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Research paper

First study about the development of adult *Echinococcus canadensis* G6 genotype of goat origin in experimentally infected dogs

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ABSTRACT

Echinococcus granulosus sensu lato (*E. granulosus* sl) must be considered as a species complex, comprising *Echinococcus granulosus* sensu stricto (*E. granulosus* ss, genotypes G1-G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5) and *Echinococcus canadensis* (G6-G10) although the species status of *E. canadensis* is still controversial. These genotypes closely match the intermediate hosts associated strains described in earlier times among which *E. canadensis* G6 corresponds to the camel strain. As there are no studies concerning the development of adult stages of the G6 genotype from non-camel origin, the aims of the present study were: to characterize for the first time the development of *E. canadensis* G6 in dogs experimentally infected with protoscoleces derived from goats, to describe the resultant adult morphology, to evaluate the growth of their rostellar hooks from larval to adult stages and to determine the prepatent period of the strobilar stage of *E. canadensis* G6 derived from goats. The development of the strobilar stage of *E. canadensis* G6 genotype of goat origin was examined by studying the growth (variation of the total worm length) and segmentation in experimentally infected dogs at 14, 25, 35 and 56 days post infection. A morphological characterization of 35-day-old worms as well as of larval and adult rostellar hooks was also carried out by conventional optical microscopic observations and/or by scanning electron microscopy. The prepatent period of the strobilar stage was assessed by microscopic examination of faeces from 2 infected dogs. Our results were compared with published data from the camel and other strains. The roles of the host, genotype and species in morphological and developmental features as well as the taxonomic position of *E. canadensis* G6 were discussed. The prepatent period of *E. canadensis* G6 genotype of goat origin was determined as at least, 41 days. The obtained results contribute to increase the knowledge about the biology and genetics of *E. granulosus* sl complex and are also of practical usefulness for the design of disease control strategies.

1. Introduction

Echinococcus granulosus sensu lato (*E. granulosus* sl) is the etiologic agent of cystic echinococcosis, one of the most widespread helminth zoonoses in the world. Canids are the definitive hosts for the adult tapeworms while a variety of domestic and wild herbivorous mammals harbour the larval stage in their livers, lungs or other organs. Humans are accidentally involved in the parasite cycle also as intermediate hosts (IH). The disease is endemic in Argentina and highly endemic in the province of Neuquén, where at least goats, pigs, sheep, cattle and humans can be infected (Pierangeli et al., 2007; Soriano et al., 2010, 2013).

Considerable genetic and phenotypic variation has been demonstrated in *E. granulosus* sl To date 10 distinct genotypes (that closely match the different strains described in earlier times) have been identified using molecular tools: 2 sheep strains (G1 and G2), 2 bovid strains (G3 and G5), a horse strain (G4), a camel strain (G6), a pig strain (G7) and 2 cervid strains (G8 and G10) (Bowles et al.,

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1992; Lavikainen et al., 2003, 2006). The existence of a human-specific genotype (G9) was proposed by Scott et al. (1997), but further analysis of sequence data revealed no significant differences between the G7 and G9 genotypes and in consequence the status of the G9 genotype remains uncertain (Kedra et al., 1999). Recent taxonomic revisions based on mitochondrial markers indicate that *E. granulosus* sl must be considered as a species complex, comprising *E. granulosus* sensu stricto (*E. granulosus* ss, genotypes G1-G3), *Echinococcus equinus* (G4), *Echinococcus ortleppi* (G5) and *Echinococcus canadensis* (G6-G10) (Nakao et al., 2007, 2013; Moks et al., 2008). However, the species status of *E. canadensis* is still controversial and it has also been proposed that the G6-G10 genotypes should be ranked as two separate species: *Echinococcus intermedius* (G6/G7) and *E. canadensis* (G8/G10) (Thompson, 2008; Saarma et al., 2009) or as three separate species: *E. intermedius* (G6/G7), *Echinococcus borealis* (G8) and *E. canadensis* (G10) (Lymbery et al., 2015). For practical reasons the nomenclature proposed by Nakao et al. (2007) has been adopted in this work. These strains/genotypes can differ in several characteristics, such as morphology, growth and development rates in the definitive hosts or infectivity to intermediate hosts, all of which have relevant implications for control strategies.

