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Factors affecting the detachment rhythm of engorged *Boophilus microplus* female ticks (Acari: Ixodidae) from Charolais steers in New Caledonia

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Abstract

As in most parts of the world where the cattle tick *Boophilus microplus* is established, resistance of ticks to acaricides occurs in New Caledonia. In order to implement laboratory resistance tests on larvae, engorged females collected in suspected farms are necessary. Investigations on the detachment schedule of the engorged females were conducted to explain certain field situations such as the lack or scarcity of engorged females on highly infested cattle driven from the pasture to the pen in the morning. Three experiments on Charolais steers naturally infested on pastures showed that: (1) engorged female burdens at sunrise are similar whether the steers spend the night in pasture or in a pen; (2) compared with steers maintained in a pen, morning detachment of females increases when the steers stay on the pasture or move from the pasture to the pen; (3) detachment rhythm of engorged females on steers staying the morning in a pen, is not influenced by feeding activity or exposition of steers to sun; (4) detachment occurs earlier for females attached on anatomical sites exposed to sun, and earlier from these sites for the steers in pasture or walking than for steers in a pen.

Keywords: Boophilus microplus; Ixodidae; Arthropoda; Cattle; Charolais; Infestation rate; New Caledonia

1. Introduction

Introduction of the cattle tick *Boophilus microplus* in New Caledonia occurred during the last World War. Fortunately, the few ticks imported from Australia were free of the tick-borne diseases usually transmitted by this tick species. This relatively favorable situation has been highlighted by the quite successful use of Limousin and Charolais cattle breeds in

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the semi-extensive breeding conditions and the tropical environment of New Caledonia. However, and as for all the breeds originating from temperate countries, Limousin and Charolais are highly susceptible to ticks and to their direct impact when infestation is high and/or permanent. Moreover, the climatic conditions of New Caledonia are suitable for the accomplishment of at least four generations per year, resulting potentially in exploding tick populations everywhere livestock is produced and all the year round. To face up to this situation, the farmers spray or dip their cattle with an acaricide—currently deltamethrin widely used for the last 10 years—at least 10 times a year. Due to this intense selection pressure, tick resistance to deltamethrin appeared and increased in the last 5 years. To confirm any suspicion of resistance in a farm, laboratory tests are required by the veterinary services in order to substitute a recently used acaricide, amitraz, for deltamethrin.

The standard FAO test no. 7 needs the use of fresh larvae produced by engorged females. These females are usually easy to collect on infested cattle—amongst other tick stages—in the early morning, the time of the day when they are known to drop-off (Wharton and Utech, 1970). However, during a survey conducted in 1998 (Bianchi and Barré, personal observation), it was observed that in some unexplained situations, and despite early morning inspection of cattle, no engorged females (in 10% of 148 farms sampled) or only very few, were obtained from animals. Infestation level and delay after acaricide treatment did not seem to be related with this phenomenon for it occurred even when cattle were highly infested with immatures and semi-engorged females, and when the visit took place long enough after the previous acaricide treatment (4–5 weeks) to allow females to take their blood meal and reach their ultimate engorged stage.

It may be hypothesized from these observations that the time of the day is not the only factor that interacts with the presence/absence of engorged females and their rhythm of detachment. The influence of sun light (Kitaoka, 1962; Wharton and Utech, 1970) and feeding activity (George et al., 1998) for example, have been suspected. In fact, even though the biology of *B. microplus* has been well documented for the last 50 years, particularly in Australia (Hitchcock, 1955; Sutherst and Wharton, 1973), little information concerning the factors of variation and the mechanisms of detachment of engorged females has been produced.

The objective of this study was to investigate some of the factors that could modulate the detachment of the engorged *B. microplus* females. From a practical point of view, this knowledge will make possible or facilitate the collection of these females on animals for different purposes, including the implementation of acaricide resistance tests. A particular attention has been focused on a few simple factors such as: time of the day, night and day localization of cattle, movements of the animals and attachment sites on the cattle of the engorged ticks.

