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Paramphistomosis of ruminants: An emerging parasitic disease in Europe

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1 **Abstract**

2 Whilst historically regarded as being of minor importance in European livestock, recent
3 evidence suggests that the prevalence of paramphistomosis is greater than that of fasciolosis
4 in parts of the UK. In order to address this emerging threat to ruminant farming systems, and
5 associated risks for food security posed by rumen fluke infection, it is imperative that we
6 develop a better understanding of the basic biology of this parasite and how it interacts with
7 its ruminant host. In this opinion article we will review recent progress in tracking the spread
8 of rumen fluke infection in Europe, and propose some research questions that should be
9 addressed if we are to develop tools to diagnose and treat paramphistomosis more effectively
10 in the future.

11

12 **The paramphistomes**

13 Paramphistomes (see Glossary), commonly known as rumen or stomach flukes, are digenean
14 parasites that infect ruminant animals across a wide geographical range of countries as far
15 apart as Mexico, India, Finland and Australia [1–4]. Paramphistome parasites belong to
16 several different genera within the family Paramphistomidae, and all require an intermediate
17 snail host and a definitive ruminant host within their lifecycle. Within the intermediate host
18 the paramphistomes develop through three larval stages (sporocysts, rediae and cercariae) and
19 undergo asexual reproduction before cercariae emerge from the snail and encyst as
20 metacercariae. Infection of the definitive host occurs upon ingestion of vegetation harbouring
21 dormant encysted metacercariae. The metacercariae then excyst in the small intestine
22 (duodenum) before migrating through the upper gastro-intestinal tract (GIT) to the rumen-
23 reticulum, where the mature parasites reside. Figure 1 illustrates this lifecycle for the species
24 *Calicophoron daubneyi*.

25 Mature paramphistomes are pink pear-shaped organisms, typically 0.5-1.0 cm long,
26 that are firmly attached to the host rumen wall by a posterior muscular acetabulum (Figure 2).
27 They have a blind-ended, bifurcated gut with an oral opening protruding into the rumen at
28 their narrower anterior end [5]. Immature stages of the parasite are much smaller at less than
29 a millimetre in length and appear within the small intestine, as early as 9 days after exposure
30 to infected pasture, as small nodules/dark spots, in association with haemorrhagic lesions of
31 the superficial mucosal tissue [6,7]. Small rumen flukes, which are bright red in colour and
32 around 2-3 mm in length but with similar external morphology to mature specimens, have
33 been described within the rumen from day 38 post-exposure to infected pasture [6] as they
34 complete their migration from the duodenum.

35 It is the newly excysted and migratory stages of the parasite that are thought to be
36 responsible for clinical paramphistomosis; a condition regarded as a leading cause of
37 livestock morbidity which results in economic loss in tropical and sub-tropical regions
38 [1,4,8]. Clinical symptoms include lethargy/recumbency, dehydration, severe scour and sub-
39 mandibular oedema, which occur as immature parasites excyst and penetrate the duodenal
40 mucosa causing significant damage to the tissues [9]. In cases with large burdens of immature
41 rumen fluke, mortality may occur as a result of the damage caused to host intestinal tissue
42 and symptoms of haemorrhagic enteritis including anaemia and hypoproteinaemia [7,10].
43 Mature paramphistome infections have been associated with ruminal papillae atrophy and
44 ulceration, at the point of fluke attachment [11,12], and negative impacts on production
45 measures such as milk yields and growth rates have also been reported [13,14].

46 In this article we will highlight the growing prevalence of paramphistome infections
47 in Western Europe, and the sparsity of data on the basic biology of these parasites. Key areas
48 for future research will be proposed that support the development of diagnostic tools and

49 treatment options to enable the sustainable control of this emerging parasitic infection in the
50 future.