The development of intestinal stages of the cervid, sheep, horse, cattle, camel and pig strains have been described by a few studies that included experimental infections of dogs with protoscoleces (PSC) obtained from their "natural" hosts before the methods of molecular genotyping were of current use (Sweatman and Williams, 1963; Thompson, 1977; Thompson et al., 1984; Kumaratilake et al., 1986; Saad and Magzoub, 1988; Eckert et al., 1989, 1993; Dubinský et al., 1998; Hosseini and Eslami, 1998). There are no studies concerning the development of adult worms derived from PSC obtained from "non-natural" hosts and neither are published reports about experimental infections of definitive hosts with PSC genetically characterized. Previous studies carried out in the Province of Neuquén, Patagonia, Argentina, showed that local goats act as the most important reservoir of *E. canadensis* G6 (camel strain) and that this strain/genotype is responsible for all the fertile cysts in this IH (Soriano et al., 2010). Based on these previous data, the aims of the present study were: 1) to characterize the development of *E. canadensis* G6 in dogs experimentally infected with PSC of goat origin, 2) to describe the morphology of the resultant adult worms, 3) to evaluate the growth of their rostellar hooks from larval to adult stages and 4) to determine the prepatent period of the strobilar stage of *E. canadensis* G6 derived from goats.

2. Materials and methods

2.1. Experimental design

In the present study, an experimental, prospective, longitudinal and post-test design was applied. The development of the strobilar stage of *E. canadensis* G6 genotype of goat origin was examined by studying the growth (variation of the total worm length) and segmentation in experimentally infected dogs at 14, 25, 35 and 56 days post infection (p.i.). A morphological characterization of 35-day-old worms as well as of larval and adult rostellar hooks was also carried out. The prepatent period of the strobilar stage was assessed by microscopic examination of faeces from 2 infected dogs. As an internal control of the experiments, the genetic identity of samples of worms obtained from each dog was verified.

2.2. Collection and processing of metacestodal material

Protoscoleces were collected from hydatid cysts obtained from the lungs of naturally infected goats, slaughtered in abattoirs from different locations of Neuquén province, Patagonia Argentina. Each individual cyst was considered as a sample or isolate and was processed separately. PSC were aspirated and their viability was evaluated using 0.4% Tripan Blue in a dye-exclusion test and/or by flame cell activity. Only the isolates that showed a viability of at least 70% were included in the study. Each batch of PSC was divided into 3 fractions: one was used immediately for experimental infection whereas the other 2 aliquots were washed 3 times with sterile saline and stored in 10% buffered-formalin at room temperature (for morphometric analysis) or conserved in 70% ethanol at 4 °C (for DNA sequencing).

Table 1
Experimental infection of dogs with *E. canadensis* G6 of goat origin.

Dog N°	Age (years)/sex	Time of exposure (days)	End of the experiment
1 2	<1–2/male 3–5/female	14	Necropsy
3 4	<1–2/female 3–5/male	25	Necropsy
5 6	<1–2/male 3–5/female	35	Necropsy
7 8	<1–2/male 3–5/male	56	Arecoline purgue
9* 10*	<1–2/male 3–5/female	56	Arecoline purgue

* Uninfected.

2.3. Molecular characterization

Genotyping of larval and adult isolates was carried out by characterization of mitochondrial DNA markers. DNA extraction was performed from 70% ethanol preserved materials using the QAmpDNA Mini kit (QIAGEN, Germany). A 450 base pair fragment of the cytochrome c oxidase subunit 1 (*cox1*) gene and a 500 base pair fragment of the NADH dehydrogenase subunit 1 (*nad1*) gene were amplified from each isolate using published primer sets (Bowles et al., 1992; Bowles and McManus, 1993). PCR amplifications were performed as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s; 52 °C for 30 s; 72 °C for 30 s and a final extension step at 72 °C for 5 min. PCR products were purified by ethanol precipitation and sequenced. Sequences were aligned and compared with sequences of *E. granulosus* sl previously published using ClustalX, Bioedit and Mega4 softwares (Thompson et al., 1997; Hall, 1999; Tamura et al., 2007).

