Kinetics of exsheathment of infective ovine and bovine strongylid larvae *in vivo* and *in vitro*

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SUMMARY

The aim of the study was to investigate the longitudinal changes of exsheathment of ovine and bovine 3rd-stage strongylid larvae in an artificial rumen (RUSITEC) and to compare the results with *in vivo* data obtained from rumen-fistulated sheep. Infective larvae were incubated in nylon mesh bags in the sheep rumen or the RUSITEC apparatus for periods of 1, 6 and 12 h, respectively. The 12 h exsheathment rates in the rumen and the RUSITEC apparatus (in parentheses) were as follows: *Haemonchus contortus*: 100 % (100 %), *Ostertagia circumcincta*: 100 % (76 %), *O. leptospicularis*: 100 % (100 %), *O. ostertagi*: 53 % (59 %), *Trichostrongylus axei*: 100 % (100 %), *T. colubriformis*: 37 % (36 %), *Cooperia curticei*: 94 % (76 %), *C. oncophora*: 95 % (89 %), *Nematodirus filicollis*: 0 % (N.D.), *N. spathiger*: 11 % (15 %), *N. battus*: 7 % (5 %), *Oesophagostomum venulosum*: 17 % (9 %), *Chabertia ovina*: 7 % (2 %), *Dictyocaulus filaria*: 1 % (N.D.). Larvae of *Nematodirus* spp. and *T. colubriformis* showed a quick rise of the exsheathment rate 2 h after transfer into the abomasum. These results confirm that exsheathment generally occurs in the part of the gastrointestinal tract immediately anterior to the habitat of the adult parasite. The overall similar course of exsheathment in both systems indicates that the essential stimuli for exsheathment were generated and maintained under *in vitro* conditions of the artificial rumen. In both systems, the bicarbonate concentration and the pH reflected a similar status of the H_2CO_3/HCO_3^- buffer system, which is known to provide the essential stimuli for larval exsheathment of the abomasal species. These results give evidence that the RUSITEC system represents a valid system for studying the kinetics of exsheathment of strongylid nematodes under *in vitro* conditions. For 7 of the species investigated the obtained results represent the first data on larval exsheathment *in vivo* and *in vitro*.

Key words: nematodes, trichostrongyles, exsheathment, ecdysis.

INTRODUCTION

In strongylid nematodes, the infective stage forms a 'bridge' by which the parasite passes from one environment to another (Rogers, 1960). After hatching from the egg, the development of the 1st-stage to the 3rd-stage larvae involves 2 moults. The process of moulting is controlled by the larval endocrine system and it is suggested that the delayed exsheathment of the second larvae is due to the suspension of the normal endocrine mechanisms (Rogers & Sommerville, 1957). After ingestion of the larvae, a stimulus from the host is necessary to restart development. This stimulus causes secretion of exsheathing fluid, a process that determines the site of exsheathment in the gastrointestinal tract (Sommerville, 1957; Rogers, 1966). For the abomasal nematodes, components of the bicarbonate/carbonic acid buffer system provide the essential stimuli for exsheathment (Rogers, 1960; Petronijevic, Rogers & Sommerville, 1985), explaining that conditions for subsequent infection are not fulfilled in the premature rumen of new-born animals in which this buffer system is not yet established (Satrija, Nansen & Christensen, 1991). Further investigations on the stimuli and underlying mechanisms that are involved in exsheathment of larvae of the various strongylid species are limited due to the lack of *in vitro* systems mimicking the situation in the gastrointestinal tract of ruminants. Therefore, the aim of the present experiment was to investigate the suitability of an artificial rumen (RUSITEC, Czerkawski & Breckenridge, 1977) to study the kinetics of exsheathment using 3rd-stage larvae of ovine and bovine gastrointestinal and lung nematodes, and to compare the results with *in vivo* data obtained with fistulated sheep.