2. Materials and methods

2.1. Animals and tick counts

A total of 12–14 Charolais steers, 3 years old, weighting 615 ± 62 kg were naturally infested on pasture during the austral autumn 2001 (between April and June). At the beginning of the current experiments (day 1), these infested steers, all very placid and easy to handle, were individually driven to a crush and immobilized for tick counts. The ticks recorded were the engorged females (called F8 regarding their body length ≥8 mm) and the semi engorged females (or "standard females" called here F4–8: length >4 and <8 mm) counted on the right body side of the steers. All the counts were done by the same technician and the F4–8 counts were made the first day of experiment 1 only. The counts were carried out for 25 anatomical parts of the body. The counts on these 25 sites were then pooled into 3 zones whether they were:

- exposed to the sun: neck, dewlap upper and lower, shoulder upper and lower, withers, back line, ribs, flank, leg upper and lower, external hock, tail, upper perineum;
- protected against sun: axilla, belly, groin, internal hock and leg, lower perineum, scrotum;
- semi-protected from the sun: head with ears, arm, foreleg, feet.

2.2. Farm, pastures and pens

The "Station de Port-Laguerre" is located on the dry west coast of New Caledonia (20°S) with 1300 mm annual rainfall. Two pastures with some trees providing shade were used. The first pasture (P1) was located at about 1 km (20 min walk) from the pens and the crush. The second pasture (P2) was adjacent to the crush (same distance from the crush than the pens). Two identical open pens partly covered with trees were used. When the steers were in these pens, they were watered and fed ad libitum with *Chloris gayana* hay.

2.3. Experiment 1: effect of "night in pasture P1 then 20 min walk to the crush versus night in the pen"

- Day 1 (17/04/2001: clouds): 12 steers maintained for 3 months on infested pastures were driven to the crush at 7:30 h and checked for F8 and F4–8. Then six steers were randomly chosen to stay until day 2 in the pens (group 1), while the other six steers went back to the pasture P1 (group 2). At this first control, and in order to confirm the initial similarity of the two groups for tick burdens, F4–8 were counted and found identical ($P = 0.873$ with a Mann–Whitney test).
- Day 2 (18/04/2001: clouds): group 2 was driven from pasture P1 to the crush at 7:30 h and checked for F8. F8 of the "pen" group 1 were checked in the same conditions. Then the day 1 situation was repeated, with inversion of the groups: group 2 stayed in the pens, group 1 moved to the pasture till day 3.
- Day 3 (19/04/2001: clouds): group 1 was driven from the pasture to the pens and F8 checked on groups 1 and 2. Then, the two groups stayed in the pens till day 4.

2.4. Experiment 2: effect of "20 min morning walk versus no walk" for steers kept in pens night and day

- Day 4 (20/04/2001: sun): the 12 animals in the pens were checked for F8 7 times from sun rise (6:00 h) till the end of the afternoon (15:00 h). After the second tick count, six steers were randomly chosen and had to walk (group W) in a corridor for 20 min (from 8:10 to 8:30 h). The six other steers remained undisturbed in the pens (group P).

Table 1
Experiment 3: description of the 5 treatments applied to 14 Charolais steers during the 3 days of experiment 3 to evaluate the effect of different factors (pasture, pen, moving and daytime) on engorged females detachment

Treatment	Night time	Day time	Number of repetitions
1	Pen	Pen	3 (days 1–3)
2	Pen	Pasture P2	1 (day 2)
3	Pen	40 min walk then pen	1 (day 3)
4	Pasture P2	Pen	2 (days 1–2)
5	Pasture P2	Pasture P2	2 (days 1–3)

2.5. Experiment 3: effect of the pasture, pen, moving and daytime on F8 counts and their contribution on the kinetic of detachment from sun exposed/protected anatomical sites

To differentiate the "pasture effect" from the "walk effect", a third experiment was conducted later during three consecutive days (Table 1). Three different treatments per day were applied to the 14 steers (3 groups of 4, 5 and 5 steers). Furthermore, after the last F8 count of every day, the 14 steers were randomly allotted to each of the 3 treatments of the next day. Some treatments were repeated for 2–3 days and so, a total of five different treatments was applied, all things considered. The F8 burdens were checked four times in the day: at 6:20 h (sunrise), 8:30, 11:30 and 16:00 h.