2.4. Experimental infections

Ten mixed breed dogs, aged between 6 months and 5 years old, were kept in individual kennels and treated orally with praziquantel (5 mg/kg) and albendazole (50 mg/kg) one week before the infection to eliminate potential intestinal helminth infections. During the anthelminthic treatment and the complete experimental period the animals were maintained on commercial dried food and water *ad libitum*, according to biosafety and ethical regulations. Two dogs were kept uninfected to be sure that no accidental infection with *E. canadensis* or other intestinal parasites could occur during the course of the experiment, and 8 were orally infected with a dose of approximately 20,000 PSC (viability 70% or higher) obtained from a single and identified hydatid cyst in each case (Table 1). Dogs 1–6 were euthanized by the i.v. injection of an overdose of sodium pentobarbital at different times p.i. Due to biosafety and ethical reasons, necropsy was avoided on day 56 p.i. Instead, dogs 7–10 were dosed with 4 mg/kg of arecoline hydrobromide according to standard procedures (Schantz, 1973).

2.5. Collection of intestinal Echinococcus stages

Intestinal *Echinococcus* stages were collected following previously described procedures, with minor modifications (Thompson, 1977; Eckert et al., 1989). After necropsy, the abdominal cavity of

Table 2
Segmentation and total length of *E. canadensis* G6 of goat origin in experimentally infected dogs.

Dog number	Days post infection	Percentage of worms at different stages of segmentation (%)						Total length (mm) Mean ± SD (95% CI) [range]
		S+n	S+b	S+1	S+2	S+3	S+4	
1	14	81	19	0	0	0	0	0.36 ± 0.07 (0.31–0.43)
2	14	76	24	0	0	0	0	[0.25–0.45]
3	25	0	0	57	43	0	0	0.81 ± 0.22 (0.69–0.92)
4	25	0	0	48	52	0	0	[0.46–1.15]
5	35	0	0	4	73	23	0	1.62 ± 0.34 (1.55–1.68)
6	35	0	0	2	71	25	2	[0.84–2.74]
7	56	0	0	0	17	71	12	3.69 ± 0.77 (2.73–4.65)
8	56	0	0	0	11	73	16	[3.02–4.92]

N = 100 worms/dog. S + n: scolex + neck; S + b: scolex + band; S + 1-4: scolex + N° segments.

dogs 1 and 2 (14 days p.i.) and 3 and 4 (25 days p.i.) was opened and the small intestine was removed, incised longitudinally and transferred to a large beaker where it was incubated in Hank's balanced salt solution (HBSS) at 37 °C for 30 min. During this time, the worms freed themselves from the gut mucosa. Two samples of 5 ml each were taken from the well mixed solution containing suspended worms and fixed in 10% formaldehyde. These samples were used to estimate the total worm number in each case, by counting the worms present in aliquots of known volume. Thereafter, the worms were collected from the sediment in the incubation medium and allowed to relax gradually in HBSS at room temperature for 30 min before being fixed in 10% formaldehyde (for morphological studies) or 70% ethanol (for genotyping).

The same procedure was followed with dogs 5 and 6 on day 35 p.i. but, in addition, a 6 cm length segment of intestine was isolated by being ligated, separated from the rest of the intestine and injected with 2.5% glutaraldehyde in PBS pH 7.2 (Glu/PBS) as a fixative. After 2 h at room temperature, this portion of the gut was longitudinally opened and immersed in Glu/PBS for scanning electron microscopy (SEM) studies.

On day 56 p.i. purge samples from dogs 7–10 were collected in plastic containers for macro and microscopic examination. Adult worms present in purge material of dogs 7 and 8 were collected by aspiration, washed in sterile saline and stored in 10% formaldehyde (morphometric studies) or 70% ethanol (genotyping). Purge materials from dogs 9 and 10 were processed as fecal samples for coprological examination (see description below).

