis.

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85 ## 1. Introduction

86

87 Echinococcosis is a health-threatening parasitic zoonotic disease caused by the
88 larval stage of *Echinococcus* tapeworms [1]. Cystic echinococcosis (CE) and alveolar
89 echinococcosis (AE) in humans, livestock and small mammals are triggered by the
90 involuntary consumption of *Echinococcus granulosus* and *Echinococcus*
91 *multilocularis* eggs, respectively, which are excreted in the feces of the definitive
92 hosts, i.e. carnivores. Naturally, the transmission occurs between definitive hosts
93 (primary dogs and foxes) and intermediate hosts (livestock and small mammals),
94 whilst humans are accidental hosts. Human infection can occur through direct contact
95 with the definitive host or indirectly through contamination of food or possibly water
96 with parasite eggs [2]. *Echinococcus granulosus* is distributed worldwide, with only a
97 few areas such as Iceland, Ireland, and Greenland, which are considered free of
98 autochthonous human cases [3,4]. *Echinococcus multilocularis* is confined to the
99 northern hemisphere, but within that range displays a wide distribution [5]. In humans,
100 metacestode infection causes severe disease and possibly death. It also results in
101 economic losses from treatment costs, lost wages and livestock-associated production
102 losses. It has been recognized as one of the world's public health issues.

103

104 Both CE and AE are endemic in the pasture areas of western China, threatening
105 more than 50 million people with a global echinococcosis prevalence of 0.28% in
106 humans, 4.68% in livestock and 4.25% in dogs. The number of patients was estimated

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107 to be 166,098 [6]. All provinces (autonomous regions, municipalities and special
108 administrative regions) have recorded cases of echinococcosis. Echinococcosis is
109 indigenous in endemic provinces and imported in non-endemic provinces.
110 Echinococcosis has been listed as a key parasitic disease in China [7]. China is
111 believed to be accountable for 40% of the world CE Disability Adjusted Life Years
112 (DALYs) [8]. A national control project has been implemented in echinococcosis
113 endemic areas in western China since 2006. Dog management and monthly treatment
114 with praziquentel are two major intervention measures implemented to prevent human
115 and livestock infections. Therefore, the detection of *Echinococcus* infections in dogs
116 is a very important indicator to assess control efficacy and risk of disease transmission
117 [9].
118
119 Diagnosis and detection of *Echinococcus granulosus* (*sensu lato*) infection in
120 animals is a prerequisite for epidemiological studies and surveillance of
121 echinococcosis in endemic, re-emergent or emergent transmission zones. Testing dog
122 fecal samples by coproantigen ELISA, often combined with mass ultrasound
123 screening programs for human CE, has been the preferred approach for monitoring
124 and surveillance in resource-poor endemic areas and during control schemes [10].
125 Dogs infection rates are very high and sensitive indicators to assess the risk and
126 burden of echinococcosis and to evaluate the effect of control measures [11].
127

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128 Currently, 2 sandwich ELISA kits and 1 ELISA test for the detection of
129 coproantigen as well as a gold immunofiltration assay are commercially available in
130 China. In this work, we evaluated the relative performance of these four kits
131 representing three different technologies, in particular sensitivity and specificity, in
132 the detection of *Echinococcus granulosus* infections in dogs in order to provide a
133 reference for practical implementation in control projects.

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135 **2. Materials and methods**

136

137 **2.1. Kits assessed.** This study assessed 4 kits currently used for the prevention and
138 control of echinococcosis in China. The kits were randomly coded as A, B, C and D.
139 The information on the kits is provided in Table 1. These kits were provided by
140 Xinjiang Tecon Animal Husbandry Bio-Technology Co., Ltd, Zhuhai S.E.Z. Haitai
141 Biological Pharmaceuticals Co.,Ltd. and Shenzhen Combined Biotech Co., Ltd (Table
142 1). Two kits were sandwich ELISA tests (A and B), one is an ELISA test (D) and one
143 is a Gold Immunofiltration assay (C) (Table 1).