2.6. Statistical analysis

- For each visit, a F8 presence rate, defined for each steer as the ratio of the number of F8 at this visit/number of F8 at the initial sunrise visit (considered by definition to be 100%), has been used.
- For each visit, a F8 instantaneous detachment rate was defined for each steer as the ratio of: (the number of F8 at the previous visit − number of F8 at this visit)/(number of F8 at the previous visit).
- A general linear model has been used to test each factor (day, treatment, hour, F8 localization) on the number of F8 (on the whole right lateral half of the body or on the three anatomical zones of the right side), the presence rate and the instantaneous detachment rate when the data were homogeneously distributed (Levene test) (SPSS, 1997, version 9.0).
- The non-parametric test of Kruskall–Wallis (or Mann–Whitney test to study factors with two levels or two levels of a multilevel factor) has been used when the data were not homogeneously distributed. In this latter case, each factor has been studied separately (SPSS, 1997, version 9.0).

3. Results

3.1. Sites infested

For all experiments and steers, a total of 5789 engorged females were counted at sunrise. Their distribution on the different anatomical sites is summarized in Table 2. The most

Table 2
Arithmetic mean ± S.D. and frequency of engorged females on 25 anatomical sites of 14 Charolais steers at sunrise (right side)

Zones	Sites	Arithmetic mean ± S.D.	Frequency for the site (%)
Protected from sun (P)	Belly	19.9 ± 22.1	14.5
	Lower perineum	3.8 ± 3.8	2.7
	Axilla	3.5 ± 4.3	2.6
	Internal hock and leg	2.7 ± 2.9	1.9
	Scrotum	2.0 ± 2.8	1.5
	Groin	1.5 ± 1.7	1.1
Exposed to sun (E)	Neck	39.1 ± 46.5	28.3
	Upper shoulder	17.1 ± 20.3	12.4
	Lower shoulder	7.3 ± 8.6	5.3
	Upper dewlap	6.4 ± 10.6	4.6
	Lower dewlap	6.1 ± 11.8	4.4
	Ribs	5.2 ± 6.3	3.8
	Flank	3.0 ± 4.0	2.2
	Lower leg	2.3 ± 3.1	1.7
	Withers	2.1 ± 3.1	1.5
	External hock	1.4 ± 1.8	1.0
	Upper leg	0.4 ± 0.9	0.3
	Tail	0.4 ± 0.7	0.3
	Back line	0.2 ± 0.5	0.1
	Upper perineum	0.2 ± 0.6	0.1
	Anus	0.0 ± 0.2	0.0
Semi-protected from sun (S)	Head with ears	9.5 ± 13.7	6.9
	Arm	2.4 ± 3.4	1.7
	Foreleg	0.7 ± 1.4	0.5
	Foot	0.6 ± 1.5	0.4
Total		137.8 ± 176.8	100

infested sites (80% of the total) were the neck (28.3%), the shoulder (17.7%), the belly (14.5%), the dewlap (9%), the head and ears (6.9%) and the ribs (3.8%). The 17 other sites were infested by less than 20% of all the engorged females.

3.2. Experiment 1: comparison of morning F8 burdens for steers kept in pens during the night and steers grazing on pastures and driven to the crush

In the early morning of days 2 (for group 1) and 3 (for group 2), after the six steers had spend the night in the pen, means of 11.7 ± 7.8 and 13.7 ± 10.1 F8, respectively, not significantly different ($P > 0.687$ with the Mann–Whitney test), were counted (Table 3). For these two consecutive days, means of F8 were also equal: 0.33 ± 0.5, for the six steers that spent the night in the pasture and were moved to the crush. For each day, the difference between the two treatments (pen versus pasture + 20 min walk) were highly significant ($P = 0.003$). These results indicate that morning burdens of F8 are significantly higher on steers that spent the night in a pen than on steers that graze on pasture and move to