2.6. Morphological studies

A total of 100 worms per dog were analyzed. The total worm length and segmentation were determined at 14, 25, 35 and 56 days p.i. using an optical microscope with a calibrated eyepiece micrometer and amplifications of 50× and 100×. In addition, the length of the terminal segment, the ratio of the terminal segment to the rest of the strobila and the position of the genital pore were evaluated. The morphology of 25 and 35-day-old worms was also examined by SEM.

For hook measurements, PSC and dissected rostella of 35-day-old adult worms were mounted in polyvinyl lactophenol on a glass slide and sufficient pressure was applied to the cover slip in order to flatten but not damage the hooks. Measurements of the total length of large (LTL) and small (STL) hooks as well as the blade length of large (LBL) and small (SBL) hooks were made as already described (Hobbs et al., 1990; Soriano et al., 2013). Morphometric data were collected from 3 large and 3 small hooks from each of 6 PSC for each hydatid cyst, and from 5 large and 5 small hooks per rostellum from each of 10 adults for dogs 5 and 6.

2.7. Scanning electron microscopy

Samples of 25 and 35-day-old 10% formaldehyde fixed worms as well as thin sections of small intestinal tissue from dogs necropsied at day 35 p.i. were examined by SEM. In all cases the samples were dehydrated in graded series of absolute ethanol (10–100%, 15 min at each concentration), passed to acetone, dried by critical point and sputter-coated with gold. Observations and photomicrographs were obtained with a JEOL/JSFM 6360 LV scanning electron microscope.

2.8. Coprological exam and prepatent period assessment

Fecal samples from dogs 7 and 8 were collected daily in 10% formaldehyde, from day 30 p.i. until taenid eggs were detected. These samples as well as purge materials from dogs 9 and 10 were processed by the formalin-ethyl ether sedimentation method and examined under light microscope with 100 x and 400 x for the presence of taenid eggs (Truant et al., 1981). The prepatent period was determined as the lapse of time between infection and the first day p.i. with presence of taenid eggs.

2.9. Data analysis

Statistical analysis of the data was performed using SPSS software (version 17; SPSS, Inc., Chicago, IL, USA). Analysis of variance was used to detect any significant differences in measurable characters between worm populations at the same time p.i. When statistically significant differences were not found, the results from both experiences were pooled. Student's *t*-test for independent samples and/or for paired samples was applied, and differences were considered as statistically significant when $p < 0.05$.

3. Results

3.1. Experimental infections and genetic identity

Experimental infections were successful in 8 of 8 dogs. Worm burdens estimated of dogs 1–6 were >10,000 in all cases except in dog 3, where the estimated total worm number was between 1000 and 10,000. No significant differences in the susceptibility to infection were detected by age, neither by sex. With the exception of *Echinococcus*, no other intestinal parasites were detected at necropsy or in purge materials of infected dogs. Dogs 9 and 10 were free of intestinal parasites at the end of the trial. All the metacestodal isolates obtained from fertile cysts (both the ones included in the study -viability at least 70%- and those which showed a viability of less than 70%) as well as the samples of recovered worms were identified as *E. canadensis* G6 genotype by *cox1* and *nad1* partial gene sequencing.

Table 3
Strobilar morphology of 35-day-old adult *E. canadensis* G6 genotype (goat origin) and of the camel, sheep, pig, cattle and horse *E. granulosus* strains.