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MATERIALS AND METHODS

Four sheep (white alpine mountain breed), approximately 2 years old, were used in the experiment. The sheep were born and reared indoors under conditions that minimized the risk of parasitic infection. They were kept on straw and were fed hay twice daily. All sheep were fitted with a permanent rumen fistula, which allows access to the rumen contents. For the surgical procedure of rumen

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cannulation the technique described by Hertzberg *et al*. (1994) was used. Briefly, after local paravertebral anaesthesia, a circular incision in the skin (diameter 8 cm) was made, leaving about 5 cm distance to the last rib and 3 cm to the transverse process of the lumbar vertebrae. The excised piece of skin plus subcutaneous fat was removed and the underlying abdominal muscles were split by blunt dissection in the direction of their fibres to expose the peritoneum. The latter was cut and the rumen was gently drawn through the opening and sutured to the skin through the dissected abdominal muscles. After 10 days, a vertical incision was made into the rumen and a cannula inserted. The sheep were allowed to recover for several weeks before experiments were started.

Infective larvae of the following 14 ovine and bovine species were used: *Haemonchus contortus*, *Ostertagia* (*Teladorsagia*) *circumcincta*, *O. leptospicularis*, *O. ostertagi*, *Trichostrongylus axei*, *T. colubriformis*, *Cooperia curticei*, *C. oncophora*, *Nematodirus filicollis*, *N. spathiger*, *N. battus*, *Oesophagostomum venulosum*, *Chabertia ovina*, *Dictyocaulus filaria*. The larvae were cultivated from faeces using the McMaster mass larval culturing system (Parker, 1977) or were obtained as freshly cultivated larvae from other laboratories. Larvae of *Dictyocaulus filaria* were harvested with the Baermann technique and stored at room temperature until they had developed to the infective stage. Infective trichostrongylid larvae were stored at 4 °C and were used for the experiments within 3 months. Twenty-four hours before use, the larvae were adapted to room temperature (20 °C) and checked for viability and morphological disorders. Only batches with more than 95 % motile larvae were used for the experiments.

The experiments were started around 07.00 h, approximately 1 h before feeding. For each experiment, approximately 2000 larvae, kept in a few ml of tap water, were transferred into a small nylon mesh bag (mesh diameter: 5 μm). Subsequently, the bags were closed tightly with a synthetic cord, leaving a free cord end of approximately 30 cm. Through the open fistula, the bags were positioned deeply inside the rumen fluid and were fixed with the cord at the inner disc of the rumen cannula, leaving a distance of approximately 25 cm between the bag and the cannula. The cords were marked with different colours specific for the species tested. Six bags with larvae were prepared for each species and 2 replicates were removed from the rumen after 1, 6 and 12 h respectively. Each species was tested in 1 sheep. A possible influence of the individual sheep on larval exsheathment was investigated when 1 month old *H. contortus* larvae, originating from the same batch, were tested in parallel in 3 sheep. Statistical comparison of batches tested in different sheep was made by ANCOVA analysis using GraphPad Prism 3.00 for Windows.

Larvae from the cultivated bags were rinsed with tap water into a 50 ml glass container. The viability of the larvae was checked microscopically by their motility. A viability rate higher than 90 % was required for further examination. After examination the larvae were fixed in 10 % formalin. Immediately after fixation, the percentage rate of exsheathed or partly exsheathed larvae was calculated by microscopical identification of 200 larvae using a magnification of $\times 100$.

Those species that showed only little exsheathment in the rumen were additionally investigated in the abomasum. Therefore, for each species, 6 bags with infective larvae that had been prepared as described before, were cultivated in the rumen for 6 h and 4 of them subsequently transferred into the abomasum. The remaining 2 bags were removed and evaluated. The correct position of the bags inside the abomasum was checked with a pH stomach probe (Hertzberg *et al*. 2000). The rate of exsheathment was tested after 1 and 2 h, when 2 bags were removed from the abomasum at each time. The potential influence of the age of the larvae on the exsheathment rate was tested with *H. contortus*. Therefore, freshly cultivated larvae and larvae from the same batch, which had been stored at 4 °C for 1, 2 and 3 months, were investigated in the same sheep.