Table 3
Experiment 1: arithmetic means and S.D. for engorged F8 female ticks counted early in the morning on 12 Charolais steers that spent the previous day and night in a pen (Pen) or in a pasture at a 20 min walk from the crush (Pasture P1)

Day	Group 1 (six steers)	Group 2 (six steers)
2	11.67 ± 7.79 a (Pen)	0.33 ± 0.52 b (Pasture P1)
3	0.33 ± 0.52 c (Pasture P1)	13.67 ± 10.09 d (Pen)

Significance denoted by alphabets (a vs. b), (a vs. c), (b vs. d) and (c vs. d): $P < 0.01$; (a vs. d) and (b vs. c): $P > 0.50$.

the crush. Effect of "pasture" and "walking", respectively, will be presented in the next experiments.

3.3. Experiment 2: comparison of F8 burdens for steers maintained in pens, constrained or not to walk for 20 min: detachment rhythm from different anatomical zones

- In the early morning (6:00 h) on day 4, the F8 burdens on the 12 steers of groups "pen" (P: 6 steers) and "walk" (W: 6 steers) who both spent the night in pens were similar (17.3 ± 15.0 versus 10.8 ± 7.0, respectively). The F8 presence rate on the steers of the P and W groups were also similar at 7:15 h before walk (86.9% versus 81.9%) (Fig. 1).
- At 8:30 h, shortly after the 20 min walk imposed on group W, the F8 presence rate on this group dropped significantly (37.7%) when compared to group P (75.2%) ($P = 0.013$). This rate was still significantly different at 11:30 h (14.1 and 36.5% for the groups W and P, respectively, $P = 0.043$) but the difference disappeared at 13:30 h (10.4 and 28.9% for the groups W and P, respectively, $P = 0.072$) and 15:00 h (7.0 and 16.4% for the groups W and P, respectively, $P = 0.119$). In brief, with the exception of the 7:15–8:30 h period,

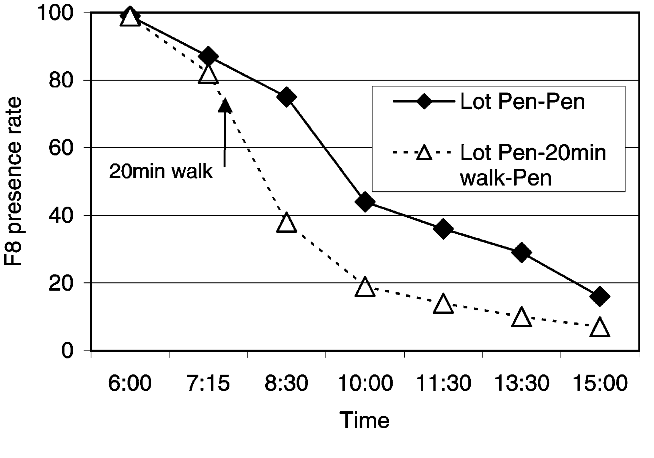

Fig. 1. Experiment 2: effect of a morning walk vs. no walk on the F8 presence rate. Group Pen–Pen: six steers spent the night and the next day in the pen; Group Pen–20 min walk–Pen: six steers spent the night in the pen, made a morning 20 min walk and returned to the pen (tick counts from 6:00 to 15:00 h).

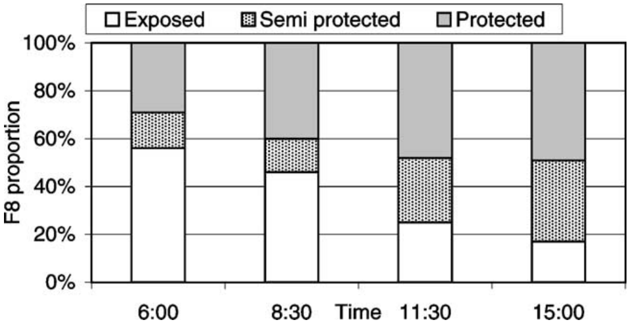

Fig. 2. Experiment 2: diurnal evolution of the distribution of engorged females on light-exposed, semi-protected and protected zones of the body ($n = 12$ Charolais steers).

shortly after the W group of animals was forced to move, producing an increase in the dropping of females, the instantaneous detachment rate was similar in the two groups.