51

52 **Paramphistomosis is an emerging infection in European livestock**

53 Historically, rumen fluke infection was rarely seen in the temperate climates of Western
54 Europe. However, morphological identifications in the 1950s suggested that
55 *Paramphistomum cervi* (or species later considered synonymous with *P. cervi*) was resident
56 in Great Britain and Ireland [15] and Italy [16]. Since then, rumen fluke have persisted as a
57 little acknowledged infection that many livestock producers remain unaware of. However, in
58 recent years, significant increases in the prevalence of rumen fluke infections have been
59 noted in ruminant livestock populations across western European countries. Using molecular
60 sequence data, the species *Calicophoron daubneyi* has been clearly identified as the primary
61 rumen fluke parasite infecting cattle, sheep and goats in Europe [17–20]. Prevalence levels as
62 high as 29-36% in Spain [21,22], 20% in France [23], and 59% in Wales [24] in cattle, and up
63 to 77% in Ireland [18] and 42% in Wales [24] in sheep have now been reported (Table 1).
64 The factors driving these increased levels are not fully understood, but the introduction of *C.*
65 *daubneyi* to Western Europe during the movement of livestock [5], the presence of a suitable
66 snail intermediate host [25,26], and climate change (milder winters and higher rainfall)
67 favouring the completion of the parasite lifecycle [27] are all thought to contribute.

68 Although clinical disease and mortality owing to rumen fluke infections remain
69 infrequent in temperate regions, fatal disease outbreaks, linked to significant immature
70 parasite burdens, have been reported in both sheep and cattle [7,10]. Additional anecdotal
71 reports from veterinarians and farmers indicate that paramphistomosis may be a rising
72 problem in some areas. With a growing geographical range, and a demonstrable potential to

73 cause significant clinical disease, interest and concern is growing among both veterinarians
74 and livestock producers regarding the potential impact of this parasite on animal health,
75 productivity and welfare, and how this threat should be tackled.

76

77 **Highly prevalent but poorly understood: how much do we know?**

78 Despite their ubiquitous presence, our current knowledge of the fundamental
79 molecular and developmental biology of rumen fluke is limited, particularly in comparison to
80 other trematodes of veterinary significance such as the liver fluke, *Fasciola hepatica*. For
81 instance, histochemical staining, as well as morphological and ultrastructural studies, have
82 detailed the anatomy of several paramphistome species [3,5,28,29], but such work has largely
83 focused on larval stages within the intermediate host or adult rumen-dwelling flukes that are
84 easier to collect, mostly belonging to tropical and sub-tropical species of paramphistome to
85 date. To our knowledge, the development of various organ systems (e.g. the tegument, gut,
86 and reproductive structures) in the newly excysted and migratory stages has not yet been
87 described for *C. daubneyi*, and only a few studies have been carried out which describe the
88 development of other paramphistomes [30]. Uncertainty also exists regarding the feeding
89 mechanisms used by the various life-cycle stages within the ruminant host. Based on a
90 transcriptome analysis, Choudary *et al.* [31] proposed that mature *P. cervi* rumen flukes may
91 obtain nutrition via damaged capillaries in the rumen wall allowing uptake of glucose and
92 other nutrients directly from the bloodstream. However, the orientation of the parasite within
93 the host – anchored by a blind-ended muscular acetabulum with their oral opening (which
94 leads to the parasite gut) protruding away from the rumen wall - would indicate that the
95 rumen contents are the primary source of nutrition for the parasite. Indeed, research dating
96 back to the 1950s [32] has shown that rumen fluke can be sustained for a prolonged period

97 outside the host, with a supply of rumen fluid. Microscopic examination of mature rumen
98 fluke showed rumen material, including Protozoa, within the parasite oesophagus, also
99 suggesting that paramphistomes may feed on components of the rumen fluid digesta or
100 microbiome [3]. Absorption of nutrients directly across the tegument is thought to be unlikely
101 given its thickness in paramphistomes and a lack of mitochondria in the outer tegumental
102 syncytium required for active transport mechanisms [28]. In contrast, ingested host tissue has
103 been observed within the gut of an immature rumen fluke collected from the small intestine
104 of a calf at post mortem [10]. The proposed switch from feeding on host tissue, in the small
105 intestine, to digesta (or microbial contents) in the rumen as these parasites develop poses
106 interesting questions about how they interact with their host during their migration along the
107 GIT.