	E. canadensis (G6) Goat origin[a]	Camel strain (Eckert et al., 1989)	Sheep strain (Thompson et al., 1984)	Pig strain (Eckert et al., 1993)	Cattle strain (Thompson et al., 1984)	Horse strain (Kumaratilake et al., 1986)
Total worm length (TWL) (mm) Mean ±SD [Range]	1.6 ± 0.3 [0.8–2.7]	2.2 ± 0.6 [1.0–3.8]	2.5 ± 0.8 [0.7–4.5]	1.9 ± 0.3 [1.1–2.9]	4.2 ± 0.5 [3.4–6.9]	2.4 ± 0.6 [1.4–3.9]
Length of terminal segment (LTS) (mm) Mean ±SD [Range]	0.8 + 0.2 [0.3–1.4]	1.2 ± 0.3 [0.6–2.2]	0.8 ± 0.3 [0.4–2.0]	1.0 ± 0.2 [0.5–1.5]	2.7 ± 0.5 [2.1–3.5]	0.6 ± 0.1 [0.4–0.7]
Average percentage of LTS in relation to TWL (%)	49	54	25	52	64	25
Maximal number of segments	4	3	4	3	3	3
Percentage of worms with 3 or more segments (%)	15	20	17	95	65	75
Position of sexually mature segment	Terminal	Terminal	Terminal, penultimate or antipenultimate	Penultimate	Penultimate	Penultimate
Position of genital pore in mature segment	64% anterior	60% anterior	100% posterior	60% anterior	100% posterior	100% posterior
Prepatent period (days)	41	"about 40"	45	35	35	> 45

[a] Data from this study.

3.2. Developmental characteristics of intestinal stages

The total worm length and segmentation of the intestinal stages are shown in Table 2. As no significant differences were found in measurable parameters between worm populations at the same time p.i., the results from both experiences were pooled in each case. At 35 days p.i. the parasites have reached an average total length of 1.62 ± 0.34 mm, whereas at 56 days p.i. the average total length was 3.69 ± 0.77 mm. Linear regression analysis showed that the global growth rate for the intestinal stages of *E. canadensis* G6 of goat origin was about 80 μm/day (regression equation: $y = 0.0823x - 1.1202$; $R^2 = 0.9682$). Taking into account the average length of the parasites at the different times p.i., partial growth rates were estimated as 60 μm/day between days 14 and 35 p.i. and 98.5 μm/day between days 35 and 56 p.i.

Nineteen to 24% of worms had started to band (to form a clear area in the region where the partition separating the scolex from the first segment will later appear) on day 14th p.i. At day 35 p.i. between 23 and 25% of the worm population had developed 3 segments and 2% had reached 4 segments in dog n° 6.

3.3. Morphological characteristics of intestinal stages

Images of intestinal worms at 14, 25, 35 and 56 days p.i. obtained by conventional light microscopic observation are detailed in Fig. 1. The morphological characteristics of 35-day-old *E. canadensis* G6 parasites obtained by experimental infection in the present work are summarized in Table 3, along with published data from camel, cattle, sheep, pig and horse isolates. SEM examination of thin sections of small intestinal tissue from dogs necropsied at day 35 p.i. showed that the worms were deeply inserted between the villi (Fig. 2).

Rostellar hooks of adult intestinal stages of *E. canadensis* G6 of goat origin (35 days p.i.) exhibited a smooth outline and were arranged in two rows, where large and small hooks alternate (Fig. 3 and Table 4). Hook measurements of adult worms obtained from dogs 5 and 6 and hook measurements of PSC used to infect those dogs were compared. Student's *t*-test for paired samples analysis indicated a statistically significant increase in the total length of large and small hooks whereas blade lengths remained invariable in both cases. The average growth of LTL and STL from PSC to 35-day-old worms was 3.83 ± 0.02 μm and 2.24 ± 0.55 μm respectively.

3.4. Prepatent period of the strobilar stage

Taenid eggs were first detected in fecal samples from dogs 7 and 8 by days 41 and 42 p.i. respectively. In consequence, the prepatent period of the strobilar stage was assessed as, at least, 41 days.

4. Discussion

Most of the studies on the development of adult stages of *E. granulosus* sl have been carried out before the introduction of molecular tools for genetic characterization. In such studies, PSC were usually pooled from several hydatid cysts of the same origin (camels, sheep or cattle, among others) before experimental infections, as the concept of strain was strongly associated with the IH. However, further molecular researches have demonstrated that several IH are susceptible to the infection with different *E. granulosus* sl genotypes (Cardona and Carmena, 2012; McManus, 2013). In consequence, the use of pooled PSC for experimental infections could be a potential source of error as different genotypes could be present. For the first time, in the present work each dog was infected with PSC genetically identified as *E. canadensis* G6 genotype, obtained from a single hydatid cyst of goat origin in each case. This is also the first report about the development of adult *E. canadensis* G6 derived from PSC obtained not from camel but from a "non-natural" host.