- At day 4, the percentage of F8 on the 3 anatomical zones of the 12 steers and its evolution along the day were significantly different (Fig. 2). At sunrise (6:00 h), the sun-exposed sites bore 56% of the engorged females present on the whole body but females dropped rapidly from these sites and this rate decreased and reached 17% at 15:00 h. Considering these sun-exposed sites the 39% loss was more important for the six steers moved for a walk (60% at 6:00 h to 11% at 15:00 h, difference 49%) than for the steers kept in the pen (51–20%, difference 31%) (Fig. 3). To the contrary females remain longer on the semi-protected and the protected sites (Fig. 2). Consequently, the percentage of F8 on these two sites compared to the whole body increases of a magnitude of 18% (16% at

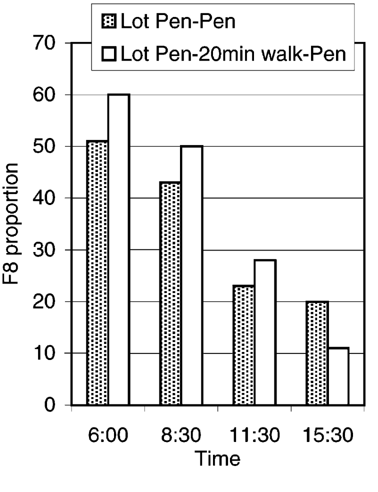

Fig. 3. Experiment 2: effect of a morning 20 min walk on the diurnal evolution of the distribution of engorged females on light-exposed zones of the body ($n = 12$ Charolais steers).

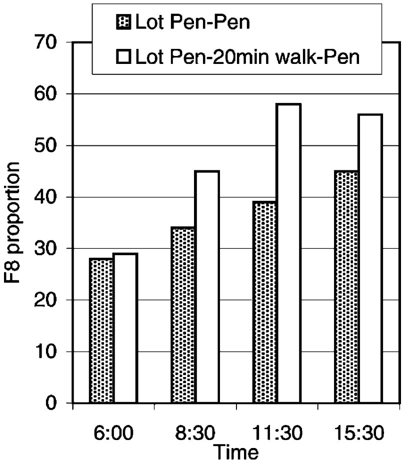

Fig. 4. Experiment 2: effect of a morning 20 min walk on the diurnal evolution of the distribution of engorged females on light-protected zones of the body ($n = 12$ Charolais steers).

6:00 h to 34% at 15:00 h) and 20% (29% at 6:00 h to 49% at 15:00 h—not significantly different) for semi protected and protected sites, respectively. The increasing rate of ticks on these zones during the day was accentuated when steers walk for 20 min (Fig. 4). These results indicate that the kinetic of detachment of engorged females (i) is dependent of the anatomical zones where they are attached, (ii) is more rapid for ticks attached on light exposed zones of the body and (iii) is facilitated by movements of animals.

3.4. Experiment 3: effect of the pasture, pen, moving and daytime on F8 counts and their contribution on the kinetic of detachment from sun exposed/protected anatomical sites

This last experiment completes the previous ones. The "pasture P2 group" is close to the pen and crush, resulting in a great reduction of the movement of steers grazing on the pasture or walking to the crush, in order to separate the "pasture effect" from the "movement effect" on the detachment rhythm. When treatments were repeated (i.e. treatments 1, 4 and 5 see Table 1), the results (expressed by sunrise F8 burdens, F8 presence and detachment rate, anatomical distribution) were similar for the 3 days. Consequently, the effect of the observation day was neglected. Then, the repetitions were pooled and the comparisons were made between the five treatments only.