144

145 **Table 1. Major features of the assessed tests for *Echinococcus granulosus***
146 **diagnosis in dogs**

147

Assay code	Assay type	Sample Volumes	Extra supplies	Time required	Product form
A	Sandwich ELISA, 2 MAbs	100 µL	No	135 min	kit
B	Sandwich ELISA, 2 MAbs	100 µL	No	135 min	kit
C	Gold immunofiltration	300 µL	No	130 min	kit
D	ELISA	100 µL	No	150 min	kit

148

149 **2.2. Specimen collection.** A total of 34 positive canine fecal specimens were
150 collected from dogs in Xinjiang, Qinghai and Gansu Positive cases were identified by

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151 demonstration of the presence of adult worms in the intestine, which is considered as
152 the "gold" standard for the identification of *Echinococcus* infections [12]. Hence, we
153 detected *E. granulosus* through autopsy in 34 specimens with a minimum parasite
154 load of 5 and a maximum load of 25,000 (Table 2). A complement of 158 negative
155 canine fecal specimens were collected, out of which 116 were from non-endemic
156 areas in Gansu and 42 from laboratory dogs without any parasitic infection. An
157 additional 28 samples of canine fecal specimens were also collected from dogs
158 displaying other parasitic infections. Eight samples of *Taenia hydatigera*, 8 of
159 *Dipylidium caninum* and 12 of *Spirometra mansoni*, were collected in the Guangdong
160 province (Table 3). All specimens were verified by etiologic inspection.

161 **Table 2. Specific parasite load in the 34 positive samples**

Infection Level	Worm load Range	Number of infected dogs	Worm burden
I	[0-500]	9	5;60;100;200(3);300(2);400
II	[500-5,000]	10	600(2);1,100;1,500;2,100;3,100 (2);3,500(2);4,000
III	[5,000-20,000]	7	6,000(5);11,000(2)
IV	[20,000-+∞]	8	20000(3);25000(5)

162

163

164 **Table 3. Composition and origin of samples**

Category	Number of dogs sampled	Sample origin
Positive canine fecal specimens	34 (*Echinococcus granulosus* infection)	Qinghai Center for Disease Control and Prevention and The Animal Husbandry Institute of Xinjiang Uygur Autonomous Region
Negative canine fecal specimens	158 (from non-endemic areas in Gansu province)	Gansu Center for Disease Control and Prevention
Canine fecal specimens of other parasitic infection	28 (including 8 with *Taenia hydatigera*, 8 with *Dipylidium caninum*, 12 with *Spirometra mansoni*)	Sun Yat-Sen University of Guangdong province

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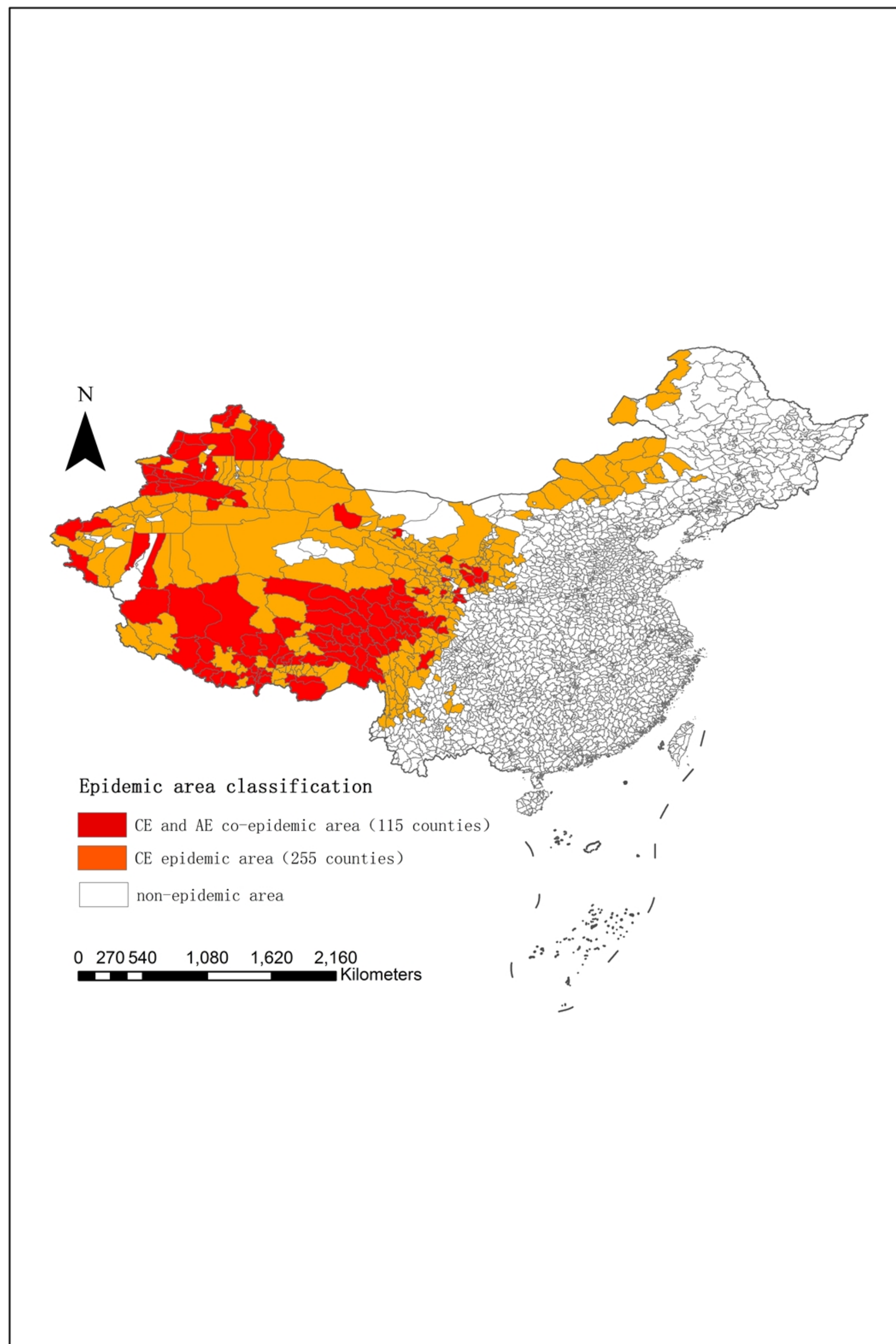
166