For measuring the bicarbonate concentration and the pH, samples of the rumen fluid were obtained from the sheep currently used in the experiment. Therefore, at the time when the bags with larvae were deposited in the rumen, a plastic tube (50 ml) was introduced deeply into the rumen fluid and closed with a lid before it was removed from the rumen. The sample was kept at body temperature until centrifugation. The bicarbonate concentration was measured immediately after processing using the 'Bicarbonat-enzymatisch' test kit (BioMérieux) and the Cobra Mira analyser.

For the *in vitro* studies, the rumen simulation technique (RUSITEC; Czerkawski & Breckenridge, 1977) was used. Experiments were restricted to 1 of the 6 fermenters of the RUSITEC system as described by Kelly (1996). Larval exsheathment was investigated during 6 successive experiments using 2 species after each other. The same spectrum of parasite species was tested that had been used *in vivo*, with the exception of *N. filicollis* and *D. filaria*, where not enough larval material was available for the *in vitro* experiments.

The fermenter was filled with 850 ml of rumen fluid from the cannulated sheep and with 100 ml of McDougall buffer (McDougall, 1948). The fermenter was fitted with an additional access for application of the nylon bags containing the larvae. The first bags were introduced into the fermenter after a 24 h stabilization period. The buffer flow rate was 550–640 ml per day. The carbon dioxide concentration of the gas phase in the fermenter was

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Table 1. Exsheathment rates (%) of infective strongylid larvae during *in vivo* and *in vitro* incubation (data of 2 series and mean) in the rumen of sheep and in the RUSITEC-system

Species*		0 h	1 h	6 h	12 h
O. leptospicularis	*in vivo*	0/0	93/99 (96)	96/97 (97)	100/100 (100)
(*ov*)	*in vitro*	0/0	77/86 (81)	89/93 (91)	99/100 (100)
O. circumcincta	*in vivo*	0/0	70/83 (77)	77/90 (84)	100/100 (100)
(*ov*)	*in vitro*	0/0	11/20 (16)	57/66 (62)	69/83 (76)
O. ostertagi	*in vivo*	0/0	14/15 (15)	37/42 (40)	50/55 (53)
(*bov*)	*in vitro*	0/0	16/21 (19)	43/51 (47)	56/62 (59)
H. contortus	*in vivo*	0/0	86/93 (90)	98/99 (99)	100/100 (100)
(*ov*)	*in vitro*	0/0	66/73 (70)	92/96 (94)	99/100 (100)
T. axei	*in vivo*	0/0	72/78 (75)	89/92 (91)	100/100 (100)
(*ov*)	*in vitro*	0/0	64/73 (69)	85/90 (88)	100/100 (100)
T. colubriformis	*in vivo*	0/0	16/25 (21)	26/34 (30)	33/40 (37)
(*ov*)	*in vitro*	0/0	17/22 (20)	18/24 (21)	32/39 (36)
C. curticei	*in vivo*	0/0	10/15 (13)	61/68 (65)	91/96 (94)
(*ov*)	*in vitro*	0/0	12/19 (16)	57/64 (61)	72/80 (76)
C. oncophora	*in vivo*	0/0	57/64 (61)	71/75 (73)	93/96 (95)
(*bov*)	*in vitro*	0/0	49/56 (53)	61/75 (68)	85/92 (89)
N. filicollis	*in vivo*	0/0	0/0	0/0	0/0
(*ov*)	*in vitro*	N.D.	N.D.	N.D.	N.D.
N. spathiger	*in vivo*	0/0	0/0	4/7 (6)	8/13 (11)
(*ov*)	*in vitro*	0/0	0/1 (1)	10/15 (13)	12/17 (15)
N. battus	*in vivo*	0/0	0/0	0/1 (1)	5/8 (7)
(*ov*)	*in vitro*	0/0	0/0	0/0	2/8 (5)
Oe. venulosum	*in vivo*	0/0	3/8 (6)	5/9 (7)	13/20 (17)
(*ov*)	*in vitro*	0/0	5/10 (8)	6/13 (10)	6/12 (9)
Ch. ovina	*in vivo*	0/0	0/1 (1)	1/2 (2)	6/7 (7)
(*ov*)	*in vitro*	0/0	0/1 (1)	0/1 (1)	1/2 (2)
D. filaria	*in vivo*	0/0	0/1 (1)	0/0	1/1 (1)
(*ov*)	*in vitro*	N.D.	N.D.	N.D.	N.D.