- Considering the F8 burdens of the steers at the first 6:00 h sunrise control, there was no difference ($P = 0.353$) for the number of engorged females counted, whether the steers spent the night in the pasture or in the pens: 118.7 ± 116.9 ($n = 19$) and 153.7 ± 122.5 ($n = 23$) F8 per animal, on pasture and in pen, respectively, referred to as 100% of the F8 in Fig. 5.
- Considering the presence and the detachment rate of F8, there were significant and rapid differences (as soon as the second morning control), whether the steers spent the first 2 h of the day in the pasture (treatments 2 and 5) or in the pens (treatments 1 and 4) and whether

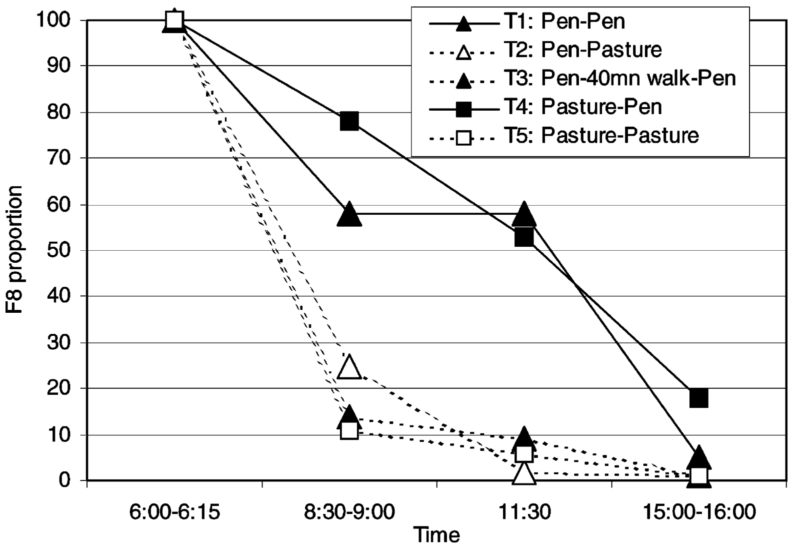

Fig. 5. Experiment 3: effect of different treatments on the F8 presence rate during the day (14 steers per treatment).

the steers spent the night in the pasture (treatment 4 and 5) or in the pens (treatments 1 and 2). In these two situations, all the steers were directly exposed to morning sun and allowed to feed with hay (pen) or grass (pasture).

- Regarding F8 burdens, presence rate and detachment rate, treatment 3 which allowed the "pen" steers to move for a limited period (pen–40 min walk–pen), produced the same effect as treatments 2 (pen–pasture) and 5 (pasture–pasture) in which steers spent the first 2 h of the day on pasture (Fig. 5).
- According to the results of experiment 2, the repartition of the engorged females and their percentage on the three anatomical zones were significantly different over the day. During the day, the sun-exposed sites began with 60% of the F8 at 6:15 h to finish with 45% at 16:00 h. At the second control, this drop-off was significantly more important for the steers in day pasture (treatments 2 and 5) than for those in the day pen (treatments 1 and 4). The treatment 3 (pen–40 min walk–pen) was intermediate between the two others. At the third and fourth control, there were no more significant differences between the F8 repartition on anatomical sites related to the treatment.

On the contrary, the sites protected from the sun increased their percentage of F8 ticks (31% at 6:15 h to 44% at 16:00 h). The semi-protected sites held the same ratio of F8 all during the day (9% at 6:15 h and 11% at 16:00 h).

4. Discussion

The whole body surface of cattle is suitable for *B. microplus* attachment. However, some sites are more infested than others. Preferred attachment sites identified in this study were those classically observed and reported in the literature for this genus: neck, shoulder, belly, dewlap (Macleod et al., 1977; De la Vega et al., 1984). These sites are those where to focus one's attention when ticks have to be collected for various purposes including implementation of acaricide resistance tests. Considering the morning abundance of these easily visible stages on these exposed sites (particularly neck and shoulders), they may also be used by farmers as an indicator of the tick burden of the herd, and a signal to decide on the timing of acaricide treatment.