108 Perhaps due to their lack of recognition and poorly understood pathogenicity,
109 no anthelmintic drugs (asides from a single formulation of oxclozanide licensed only in
110 France: Douvistome) are currently available with a label claim for rumen fluke control in
111 Western Europe. Numerous studies, both *in vitro* and *in vivo*, have attempted to verify the
112 efficacy of existing anthelmintics against a range of rumen fluke species [33–35]. Currently,
113 oxclozanide (normally marketed as a treatment for liver fluke) is the drug of choice to
114 control both immature and mature paramphistome infections, although this drug does not
115 appear to have been tested against immature *C. daubneyi* specifically [36]. Additionally,
116 research into diagnostic tools for paramphistomosis has been very limited [37,38], and
117 currently no diagnostic test has been developed for the identification of pre-patent infections,
118 leaving faecal egg counts (FEC) or post mortem as the only options to identify chronic or
119 acute disease respectively. Research on other trematodes has benefited from significant
120 transcriptome and genome [39] datasets that facilitate comparative studies between life-cycle
121 stages and species, as well as proteomics investigations to identify putative drug or vaccine

122 targets [40–42]. For the paramphistomes, only a single transcriptome assembly for adult *P.*
123 *cervi* has been published, but the assembled contigs have not been made publically available
124 [31]. Similarly, proteomic data for paramphistomes are sparse. Due to the lack of
125 transcriptome/genome datasets most studies so far have simply described the proteome
126 profile of excretory/secretory or somatic proteins (visualised by SDS-PAGE) and identified
127 antigenic components by Western blot using sera from paramphistome infected animals
128 [38,43–45]. These studies have focussed on tropical paramphistome species and, to date,
129 there are no published studies on the proteome of *C. daubneyi* found in Europe.

130

131 **New developments and discoveries**

132 The renewed interest in paramphistomes in temperate climates has sparked several new
133 studies and publications in the last few years. Since Gordon *et al.* [20] proposed *C. daubneyi*
134 as the primary rumen fluke species infecting livestock in Britain in 2013, a number of studies
135 have been conducted in the UK and Ireland, all of which have confirmed this finding using
136 molecular approaches to species identification [46–48]. Studies across Western Europe, in the
137 Netherlands [49], Belgium [50], Italy [51] and Spain [17] have also confirmed, using
138 molecular tools, the predominant rumen fluke species identified in livestock to be *C.*
139 *daubneyi*. A study in Ireland [18] confirmed a high prevalence of *C. daubneyi* in Irish sheep
140 flocks, but also identified infection with another rumen fluke species, *Paramphistomum*
141 *leydeni*, in a single animal. One case of *P. leydeni* infection in cattle has also been reported in
142 the Netherlands [49]. This suggests that occasional infection from ruminant wildlife reservoir
143 hosts, where *P. leydeni* has been identified previously [52], may also occur in livestock.

144 Looking for possible interaction between endemic *F. hepatica* and *C. daubneyi* on
145 Welsh farms, Jones *et al.* [24] reported a significant negative correlation between infection

146 levels based on FECs for each parasite; lower egg counts were seen for *C. daubneyi* in
147 animals with a high FEC result for *F. hepatica*, and *vice versa*. Furthermore, it has now been
148 confirmed that *C. daubneyi* and *F. hepatica* share the same key host snail; *Galba truncatula*
149 [26]. Studies of the interactions between these two species at the intermediate host level will
150 be important to inform our understanding of the dynamics of trematode infection and possible
151 co-infection within *G. truncatula* populations, and to better understand the ecology and
152 epidemiology that underpin the risk factors for livestock infections [24]. It appears that
153 successful co-infections (where cercariae and infective metacercarial cysts are produced)
154 between these two species within an individual intermediate host are normally not seen under
155 field conditions, even when both *C. daubneyi* and *F. hepatica* are present in the same snail
156 population [53,54]. Successful co-infections producing metacercariae have been
157 demonstrated in the laboratory [55,56], and reported from a small number of naturally
158 infected lymnaeid snails in France [56]. However, co-infection prevalence in the snail
159 population included in this study was <1%, further indicating the rarity of such co-infections.
160 Transmission of the rumen fluke *P. leydeni* within wildlife, and occasionally livestock
161 ruminant populations, is thought likely to occur via a snail other than *G. truncatula* [49].
162 These findings raise intriguing questions regarding the epidemiology of, and potential
163 interaction between, liver fluke and rumen flukes in their common environment and shared
164 host range. Such interactions may have significant implications for parasite control.