167

168 **Figure 1. Distribution of echinococcosis by county level in China**

169 AE: Alveolar echinococcosis

170 CE: Cystic echinococcosis

171

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172 **2.3. Sample preparation.** A double-blind method was used in the detection process.
173 All information was kept confidential. Experimenters did not know what they were
174 testing, they only received code numbers as sample identifiers. Each one of the four
175 kits was handled by a different group. In order to ensure that the concentration of
176 sample in the different groups was the same, the preliminary preparation of the
177 samples was performed by the senior experimenter of the organization. Samples were
178 stored at -80℃ upon collection. Fecal specimens were defrosted and 3g of each
179 sample were diluted in Phosphate Buffer Saline (PBS) at pH 7.2～7.4, to the final
180 concentration of 1g/mL and centrifuged at 3000 G for 30 minutes. After
181 centrifugation, 2 mL of supernatant were collected. For two groups of parallel
182 samples for each test, 6 sample batches of 100μL and 2 sample batches of 300μL
183 were prepared. In order to avoid any mutual confirmation of results, all samples were
184 randomly encoded. The information was kept confidential.
185

186 **2.3. Detection tests.** All samples were tested by each kit in double according to the
187 manufacturer's instructions. The operator of each detection kit was assigned by the
188 company. Parallel detection tests with the four different kits were conducted
189 simultaneously in the same laboratory.
190

191 **2.4. Data analysis.** Data were analyzed using the SPSS 20.0 software package (IBM,
192 Armonk, USA). The indicators considered for analysis were: accuracy, reliability,
193 sensitivity, specificity, positive predictive value, negative predictive value, Youden's

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194 index, cross reaction rate, consistency rate and weighted consistency rate. Data were
195 tested using a chi-square test. Each index is the average of the test results of two
196 groups of parallel samples. Definitions and calculation methods of relevant indicators
197 are as follows.
198 **Sensitivity:** Proportion of known infected fecal samples that tested positive in an
199 assay（Infected fecal samples that tested negative are considered as false negatives.）
200 **Specificity:** Proportion of uninfected reference fecal samples that tested negative in
201 an assay. (Uninfected fecal samples that tested positive are regarded as false
202 positives). This type of specificity is denominated **specificity 1**. Specificity tests
203 which referring to reference fecal samples not infected with *Echinococcus* but
204 harboring other parasites is denominated **specificity 2.**
205 **Cross reaction rate:** Proportion of samples uninfected with *Echinococcus* but
206 harboring other parasites reference fecal samples, which tested positive in an assay.
207 **Positive predictive value (PV+):** PV+ is an indicator of the probability that
208 individuals with positive testing results do have the disease.
209 **Negative predictive value (PV-):** The PV- is an indicator of the probability that
210 individuals with negative testing results do not have the disease.
211 **Youden's index**：Youden's index expresses the total ability of a reagent to detect true
212 positive and true negative samples.
213 **Consistency rate**：Proportion of samples with the same test results of reagents as the
214 real results.
215 **Kappa Value:** Kappa value is used to analyze and evaluate the consistency of two
216 parallel samples detected by one detection method. Considering the influence of

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217 opportunity factors on consistency rate.