Protoscoleces of *E. canadensis* G6 of goat origin are readily infective to dogs, as 8/8 animals were successfully infected in the present trial. Similar infection rates were observed by other authors in experimental infections of dogs with PSC of camel origin (Eckert et al., 1989; Saad and Magzoub, 1988).

Morphological data obtained in this work demonstrated that adult *E. canadensis* G6 derived from goats have a long terminal segment in comparison with the total worm length, that the position of the sexually mature segment is always terminal and that the genital pore in the mature segment is more frequently anterior (Table 3, Fig. 1). These morphological characteristics are identical to those described for *E. canadensis* G6 of camel origin and similar to the ones reported for *E. canadensis* G7 derived from pigs, with the exception of the position of the mature segment in the last case

Fig. 1. Intestinal stages of E. canadensis G6 of goat origin at different days post infection in dogs. a: 14 days; b: 25 days; c: 35 days; d: 56 days. ▬ 100 μm; arrow: genital pore.

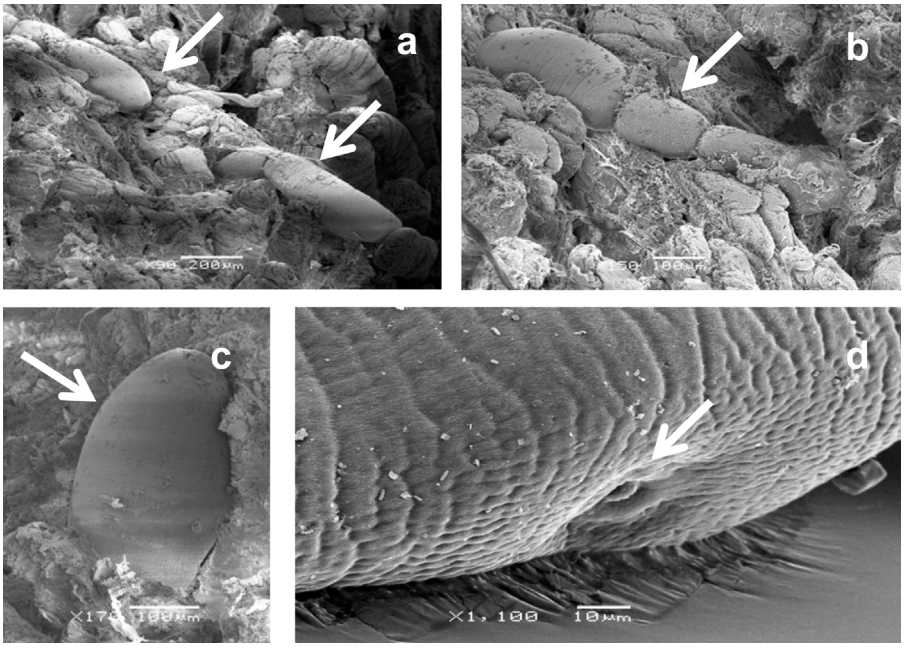

Fig. 2. Scanning electron microscopy study of 35-day-old *E. canadensis* G6 of goat origin. a, b, c: adult worms deeply inserted between intestinal villi; d: detail of the genital pore. Bars: a = 200 μm, b and c = 100 μm, d = 10 μm.

(Eckert et al., 1989, 1993). On the other hand, these morphological features remarkably differ from those described for the sheep (G1), cattle (G5) and horse (G4) isolates (Table 3). There are also differences between the adult G6 and G7 morphological characteristics and the limited available data from adult worms of cervid (G8 and G10) origin (Thompson et al., 2006; Lymbery et al., 2015).