* (*ov*): ovine origin; (*bov*): bovine origin.

measured daily by a Dräger tube (Dräger Tube CH 20301, carbon dioxide 5%/A, Drägerwerk AG, Germany). Additionally, pH was determined by a pH meter (model 632, Metrohm, Herisau, Switzerland) in rumen fluid samples directly drawn from the fermenter. Then, HCO_3^- concentrations were calculated using the formula of Henderson-Hasselbalch ($pH = pK + \log([HCO_3^-]/[H_2CO_3^-])$):

$$HCO_3^- = \text{volume of } CO_2 \text{ in } \%/2{\cdot}226 \times (1 - 1/(10^{pH-pK} + 1)).$$

Exsheathment of infective larvae was studied using the same technique and protocol as in the *in vivo* experiments.

The experiments were approved by the ethical committee of the Cantonal Veterinary Office, Zurich.

RESULTS

The exsheathment rates of 3rd-stage larvae, measured in the rumen and in the RUSITEC apparatus, are shown in Table 1. All ovine abomasal species showed substantial exsheathment within 1 h of incubation in the rumen fluid and exsheathment was complete in *O. circumcincta*, *O. leptospicularis*, *H. contortus* and *T. axei* within 12 h of incubation. Among those ovine species living in the small intestine, members of the genus *Nematodirus* showed no or very little exsheathment *in vivo* after 12 h, whereas almost complete exsheathment was observed in *C. curticei* after the same period. Little exsheathment was detected in the strongylid species *Oe. venulosum* and *Ch. ovina* after 12 h of incubation.

When larvae of ovine species with a poor ruminal exsheathment rate were transferred into the abomasum, a marked increase of exsheathment resulted in *N. battus* (47%), *N. filicollis* (68%) and *T. colubriformis* (71%) (Fig. 1). In contrast, no response was observed with larvae of *Oe. venulosum*, *Ch. ovina* and *D. filaria*.

From the bovine species, *C. oncophora* showed almost complete exsheathment, whereas only about half of the *O. ostertagi* larvae lost their sheath after 12 h of incubation in the rumen.

Haemonchus larvae originating from the same batch, which were investigated in 3 different sheep, exhibited no significant differences ($P > 0{\cdot}05$) in their exsheathment rates after 1, 6 and 12 h incubation in the rumen. The same was true with freshly cultivated *Haemonchus* larvae that were tested at monthly intervals after cultivation for a period of 3 months ($P > 0{\cdot}05$). In all assays, the motility of the larvae was generally above 95% after 12 h incubation.