218

219 $\text{Sensitivity} = \frac{TP}{TP + FN} \times 100\%_{(1)}$;

220 $\text{Specificity} = \frac{TN}{FP + TN} \times 100\%_{(2)}$;

221 $PV+ = \frac{TP}{TP + FP} \times 100\%_{(3)}$;

222 $PV- = \frac{TN}{TN + FN} \times 100\%_{(4)}$;

223 $\text{Youden's index} = \text{Sensitivity} + \text{Specificity} - 1_{(5)}$;

224 Cross reaction rate=1- specificity $2_{(6)}$;

225 $\text{Consistency rate} = \frac{TP + TN}{N} \times 100\%_{(7)}$;

226 $\text{Kappa Value} = \frac{N(TP+TN) - (R1C1+R2C2)}{N^2 - (R1C1+R2C2)} \times 100\%_{(8)}$;

227 N: total number of samples; TP: true positive; FP: false positive; TN: true negative;

228 FN: false negative; R1: sum of the first row; R2: sum of the second row;

229 C1: sum of the first column; C1: sum of the second column.

230

231 Sensibility and specificity normality were confirmed by Kolmogorov Smirnov

232 normality test, thereafter significant differences between assays were assessed by

233 Student's T-test.

234

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236 ## 3. Results

237

238 **3.1 Sensitivity assessment.** The sensitivity of each detection kit was assessed using
239 the 34 feces obtained from *Echinococcus* infected dogs listed in Table 4. We
240 randomly selected 2 parallel groups including 34 fecal specimens and calculated the
241 average as the sensitivity of each detection method. The kit B displayed the highest
242 average sensitivity, i.e. 83.82%, while D showed the lowest average sensitivity, i.e.
243 51.47% (Tables 4 and 5). The average sensitivity of kits A and C was 76.47% and
244 70.59%, respectively. When the sensitivity was calculated according to the worm load,
245 strong variations were observed (Table 5). The sensitivity varied widely depending on
246 the worm count. For kit A, the sensibility varied from a lowest of 44.44% for a worm
247 burden class of 500 or less to a maximum of 100% for a worm burden of 5,000 to
248 20,000. The sensitivity decreased sharply to 81% for a worm burden above 20,000
249 (Table 5). For the other three kits the calculated sensitivity increased along with the
250 worm load. The lowest sensitivity for a worm load below 500 was 72.22%, 44.44%
251 and 11.11% for kits, B, C and D, respectively (Table 5). The highest sensitivity was
252 observed for a worm burden above 20,000 with 93.75% for kits B and C, and 81.25%
253 for kit A and D (Table 5)

254

Table 4 .Summary of evaluation results of relevant indicators

Assay code	Randomized ID	Sensitivity		Specificity		Positive predictive value		Negative predictive value		Youden's index		Consistency rate		Kappa value	Repeatedly
A	1	82.40% （28/34 ）	76.47%	89.20% （141/158）	87.97% -12.03%	62.22% (28/45)	57.78%	95.92% (141/147)	94.56%	0.72	0.64	88.02%	85.94%	0.69	88.18% （194/220）
	5	70.60% （24/34）		86.70% （137/158）		53.33% (24/45)		93.20% (137/147)		0.57		83.85%			
B	3	82.40% （28/34）	83.82%	72.80% （115/158）	74.68% -25.32%	39.44% (28/71)	41.69%	95.04% (115/121)	95.54%	0.55	0.59	74.48%	76.30%	0.65	84.55% （186/220）
	4	85.30% （29/34）		76.60% （121/158）		43.94% (29/66)		96.03% (121/126)		0.62		78.13%			
C	7	67.60% （23/34）	70.59%	63.30% （100/158）	63.61% -36.39%	28.40% (23/81)	29.44%	90.09% (100/111)	90.95%	0.31	0.34	64.06%	64.84%	0.63	82.73% （182/220）
	8	73.50% （25/34）		63.90% （101/158）		30.49% (25/82)		91.82% (101/110)		0.37		65.63%			
D	2	52.90% （18/34）	51.47%	74.70% （118/158）	75.63% -24.37%	31.03% (18/58)	31.26%	88.06% (118/134)	87.87%	0.28	0.27	70.83%	71.35%	0.64	87.73% （193/220）
	6	50.00%		76.60%		31.48%		87.68%		0.27		71.88%			