165 Recent studies have provided a valuable first look at the host immune response to
166 chronic paramphistome infection. Histological examination demonstrated localised tissue
167 damage and infiltration of inflammatory cell populations (mainly CD3+ T cells) at the site of
168 attachment of mature rumen fluke to the ruminal papillae [12,57]. Cytokine analysis
169 suggested a localised Th1 immune response with upregulation of IFN γ and IL-10 in response
170 to paramphistome infection in the rumen [57]. This evidence of an active immune response

171 during chronic paramphistomosis is interesting in light of the supposed ‘well-tolerated’ status
172 of mature rumen fluke by the host [48]. Whilst this tolerance is widely referred to, it is
173 unclear what this really means for the host and any mechanism actively used by rumen fluke
174 to promote this has not been characterised. Whilst the limited penetration by the adult
175 parasites into host tissue will contribute to this, it is also likely that rumen flukes, like other
176 helminth parasites, secrete immunomodulators to promote their survival in the mammalian
177 host [58–61]. Alternatively, given the rich diversity of commensal gut microbes within the
178 rumen fluid, these parasites may benefit from microbiome-induced immune modulation [62]
179 but further research is required to investigate any such interactions. There is some evidence
180 that the host immune response may confer a degree of protection against subsequent
181 paramphistome infections [4,6], but it is not yet clear how long-lasting any effect may be, and
182 if this is sufficient to prevent clinical disease upon repeat infections in the same or future
183 grazing seasons.

184 Whilst production effects caused by tropical paramphistome species have been
185 reported [1,13], it is not yet clear if sub-clinical disease, specifically due to chronic *C.*
186 *daubneyi* infection, has a significant impact on animal productivity or welfare in temperate
187 areas. Although limited by a small sample size (n=6), an abattoir study by Bellet *et al.* [47]
188 did suggest a negative correlation between rumen fluke infection and cold carcass weights
189 and fat coverage at slaughter in British cattle. Malrait *et al.* [50] also identified an association
190 between rumen fluke infection and poor faecal consistency scores in Belgian cattle.

191 Based on FEC reduction tests in cattle, oxclozanide was shown to be the most
192 effective existing flukicide (compared with albendazole, netobimin and closantel) against *C.*
193 *daubneyi* infection [35]. Closantel displayed a slightly lower efficacy against mature *C.*
194 *daubneyi* but in other studies this drug was ineffective against paramphistomes [33,50]. To
195 date, the efficacy of different anthelmintics against immature *C. daubneyi*, which are

196 responsible for most pathology in infected animals, has yet to be investigated. Conducting
197 drug efficacy tests *in vivo* against immature paramphistomes is challenging, owing to the
198 current lack of a diagnostic test for pre-patent infections or a suitable animal model for
199 experimental infection. Some efforts towards diagnostic development have been made
200 recently: Anuracpreeda *et al.* developed a diagnostic sandwich ELISA, based on detection of
201 a 16 kDa protein from a *Paramphistomum gracile* whole worm extract [37,63,64]. This offers
202 hope for the development of antibody-based diagnostic tools (e.g. for serum, bulk milk and
203 saliva) for *C. daubneyi* in the future. Perhaps most desirable would be the development of a
204 coproantigen test similar to that available for *F. hepatica* [65]. These tests are not invasive,
205 and are able to identify current infections by direct detection of parasite antigens. This is a
206 major advantage over traditional serology based diagnostics which detect specific host
207 antibodies and therefore cannot discriminate between active infections and previous host
208 exposure [65].

209

210 **Research priorities: Playing catch-up**

211 Rumen fluke have been much neglected in recent parasitological research efforts. However,
212 there is now a pressing need to re-examine these parasites in order to understand how they
213 interact with, and impact on, their ruminant hosts in the temperate climates and intensive
214 farming practices of Western Europe (see Outstanding Questions). The success of much of
215 this basic research ultimately hinges on the generation of large scale “omics” datasets for
216 paramphistomes in general, but particularly for *C. daubneyi*. Transcriptomic and proteomic
217 studies of *C. daubneyi* should reveal host-exposed molecules that are secreted by the parasite
218 or presented on the tegumental surface. As seen with other trematodes [40,42], many of these
219 molecules are likely to be antigenic and represent important diagnostic or vaccine candidates.

220 It is now recognised that many helminths also export a variety of protein and RNA
221 molecules, including potential diagnostic/vaccine targets, packaged within extracellular
222 vesicles [58,66–69]. Their characterisation in *C. daubneyi* may provide a further source of
223 diagnostic antigens whilst revealing mechanisms of interaction between rumen flukes and
224 their ruminant hosts.