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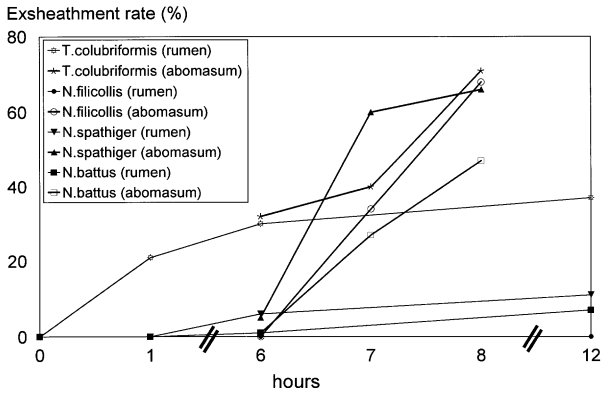

Fig. 1. Sequential exsheathment rates of infective larvae of *Trichostrongylus colubriformis*, *Nematodirus filicollis*, *N. spathiger* and *N. battus* during a 12 h incubation in the rumen of fistulated sheep or after transfer to the abomasum following a 6 h incubation in the rumen (each value represents the arithmetic mean of 2 replicates).

In the 4 selected sheep, the pH of the rumen fluid was fairly constant throughout all experiments, and varied between 6·5 and 6·9, whereas the bicarbonate concentrations ranged between 21 and 32 mmol/l.

Six series of experiments were performed with the RUSITEC system, during which the different species were investigated. The pH of the fermentation fluid remained very stable throughout all experiments and ranged between 6·75 and 6·93 (mean 6·84; S.D. 0·05). Also, the CO_2 concentration in the gas phase only fluctuated within a narrow range (58–64 %; S.D. 4), reaching 60 % as a mean of all series. The calculated content of HCO_3^- varied between 19·7 and 26·2 mmol/l (mean 22·8 mmol/l; S.D. 1·58).

In 4 of 6 ovine species which showed a substantial exsheathment rate *in vivo* (*H. contortus*, *O. leptospicularis*, *T. axei*, *T. colubriformis*), results obtained after 12 h incubation in the RUSITEC system were very similar to the results obtained in the rumen. Two species (*C. curticei*, *O. circumcincta*) exhibited slightly lower exsheathment rates *in vitro* compared with the *in vivo* data (Table 1).

In accordance with the *in vivo* results the rate of exsheathment was small in the *Nematodirus* species and the 2 strongylid species. Similar behaviour of larvae in both systems was also found for the 2 bovine species *O. ostertagi* and *C. oncophora*.

DISCUSSION

In strongylid nematodes, exsheathment of the 3rd-stage larvae marks the transition from the free-living to the parasitic phase, where the larvae start to feed from the host. In the present study, the results obtained with rumen-fistulated sheep indicate that, in trichostrongylid nematodes, exsheathment generally takes place in the part of the gastrointestinal tract immediately anterior to the habitat of the adult parasite, confirming earlier observations made by Sommerville (1954, 1957). For those species residing in the abomasum, exsheathment occurred quickly after introduction of larvae into the rumen fluid reaching mean levels between 75 % (*T. axei*) and 96 % (*O. leptospicularis*) after 60 min. Given that the mean turnover of fluid in the rumen is 8–12 h (Kuenzle & Jenny, 1979), and that the transport of the larvae is only slightly retarded compared with the fluid transport (Dakkak, Fioramonti & Bueno, 1981), it is likely that, under physiological conditions, most of the infective larvae of the species investigated will be prepared for subsequent invasion of abomasal glands. In studies with *H. contortus*, Dakkak *et al.* (1981) observed that the first infective larvae entered the abomasum 10–20 min after oral inoculation and, in accordance with the present results, the majority of larvae was exsheathed after 60 min. For *H. contortus* and *T. axei*, Sommerville (1957) calculated a 50 % exsheathment rate of 34 and 24 min respectively, whereas, in the present study, *T. axei* larvae showed a slightly retarded exsheathment in comparison with *H. contortus*. Incubation in the rumen for more than 12 h did not improve exsheathment in all species investigated (data not shown). Instead, the larval mortality rate rapidly increased between 12 and 24 h, indicating that a persistence of the larvae in the rumen for longer than 12 h is not beneficial for their survival. Within a range of 3 months, the age of the *H. contortus* larvae was without influence on the exsheathing rate which is in agreement with previous observations (Rogers, 1960; Silverman & Podger, 1964). Furthermore, between-sheep-differences of exsheathment rates obtained with *H. contortus* were remarkably low, and this was also the case for several other species in which only the 12 h data were determined (data not shown). Therefore, serial data obtained from single sheep well represented the overall kinetics of larval exsheathment, and differences were in a similar range as variations between batches tested in the same sheep.