	(17/34)		（121/158）		(17/54)		(121/138)		
Result		X^2=9.31		X^2=24.90		X^2=40.20	X^2=9.46	X^2=23.44	X^2=3.68
p value		$P<0.05$		$P<0.05$		$P<0.05$	$P<0.05$	$P<0.05$	$P>0.05$

258
259
260 **Table 5. Effect of the worm load sensitivity**
261

Assay code	Randomized sample ID	Number of positive tests										Number of false negatives	Worm count for false negatives
		[0-500]		[500-5,000]		[5,000-20,000]		[20,000-+∞]		Total			
		n=9	Sensitivity (%)	n=10	Sensitivity (%)	n=7	Sensitivity (%)	n=8	Sensitivity (%)	N=34	Average sensitivity (%)		
A	1	5	44.44	9	85.00	7	100.00	7	81.25	28	76.47	6	5;60;100;400;1,500;20,000
	5	3		8		7		6		24		10	5;60;100,;200;300;400; 2,100;3,100;20,000;25,000
B	3	6	72.22	8	80.00	6	92.86	8	93.75	28	83.82	6	5;60;200;,600(2);11,000
	4	7		8		7		7		29		5	60;200;600(2);20,000
C	7	3	44.44	7	65.00	6	85.71	7	93.75	23	70.59	11	5;100;200(3);400;600(2);1,100; 6,000.25,000

	8	5		6		6		8		25		9	5;100;200(2);600(2);1,100;3,100;6,000
	2	1		5		5		7		18		16	5;60;100;200(3);300;400;600(2);1,500; 2,100;3,100;6,000(2);20,000
D			11.11		50.00		71.43		81.25		51.47		
	6	1		5		5		6		17		17	5;60;100;200(3);300;400;600(2);1,500; 2,100;3,100;6000(2);20,000(2)

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263 **3.2. Assessment of false positive answers (specificity 1).** The level of non-specific
264 reactions was assessed for each detection kit on 158 feces obtained from
265 *Echinococcus*-negative dogs (Table 4). We randomly selected 2 parallel groups from
266 the 158 fecal specimens. The lowest level of non-specific reaction was shown by the
267 kit A, i.e. 12.03%, while the kit C displayed the highest level of non-specificity, i.e.
268 36.39%. Kits B and D yielded intermediate values, i.e. 25.32% and 24.37%,
269 respectively (Table 4).
270
271 **3.3. Cross-reactivity assessment with other tapeworms (specificity 2).** Kit A
272 displayed no cross-reactivity at all with any of the control parasites, i.e. *T. hydatigena*,
273 *D. caninum*, and *S. mansoni* (Table 6). Kits B and C displayed the highest level of
274 cross-reactivity, i.e. 23.21%, whereas kit D showed an intermediate level of 16.07%.
275 Kit C cross-reacted with all three heterologous worms. Kit B showed cross-reactivity
276 with *D. caninum* and *S. mansoni,* while kit D cross-reacted with *T. hydatigena* and *D.*
277 *caninum*.

278 **Table 6. Cross-reactivity with other parasites**

279

Assay code	Randomized sample ID	Number of positive tests				Cross reaction rate (%)
		Taenia hydatigena n=8	*Dipylidium caninum* n=8	*Spirometra mansoni* n=12	Total N=28	
A	1	0	0	0	0	0.00
	5	0	0	0	0	
B	3	0	3	3	6	23.21
	4	0	4	3	7	
C	7	3	2	2	7	23.21
	8	6	0	0	6	
D	2	1	5	0	6	16.07
	6	1	2	0	3	

280 281 282 283 284 285 286 287

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288

289 **3.4. Assessment of global performance and accuracy.** The best score when using
290 Youden's index was obtained by kit A (0.64), whereas kit B reached a score of 0.59
291 (Table 4). Kits C and D obtained very low scores of 0.34 and 0.27, respectively
292 (Table 4). The Youden's index varies from 0 to 1 with 0 indicating an
293 undiscriminating, therefore useless test, while 1 is indicating a perfect test. Even with
294 the best scores, kits A and B were far from being perfect. The accuracy assessment
295 conducted to evaluate the repeatability of each test yielded scores higher than 80%
296 whatever the kit considered. However, kits A and D reached a higher score, i.e.
297 88.18% and 87.73%, respectively, compared to kits B and C, i.e. 84.55% and 82.73%,
298 respectively.