225 Further in-depth studies of these parasites within their intermediate snail host are also needed.
226 These efforts may reveal opportunities to introduce parasite control measures to prevent
227 transmission via the intermediate host [54], and inform understanding of epidemiological
228 factors impacting lifecycle stages outside the definitive host. Knowledge of the dynamics of
229 trematode infections within their intermediate hosts may further aid the identification of risk
230 factors for both *F. hepatica* and *C. daubneyi* infections [24,53] and allow risk prediction
231 models to be developed.

232 Because clinical paramphistomosis is invariably associated with burdens of immature
233 parasites in the small intestine [7,10], it is imperative to study these juvenile and migratory
234 life-cycle stages as well as the adult flukes that are more easily recovered from the rumen.
235 Comparative proteomics studies may reveal how the antigenic profile of the parasite changes
236 as it matures, and identify virulence factors involved in pathogenicity. Similar approaches, in
237 other helminth species, have identified key molecules involved in parasite feeding/infectivity
238 (such as the cathepsin L family of *F. hepatica* [70]) that are now being validated as targets for
239 parasite control using RNA interference [71,72]. Understanding how *C. daubneyi* establishes
240 infection within the small intestine, and how it feeds as it migrates along the GIT, may also
241 present similar opportunities for rumen fluke control.

242 Characterising the host immune response as infection progresses from an acute to
243 chronic stage will inform both vaccine and diagnostic development. For example, if *C.*

244 *daubneyi* secreted antigens are developmentally expressed, it may be possible to develop a
245 diagnostic method capable of discriminating between acute and chronic stage infections
246 [73,74]. Studying how the host immune system responds to infection will shed light on any
247 immunomodulatory mechanisms employed by *C. daubneyi* and how these may impact on the
248 ability of the host to respond to concurrent bacterial infections or mount an effective immune
249 response against vaccine antigens. Thus, establishing suitable animal models of infection to
250 study *C. daubneyi* immunology is a priority.

251 Given the foreseeable length of time and research effort required to develop a vaccine
252 against *C. daubneyi* infections, the identification of effective anthelmintic treatments is
253 critical. The current lack of a widely-licensed drug product for paramphistomosis, and the
254 reliance on a single compound, oxclozanide (often used off-licence), is far from desirable.
255 With widespread drug resistance now affecting the control of a large range of parasitic and
256 infectious diseases [75,76], reliance on a single drug compound raises the risk of untreatable
257 isolates emerging, and is never good policy for sustainable parasite control. Future research
258 may focus on the identification of novel compounds with efficacy against both juvenile and
259 mature rumen fluke (or repositioning of existing drugs) and the selection and validation of
260 new drug targets, using *in vitro* assays to assess anthelmintic efficacy against measurements
261 such as fluke motility [77] or egg viability [78].

262 With paramphistomosis an established threat to sustainable agriculture in the tropics
263 and sub-tropics [1,79], questions surrounding the exact sub-clinical, production or animal
264 welfare impacts of chronic paramphistome infections in temperate climates are yet to be
265 satisfactorily addressed. Comprehensive studies are now needed to establish the impact and
266 importance of *C. daubneyi* infections within the ruminant livestock systems of Western
267 Europe, in order to inform veterinarians and livestock producers of appropriate control

268 measures which may be necessary, or to alleviate concerns surrounding the emergence of this
269 relatively unknown infection.

270

271 **Concluding remarks**

272 Based on recent survey work (Table 1), rumen fluke should now be considered an emerging
273 parasitic infection of ruminant livestock in Europe. There are a number of significant
274 challenges for the agricultural industries in the coming decades, including maintaining
275 sustainable farming practices in the face of climate change and the need to feed an ever-
276 growing world population. It will be essential to understand and manage all factors which
277 may impact on livestock productivity and farming efficiency in order to overcome these
278 hurdles. With paramphistomosis an emerging factor in European ruminant production
279 systems, there is a clear need to quantify both production losses and the clinical threat from
280 rumen fluke infection as well as to develop new diagnostic tools and therapeutic options. To
281 gain a comprehensive understanding of this parasitic infection, large scale production trials,
282 along with the development of ‘omics’ datasets, are urgently needed. Given the expansive
283 data already available for other helminths, which pose a threat to livestock, a substantial
284 effort is now required to bring our knowledge of paramphistome infections up to par.