Among the species residing in the small intestine, a very low exsheathment rate was observed in the 3 *Nematodirus* species after 12 h. In contrast, approximately one third of *T. colubriformis* larvae lost their sheath after 6–12 h of incubation in the rumen, which contradicts the observations of Sommerville (1957), who could not find any larval exsheathment in the rumen with this species. The reason for this discrepancy is not clear, but could be due to the use of cellophane bags compared with nylon mesh bags in the present study. The validity of the *in vivo* results obtained with *T. colubriformis* in the present study was supported by the data measured in the artificial rumen (see below). Almost complete exsheathment occurred in *C. curticei* larvae after 12 h incubation in the rumen, confirming earlier observations made by

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Ahluwalia & Charleston (1974). As a parasite of the small intestine, *C. curticei* would be expected to exsheath in the abomasal fluid, but a recent report suggested that, at least in cattle, the organ tropism of *Cooperia* species may not be as exclusive as it was previously thought (Chollet *et al.* 2000). In Zebu cattle, these authors found higher burdens of *C. pectinata* and *C. punctata* in the abomasum in comparison with the small intestine. Therefore, the exsheathment behaviour of *C. curticei* and also of its bovine analogon *C. oncophora* (see below) may indicate that these species were formerly parasites of the abomasum which subsequently have adapted to the conditions of the small intestine. According to these observations, a large portion of *Cooperia* larvae will pass through the acid environment of the abomasum without being protected by their sheath. However, in those species that were transferred from the rumen into the abomasum, a period of 2 h in the abomasal fluid did not affect motility of the exsheathed larvae significantly. Furthermore, exsheathed *Cooperia* larvae will most likely benefit from an increased abomasal pH in the abomasum induced by those species developing in the abomasal mucosa. In contrast, infectivity could be severely impaired under these circumstances for those species that are dependent on an acid environment for exsheathment. This is particularly true for the *Nematodirus* spp. and to a lesser extent for *T. colubriformis*. A rapid rise of the exsheathment rate occurred in these species after transfer of larvae into the abomasum after 6 h incubation in the rumen, with only little (*Nematodirus* spp.) or moderate (*T. colubriformis*) exsheathment. Under *in vitro* conditions, hydrochloric acid has been defined as one of the essential stimuli for *T. colubriformis* and *N. battus* to exsheath and, in the former, the effect was enhanced by carbon dioxide in the gas phase (Rogers, 1960). Silverman & Podger (1964) concluded from their experiments that, under acidic conditions, pepsin was able to accelerate exsheathment of *T. colubriformis* in a gas phase of 100 % CO_2. Pepsin and hydrochloric acid were also found to be necessary for *T. retortaeformis* larvae to exsheath under *in vitro* conditions (Crofton, 1947). When *H. contortus* larvae were transferred directly into the abomasum of sheep, no exsheathment was observed, whereas a substantial portion of larvae lost their sheath under *in vitro* conditions that were designed to mimic the abomasal fluid (Sommerville, 1957). These results confirm the necessity of validated *in vitro* systems. With respect to the investigation of the intestinal species, this would also include a pre-cultivation of larvae under rumen conditions before they are exposed to the abomasal environment as was performed in the present study. This procedure could account for the relatively high exsheathment rate of 65 % that was observed with *N. spathiger* larvae after a 2 h incubation in the abomasum, in comparison with results from Sommerville (1957) who observed exsheathment rates below 50 % after 4 h of incubation.