299

300 **3.5. Assessment of differences between assays**

301 The relative difference between assays was assessed using a Student's T-test
302 following the positive normality assessment of the data sensitivity and specificity. The
303 difference between kits was also assessed with a student's test. For the sensitivity,
304 results from kit A are significantly different from those obtained with kits C and D but
305 not with those from kit B (Table 7). Results from kit B are significantly different from
306 those from kit C and kit D (Table 7). Results from kit C are not significantly different
307 from those obtained with Kit D (Table 7). With respect to specificity, results from kit
308 A are not significantly different from those coming from kits B and C but are
309 significantly different from those obtained with kit D (Table 8). Kit B yielded results

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310 significantly different from those from kits C and D (Table 8). Finally, results from
311 kits C and D were not significantly different (Table 8).
312
313 **Table 7. Assessment of the results difference for sensitivity**

Comparisons	DoF	T	p-value
A-B	2	3.23	0.084
A-C	2	6.21	**0.025***
A-D	2	5.96	**0.027***
B-C	2	4.93	**0.038***
B-D	2	4.61	**0.044***
C-D	2	-1.70	0.232

314 * Significant at 0.05
315 DoF: Degree of Freedom
316
317
318 **Table 8. Assessment of the results difference for specificity**

Comparisons	DoF	t	p-value
A-B	2	-0.68	0.569
A-C	2	2.24	0.155
A-D	2	4.87	**0.040***
B-C	2	4.60	**0.044***
B-D	2	14.45	**0.005*****
C-D	2	3.49	0.073

319 * Significant at 0.05
320 *** Significant at 0.01
321 DoF: Degree of Freedom
322

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324 **3. Discussion**

325

326 China is reporting the highest human prevalence rate of echinococcosis
327 worldwide. Following the 2012-2016 national survey, 368 out of 413 counties were
328 identified as endemic for echinococcosis [6]. Currently the number of endemic
329 counties rose to 370, after the disease was detected in 2017 in Dongxiang County in
330 Gansu province and in the Ulagai Management District in Inner Mongolia (Figure 1).
331 The endemic counties spread over 9 provinces or autonomous regions of Tibet,
332 Sichuan, Qinghai, Xinjiang, Gansu, Ningxia, Inner Mongolia, Yunnan and Shanxi
333 (Figure 1). The overall detection rate was 0.51% (5,133/1,001,173) [6]. The
334 prevalence was estimated to be 0.28% in endemic areas and the number of patients
335 was estimated to be 166,098 with a number of persons at risk of about 60 million [6].
336 Out of the 370 endemic counties, 158 are located in Qinghai Tibetan Plateau with a
337 prevalence of 1.28% which is 10 times higher than the prevalence in non-Qinghai
338 Tibetan Plateau areas, i.e. 0.13% [6].

339

340 These last few years, significant technical progress was made in immunological
341 diagnosis of *Echinococcus* infection in definitive hosts [13-15]. The detection of
342 parasite antigens in stool by ELISA has been developed for the detection of fecal
343 antigen released by canine *Echinococcus* [13,16-18]. *Echinococcus* antigens can be
344 detected in dog feces 5 to 10 days after being experimentally infected [19]. This
345 detection becomes negative five days after treatment with praziquantel [19]. The