In the same way, comparative analysis of rostellar hook morphometric data from this work and from other authors revealed high similarities among the *E. canadensis* G6 (goat and camel origin) and *E. canadensis* G7 (pig) isolates, as well as differences with the *E. granulosus* sl sheep (G1), cattle (G5) and horse (G4) isolates (Table 4) and with the cervid ones (Thompson et al., 2006; Lymbery et al., 2015). These results suggest that adult's morphology could

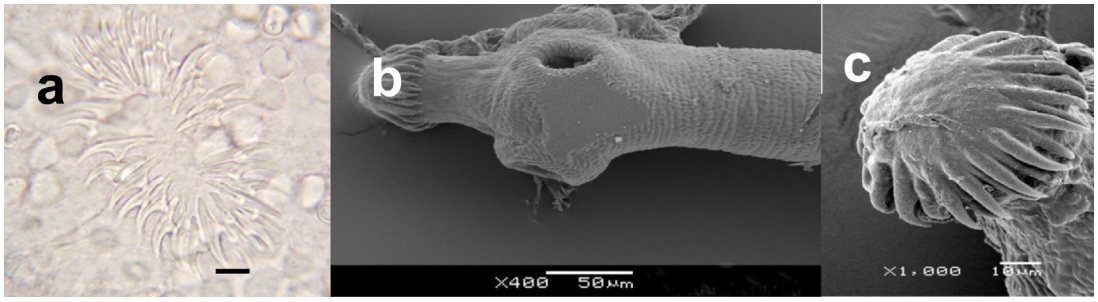

Fig. 3. Rostellar hooks of *E. canadensis* G6 of goat origin: a: PSC, optical microphotography; b and c: 35-day-old adult worms, scanning electron microphotographies. Bars: a and b = 5 µm; c = 10 µm.

Table 4
Rostellar hook characteristics of 35-day-old adult *E. canadensis* G6 genotype (goat origin) and of the camel, sheep, pig, cattle and horse *E. granulosus* strains[a].

	E. canadensis (G6) Goat origin[b]	Camel strain (Eckert et al., 1989)	Sheep strain (Thompson et al., 1984)	Pig strain (Eckert et al., 1993)	Cattle strain (Thompson et al., 1984)	Horse strain (Kumaratilake et al., 1986)
LTL (µm)	34.40 ± 1.2 [32.0–37.0]	35.4 ± 1.2 [30.4–39.0]	31.8 ± 2.5 [27.3–37.3]	35.4 ± 1.5 [31.5–38.9]	39.1 ± 0.8 [37.0–41.0]	40.2 ± 1.6 [30.0–44.1]
LBL (µm)	14.57 ± 0.7 [13.0–17.0]	14.7 ± 0.9 [13.5–17.0]	12.9 ± 1.0 [10.2–14.6]	11.8 ± 1.2 [5.3–16.6]	15.5 ± 0.4 [15.0–16.0]	14.5 ± 1.4 [9.5–17.0]
LBL x 100 LTL	42.1 ± 2.2 [34.0–46.9]	43.2 ± 2.8 [40.2–50.0]	40.1 ± 2.5 [35.8–43.9]	33.4 + 3.9 [27.0–43.0]	39.6 ± 1.1 [37.5–42.0]	34.0 ± 2.0 [32.0–36.0]
STL (µm)	27.49 ± 1.3 [25.0–30.0]	28.8 ± 2.0 [25.0–32.0]	24.6 ± 2.9 [21.4–34.7]	28.4 ± 1.7 [23.7–32.1]	32.7 ± 1.1 [31.0–34.0]	31.7 ± 1.4 [30.7–34.9]
SBL (µm)	10.38 ± 0.7 [9.0–12.0]	10.5 ± 0.6 [8.0–11.0]	9.1 ± 1.2 [7.0–12.0]	8.5 ± 0.9 [5.9–11.1]	11.1 ± 0.7 [10.0–12.0]	9.9 ± 0.7 [8.7–10.8]
SBL x 100 STL	37.6 ± 2.9 [31.0–46.2]	37.0 ± 3.2 [32.3–42.3]	37.8 ± 3.9 [30.4–45.4]	30.9 ± 3.8 [21.0–40.0]	33.9 ± 2.3 [30.9–39.0]	31.0 ± 3.0 [26.0–35.0]
Number of hooks	34.1 ± 2.1 [31.8–37.6]	32.7 ± 3.2 [30.0–37.0]	33.0 ± 3.4 [19.0–45.0]	33.0 ± 2.1 [26.0–39.0]	33.0 ± 2.0 [30.0–36.0]	38.8 ± 3.0 [28.0–36.0]
Outline of hooks	smooth	smooth	rough	smooth	smooth	smooth
Arrangement of hooks	Large and small hooks alternate 1 to 1					