From the bovine trichostrongyles tested, *C. oncophora* showed a similar 12 h exsheathment rate compared with the corresponding ovine species, and even exhibited considerably higher values in the early phase of the experiment. In contrast, the exsheathment rate of *O. ostertagi* only reached 53 % compared with 100 % of the 2 ovine *Ostertagia* species. No data are available about the *in vivo* exsheathment of *O. ostertagi* in the bovine rumen, but it is likely that higher values will occur in the specific host and that the lower rates measured in sheep may indicate a first level of host specificity at this early stage of 'infection'.

With the 2 strongylid species, *Oe. venulosum* and *Ch. ovina*, no indications for substantial exsheathment, either in the rumen or in the abomasum, were obtained. Incubation of these species in the small intestine of freshly slaughtered sheep for 2 h was also without effect (data not shown). With the related species *Oe. columbianum*, Sommerville (1957) obtained controversial results in that a moderate exsheathing rate *in vitro* (< 20 %) could not be validated by *in vivo* experiments. Further investigations are necessary to determine the exact location of exsheathment for this species and the responsible stimuli.

Larvae from the ovine lungworm, *Dictyocaulus filaria*, did not show any significant exsheathment, either in the rumen or in the abomasum in the present study. This is in apparent contradiction with results from Silverman & Podger (1964), who found exsheathment rates between 85 and 90 % during incubation of the related *D. viviparus* larvae in pepsin and hydrochloric acid at pH < 2. Later, Parker & Croll (1976) showed that pepsin was not an absolute requirement for exsheathment of *D. viviparus* larvae and suggested that intestinal enzymes of the host were responsible.

In vitro incubation in the RUSITEC system induced larval exsheathment in a very similar manner compared with the *in vivo* results, suggesting that the essential stimuli for exsheathment were generated and maintained under *in vitro* conditions. Among those species showing a high exsheathment rate, comparable temporal changes and 12 h rates were obtained in both systems for *H. contortus*, *O. leptospicularis*, *T. axei* and *T. colubriformis*, whereas in 2 species (*C. curticei*, *O. circumcincta*) *in vitro* exsheathment rates after 12 h were slightly lower than those observed under *in vivo* conditions respectively. These differences could be due to an insufficient contact of the larvae with the incubation medium, which occurred occasionally when the nylon bags were not completely covered by rumen fluid. Lower exsheathment rates in some species were also observed in earlier experiments, when the

larvae were incubated in modified Eppendorf containers where the fluid exchange was restricted to a porous lid (Hertzberg, unpublished results). According to Rogers (1960), undissociated carbonic acid is the major stimulus in the rumen for exsheathment of *H. contortus* and *T. axei* larvae, which represented the abomasal species in these earlier experiments. In the present study, the bicarbonate concentration and the pH indicated a similar status of the H_2CO_3/HCO_3^- buffer system under *in vivo* and *in vitro* conditions. In contrast to undissociated carbonic acid, bicarbonate concentrations can be measured enzymatically or with ion-selective electrodes. As, at a given pH, the bicarbonate concentration correlates with the carbonic acid concentration, this parameter may be used as an indicator for the suitability of the conditions for exsheathment.

The similar behaviour of larvae in both systems was also true for those species that did not respond to conditions in the rumen fluid. Thus, little exsheathment was observed in *N. spathiger*, *N. battus*, *Oe. venulosum* and *Ch. ovina*. It is likely also that moderate acidic conditions can be maintained with the RUSITEC system and therefore this approach may also enable further investigations on the stimuli that induce exsheathment in the intestinal species. In summary, the exsheathment data obtained from larvae of several gastrointestinal nematodes in the sheep rumen were in good agreement with previous studies and therefore formed a solid basis for subsequent *in vitro* investigations. These results indicate that the RUSITEC system represents a valid system for studying the kinetics of exsheathment of strongylid nematodes under *in vitro* conditions. The possibility of creating defined environments by changing single parameters may elucidate more details of the mechanisms of larval exsheathment in the future.

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