285

286

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493 **Figure 1: Life-cycle of the rumen fluke, *Calicophoron daubneyi***

494 Unembryonated eggs pass from the rumen, through the gastrointestinal tract and onto grazing
495 pasture in the host's faeces (1). Eggs embryonate under favourable environmental conditions
496 (adequate temperature and moisture) (2) and miracidia hatch from the egg and locate a
497 suitable snail intermediate host (typically *Galba truncatula*) (3). Within the snail host (4),
498 parasites reproduce asexually and develop through 3 larval stages (4a Sporocysts, 4b Rediae,
499 4c Cercariae), before the free-swimming cercariae are released from the snail and encyst on
500 vegetation (5). Metacercariae remain dormant, resistant to moderate environmental changes,
501 until they are ingested by the definitive ruminant host (6). Parasites then excyst in the
502 duodenum of the small intestine (7) where immature flukes feed on sub-mucosa until they are
503 ready to begin their migration to the rumen. In the rumen, mature fluke attach firmly to the
504 rumen wall, or rumen papillae, via their muscular acetabulum, and release eggs into the
505 rumen contents (8).

506

507 **Figure 2: Images of rumen tissue recovered from a naturally infected cow at slaughter**

508 The area marked 'A' shows an example of the raised nodules seen where parasites attach to
509 the rumen wall. 'B' shows an area of papillae atrophy, commonly seen alongside rumen fluke
510 infection. P, normal papilla.

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515 **Table 1: Reported prevalence of rumen fluke across Western Europe**

516

Area	Host	Method^a	Prevalence	Reference
France	Goat	FEC	58.1%	[34]
France	Cattle	PM	20%	[23]
Belgium	Cattle	PM	28%	[50]
		FEC	22%	
Ireland	Sheep	PM	14%	[80]
	Cattle	PM	52%	
Spain	Cattle	FEC	36%	[22]
Spain	Cattle	PM	6.2%	[17]
Wales	Sheep	FEC	42%	[24]
	Cattle	FEC	59%	
Ireland	Cattle	FEC ^b	57-100%	[48]
Ireland	Sheep	FEC	77%	[18]
England	Cattle	PM	25%	[47]

517 ^aDetected prevalence levels are detailed, along with the methods used to diagnose the
518 infections. FEC, faecal egg counts (farm level); PM, post mortem identification in the
519 slaughterhouse; ^b, herd level.

520

521 **Glossary**

- 522 • **Omics:** Generally, the suffix ‘-omics’ is used to describe large-scale studies which
523 analyse the interactions of biological processes and specific molecule types within a
524 particular cell, tissue type or organism, including (but not limited to) genes
525 (genomics), transcripts (transcriptomics), proteins (proteomics) and small metabolites
526 (metabolomics). Omics technologies encompass a range of tools and research
527 platforms which enable a whole systems approach to studying molecular biology, and
528 the analysis of omics data relies heavily on bioinformatics.
- 529 • **Digenean:** A subclass of organisms within the phylum Platyhelminthes and Class
530 Trematoda, the digenea are obligate parasites with complex lifecycles, involving
531 multiple hosts. Most commonly a molluscan first and vertebrate definitive host are
532 required, with many species having 2nd and 3rd intermediate hosts also. Digenea are
533 characterised by their syncytial tegument and usually two suckers – one oral, leading
534 to the gut, and one muscular, which is blind-ended. Typically digenean parasites are
535 hermaphroditic, with the Schistosomes a notable exception.
- 536 • **Paramphistome:** digenetic trematodes from the family Paramphistomidae that reside
537 in the rumen of their definitive hosts, typically sheep and cattle. The term
538 paramphistome refers to their two-mouthed appearance, with an anterior oral sucker
539 and posterior muscular sucker which is used for attachment to the host.
- 540 • **Rumen:** The rumen is the second of the four stomach chambers described in ruminant
541 mammals, although the term is commonly used to refer to the single organ formed by
542 both the reticulum (first chamber) and rumen. The primary role of this chamber is the
543 microbial degradation of ingested plant materials and for the uptake of volatile fatty
544 acids produced by microbial fermentation.