[a] Data shown as Mean ± (SD) and Range []. LTL, LBL, STL and SBL: Total length and blade length of large (L) and small (S) hooks, respectively.
[b] Data from this study.

be genetically determined by the *E. granulosus* sl genoptype instead of being influenced by the IH of origin. Moreover, the fact that *E. canadensis* G6 adult worms (camel and goat origin) are not identical but share several morphological features with *E. canadensis* G7 parasites supports, from a not molecular approach, that both genotypes (G6 and G7) could belong to a single species separated from the G8 and G10 genotypes, as has been proposed by other authors (Thompson, 2008; Saarma et al., 2009; Lymbery et al., 2015).

As indicated by our data, *E. canadensis* G6 worms derived from goats can reach a mean total length of only 1.6 ± 0.34 µm at 35 days p.i. This value is about 20% less than the mean total length described by Eckert et al. (1989) for the camel strain. In contrast, adult *E. canadensis* G6 of goat origin showed a higher degree of segmentation than those of camel origin, as 2% of worms have developed 4 segments at 35 days p.i. in dog 6 (Table 3), suggesting that growth and segmentation could be in some way influenced by the IH of origin. Further studies concerning the development of adult *E. canadensis* G6 from "non-natural" hosts (sheep, cattle) should be carried out to confirm or refuse these findings.

The assessment of the growth rates of adult worms as well as SEM evaluations of morphology and insertion of *E. canadensis* G6 intestinal stages were conducted for the first time in the present work and therefore, comparison with findings from other authors was not possible.

With regard to the growth of rostellar hooks from the metacestode to the adult stage, statistically significant differences were only observed for the LTL and STL. These results agree with and support previous suggestions that such growth is achieved by the addition of new hook material only to certain parts of the hook, whereas the blade appears to be unchanged (Hobbs et al., 1990).

The production of fully developed eggs in *E. granulosus* sl has generally been regarded to begin between 42 and 48 days p.i. in dogs; however, some strains mature earlier (Table 3). Eckert et al. (1989), estimated in "about 40 days" the prepatent period for the camel strain based on the observation of worms holding eggs with fully developed embryophores at 41 days p.i. The results of the present study confirm that estimation, considering that eggs started to be detected in dog faeces at 41 days p.i. and in consequence, the prepatent period was determined as at least, 41 days. Moreover, the fact that mature eggs *were observed in dog faeces* insures that they can effectively reach the environment and infect potential IH on the 41th day after dog infection. Taking into account that the prepatent periods assessed for *E. canadensis* G6 (camel and goat origin) seem to be identical between them and differ from the 35 days period of *E. canadensis* G7 (pig strain) and of the other species of *E. granulosus* sl, it is possible that this feature could also be genetically determined by the *E. granulosus* sl genotype. These results could be taken into account for the design of control strategies based on periodic anthelminthic treatment of dogs, especially in regions where camels are absent but *E. canadensis* G6 is identified in other IH.

5. Conclusions

The morphology of adult stages of as well as the prepatent period of the strobilar stage could be genetically determined by the *E. gran-*

ulosus sl genotype. On the other hand, growth and segmentation could be in some way influenced by the IH of origin.

Our findings support the proposal that *E. granulosus* sl genotypes G6 and G7 should belong to a single species separated from the G8 and G10 genotypes.

The results of this work contribute to increase the knowledge about the biology and genetics of *E. granulosus* sl complex and are also of practical usefulness for the design of disease control strategies.

Ethics

The International Guiding Principles for Biomedical Research Involving Animals, issued by the Council for the International Organizations of Medical Sciences, as well as local ethical regulations, were applied throughout the course of the study.

Conflict of interest

The authors declare that they have no conflict of interest.

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