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346 detection of specific antigens in the definitive host stool samples is more informative
347 than the detection of serum antibodies, because of the higher probability of being
348 associated with the current infection. Owing to their effectiveness, these tests have
349 been introduced to local echinococcosis prevention programs where they are currently
350 being implemented. ELISA has been adopted as the main diagnostic method in place
351 of the arecoline cathartic method to monitor canine *Echinococcus* infection in control
352 programs. There is thus a need to evaluate the fecal antigen tests regularly to improve
353 the quality of monitoring activities and objectively assess prevention effectiveness.
354
355 In this work, we evaluated the accuracy and reliability of four commercial kits
356 currently in use in China and results showed that the sensitivity of the four kits ranged
357 between 51.5% and 83.9% only. Out of all the samples tested, only two displayed a
358 worm burden lower than 100. Therefore, the range of sensitivity obtained in this study
359 is far below that of 92% to 100% for more than 100 worms reported by previous
360 studies for fecal antigen detection [13]. A sensitivity ranging from 29% to 79% has
361 been previously reported for a worm burden lower than 100 worms as determined by
362 necropsy or arecoline cathartic [18]. This is more in the range of what was observed
363 in this work but with a worm burden higher than 100. The sensitivity results reported
364 in this work also indicated that the threshold of 100 is not realistic, which could
365 explain the variation in results from one report to another. The minimal burden of
366 worms for assessing sensitivity should be 500. Nevertheless, owing to the quite low
367 sensitivity observed and to the important variation induced by the worm burden,

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368 further modifications and optimization must be conducted to increase the sensitivity
369 of the kits we tested.
370
371 Non-specific, or false-positive, reactions from the kits tested in our study ranged
372 from 12.03% to 36.39%, while the cross-reactivity with other parasites ranged from 0
373 to 23.21%, depending upon the kit. Youden's index is a comprehensive indicator
374 reflecting the sensitivity and specificity. Under the assumption that sensitivity and
375 specificity are equally important, the kit with the highest Youden's index is given
376 priority. In this study, the highest Youden's index was 0.64 for kit A. However, the
377 positive predictive value changes with the infection rate. In this study, the detection
378 results corresponding to the infection rate of 17.70% (34/192) were generally low,
379 indicating the occurrence of false positive results. The consistency rate is the main
380 index reflecting the reliability of kits, which mainly represents the stability of the
381 detection ability of kits. The highest consistency rate of kit A is 85.94%. Kappa value
382 is also an important index to reflect the repeatability of test results. Thus kit A shows
383 the best reliability in terms of repeatability and stability. Although quite high, there is
384 still a need for further improvement in particular because of the level of false
385 positives.
386
387 Out of the three technologies assessed, i.e. Sandwich ELISA, ELISA and
388 immunofiltration, the latter displayed the lowest performance score. Immunofiltration
389 has the advantage of being used *in situ* with a simple protocol and with results being

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390 immediately available. However, the poor performance displayed by this technology
391 does not make it a reliable and efficient choice for the monitoring of echinococcosis.
392 More developments are needed to improve this technology. This study shows clearly
393 that sandwich ELISA should be the methodology to implement for the surveillance of
394 canine echinococcosis. Kit A displayed the best weighted overall score but kit B
395 yielded a weighted overall score close to the former. Both were based on the
396 technology of sandwich ELISA with two monoclonal antibodies.
397

398 ELISA showed performances intermediate between sandwich ELISA and
399 immunofiltration and does not appear as a reliable option for surveillance.
400 Nevertheless, even if sandwich ELISA seems to be the technology of choice for the
401 surveillance of canine echinococcosis, improvements and optimization are still needed
402 to ensure proper surveillance. This study provides a reference for improving control
403 measures and assessment of the prevalence of echinococcosis in the endemic counties
404 of China. This is a key step towards elimination. Since the most sensitive indicator of
405 the risk of epidemic is the dog infection rate, these kits are tools of primary
406 importance. Therefore, we urge manufacturers to strengthen research on their
407 products in order to improve and enhance their overall quality, and in particular
408 sensitivity and specificity, for effective *Echinococcus* diagnosis and control
409 implementation in China.

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423

424 **Conflict of interest**

425 The authors declared no competing interests.

426

427 **Authors participation**

428 Conceived the study: Weiping Wu, Liying Wang, Hu Wang, Qian Wang
429 Designed the study: Liying Wang
430 Coordination of units: Weiping Wu
431 Collected samples and information: Liying Wang
432 Performed pre-experiment: Huixia Cai
433 Organization of the work: Liying Wang, Weiping Wu
434 Supervision: Qian Wang, Hu Wang, Yan Huang, Yu Feng
435 Data analysis: Liying Wang, Min Qin, Laurent Gavotte, Roger Frutos
436 Writing-original draft: Liying Wang, Xuefei Bai, Min Qin

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437 Writing-reviewing and editing: Sylvie Manguin, Laurent Gavotte, Roger Frutos

438 All authors read and approved the final manuscript.

439

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