545

546 **Trends**

- 547 • Helminth parasites impact hugely on livestock productivity by affecting growth rates,
548 fertility, meat quality, wool or milk production, and sometimes cause mortality.
- 549 • Levels of infection with the rumen fluke, *Calicophoron daubneyi*, have increased
550 dramatically in the UK and Ireland in recent years and may exceed that of liver fluke
551 (*Fasciola hepatica*) in some areas.
- 552 • There are a growing number of reports of animal morbidity and mortality associated
553 with acute paramphistomosis.
- 554 • Current diagnostic and treatment options are very limited, and improving these will
555 depend on answering basic questions about rumen fluke biology.

556

557

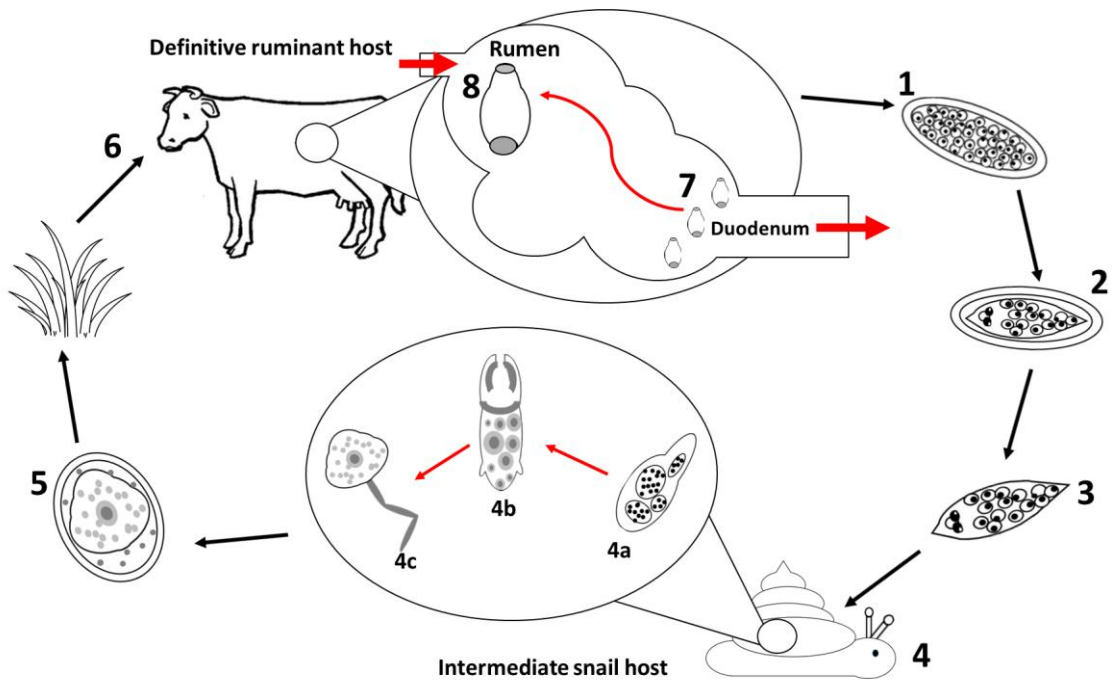
558 **Outstanding questions**

- 559 • What is the impact of chronic *C. daubneyi* infection on animal health, welfare and
560 production?
- 561 • How do immature *C. daubneyi* contribute to the pathology of infected animals?
- 562 • What is the mechanism of feeding used by immature and mature *C. daubneyi* in the
563 small intestine and rumen respectively?
- 564 • Adult *C. daubneyi* are often said to be “well tolerated” by their hosts. What does this
565 mean for the host and what (if any) parasite-derived molecules drive it?
- 566 • Can we identify new drugs or drug targets to help control rumen fluke infection?
- 567 • Since pre-patent infections are the primary cause of clinical paramphistomosis, can
568 we identify antigens specific to early stage rumen fluke infection, to enable timely
569 diagnosis and treatment?
- 570 • What immune response does the host mount against rumen fluke and is this amenable
571 to vaccine design?
- 572 • What is the outcome of potential competition between *C. daubneyi* and *F. hepatica*
573 within an intermediate snail host population? What impact does this have on
574 transmission to the definitive ruminant host?

575

576

577 **Figure 1**



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580

581 **Figure 2**



582