During each of the five nychthemeral cycles of observation in this current study, and according to literature (Wharton and Utech, 1970), engorged females were present on animals mostly from late in the night to the next midday, and detached mainly in the early morning. Using stalled animals, has already shown that the peak (34.2%) of drop-off of engorged females occurred between 6:00 and 10:00 h.

Our first experiment with steers penned at night on the one hand or in pasture on the other confirmed farmers and field technicians statement: when inspected in the early morning, the cattle that spent the night in the pasture and moved from the pasture to the stock-yard were dramatically less infested with engorged females (40 times less, Table 3), than a control group that spent the night undisturbed in a pen.

On suspecting the role of animal movement in the F8 detachment pattern, it has been stated that "the host activity may have been one factor influencing the time of the peak tick fall" and "the resumption of activity after nocturnal resting of cattle in pasture may produce the increased early morning fall of engorged ticks". Wharton and Utech (1970) observed that female ticks that reached a certain size (4–6 mm) during the day became fully engorged during the next night and drop early in the morning. Furthermore, they reported that the pattern of engorgement and dropping varied depending of the season and the place where cattle were held, and thought that environment (temperature, light, covered stalls versus open yards) should be related with this phenomenon. These authors supported the suggestion from Kitaoka (1962), that the sunshine stimulus played a predominant role in the dropping of engorged females.

Our second experiment, aimed at distinguishing a physiological effect of "pasture" related to feeding activity, from a more physical effect induced by walking, suggested that the movement of the steers by itself is one of the major factors that stimulates the detachment of the ticks. If steers were maintained in a pen at night, females detached more rapidly from the sub-group of steers that were forced to move a few minutes in the morning than from the other sub-group maintained undisturbed in the pen, confirming that it is not the pasture by itself that plays a role in the decreased number of ticks.

The use of an adjacent and small pasture, limiting steers movements as much as possible in our third experiment, allowed also to distinguish the effect of pasture on one hand from the effect of a walk on the other. In these conditions, whether the steers spent the night in the pen or in the pastures, the F8 burdens were similar at sunrise but decreased much faster in the groups that graze or walk shortly after sunrise compared with the groups that remained in the pens. Moreover, the detachment rate induced by host activity appears to be time-related: in our experiments, the effect of a 20 min walk was intermediate between no walk and a 40 min walk.

Considering the F8 presence rate, the place where the cattle were kept from sunrise till the end of the day is a major explaining factor which differentiates the pasture "model" from the pen "model". The feeding activity of the host does not explain the differences between the two models unless we consider that eating hay (in the pen) or grass (in the pasture) should imply different consequences in the feeding process itself or in the blood components of the animals. The influence of the time the cattle (confined in a stall), are fed has been suggested by the study of George et al. (1998) on *B. annulatus*. Feeding activity effect was not obvious in our experimental device, and due to the fact that pen and pasture animals were all in external surroundings, the daylight which is considered important by Kitaoka (1962), did not make a difference in both situations on female detachment.

However, the implication of external stimuli was clearly demonstrated in our experiments. The intensity of light received by particular sites of skin (in addition with the females which are attached on them), appears to play an important role in the detachment kinetics and modulate greatly the effect of movement. Various trials showed that morning detachment is faster for engorged females attached on light/sun-exposed sites than for females attached on protected sites. Moreover, animal activity has a selective effect on the detachment of females from these sites: whatever the experiment, movement induced a more rapid drop-off of females attached on exposed sites than on those attached on protected sites. It can be concluded that detachment is the conjunction of internal (tick) factors leading to complete engorgement of the female during the night, external host stimuli materialized by resumption of cattle activity in the early morning and external environmental stimuli related to light intensity on engorged ticks and/or surrounding skin.

The understanding of the appropriate way to accelerate the detachment rate of engorged ticks could have a practical interest for dairy cattle by cleaning up the cows before they return to pasture: this could avoid a too important infestation without the use of chemicals or vaccines.

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