



Optimizing long-acting acaricide use for integrated tick management of *Rhipicephalus australis*-infesting cattle in New Caledonia

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Abstract

The tick *Rhipicephalus australis*, formerly known as *Rhipicephalus microplus*, is the most economically important ectoparasite of livestock in New Caledonia, affecting cattle health and production. Decades of control attempts based on the application of chemical acaricides have exerted a strong selective pressure on *R. australis* populations, some of which have evolved resistance to these treatments. Research to develop integrated tick control programs is now focused on decreasing applications of chemicals. This study reports the implementation of a method of pasture and herd management involving minimal strategic use of long-acting acaricides, here defined as those having substantial efficacy against larvae for several weeks. Diverse parameters concerning the utilization of long-acting acaricides in association with pasture and herd management on 21 New Caledonian farms over a 5-year period were analyzed to optimize their strategic use. Longer larval acaricidal effect was achieved with a commercial pour-on formulation of fluazuron than with a commercial injectable (subcutaneous) formulation containing 3.15% ivermectin. Pasture and herd management allowed an increase in the delay between a long-lasting acaricide application and the subsequent acaricide treatment from 11.0 weeks to 17.7 weeks. However, if ticks were detected and reported by producers on the day of a long-acting acaricide application, the delay to the following treatment was reduced from 18.5 weeks to 11.2 weeks. The impact of a long-acting acaricide treatment on larval populations in pastures was greatest with a stocking rate of 5 animals per hectare grazing during 1 week. These results provide science-based evidence to cattle producers for adaptive integrated tick management in order to delay the development of acaricide resistance.

Keywords Cattle tick · *Rhipicephalus australis* · Long-acting acaricides · Integrated tick management · New Caledonia

Introduction

Ticks are economically important ectoparasites of cattle that affect their health and production directly through their parasitic lifestyle and indirectly as vectors of

pathogens causing animal diseases, several of which are zoonotic and of veterinary public health relevance (Pérez de León et al. 2020). In New Caledonia, the cattle tick *Rhipicephalus australis*, previously known as *Rhipicephalus microplus* (Estrada-Peña et al. 2012), causes major health

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problems among cattle herds (Hüe et al. 2017). Since its introduction to New Caledonia from Australia during the second world war, synthetic acaricides have been provided free of charge to cattle producers by the Caledonian Government to treat cattle infested with *R. australis* (Bianchi et al. 2003). However, the intense application of these types of veterinary drugs to treat infested cattle caused selected populations of *R. australis* to become resistant to them (Beugnet and Chardonnet 1995). Thus, the choice of acaricides provided by the Government is determined by the detection of widespread resistance to a chemical class of veterinary drugs.

Tick management was initially supervised by veterinary official services, but since 2010, it has been supervised by a producer association: the Groupement de Défense Sanitaire Animal (GDSA). Acaricides in a single chemical class were generally used during a decade before resistance appeared, after which acaricides from a different chemical class have been used. Amitraz is an acaricide belonging to the formamidine chemical class that has been used by all Caledonian breeders since 2003. Antiparasitic drugs of short-action containing the macrocyclic lactones ivermectin and moxidectin, which are used to treat cattle infested with ticks because of their endectocidal activity (Rodríguez-Vivas et al. 2014), are also used once a year when cattle breeders want to treat against internal parasites. A survey conducted in 2014 documented that 25% of *R. australis* strains were resistant to amitraz (Petermann et al. 2016). However, this acaricide is still used, even in farms where acaricide resistance is known, and some of these farms were included in the present study.

The tick control program proposed by the GDSA is based on adaptive integrated management of herds and pastures to reduce tick infestations, and includes the use of long-acting acaricides (LAA). Herein, LAA are defined as veterinary drug products with a chemical acaricide as the active ingredient(s) having lasting efficacy against cattle ticks, including *R. australis* larvae, sustained for several weeks (Davey et al. 2011; Nava et al. 2015). In New Caledonia, the rational use of LAA has not yet been fully adapted to enhance integrated tick management and allow use of desirable beef-producing European cattle breeds. Thus, resistance to the widely used acaricides is forcing New Caledonian cattle producers to change herd genetics away from more tick susceptible European breeds (Petermann et al. 2016). The genetic transition of cattle herds in New Caledonia is accomplished by introducing bulls from breeds with known tick-resistant traits (Hüe 2019). Within a herd, the complete transition to a tick-resistant cattle herd occurs over a 5- to 7-year period. LAA are mainly used to help breeders control tick populations during this herd transition period. In contrast to non-residual acaricides, LAA can be particularly useful when treated cattle are grazing in tick-infested paddocks and collect and kill tick larvae.

Two LAA currently used in New Caledonia are (1) a commercial injectable (subcutaneous) formulation containing 3.15% ivermectin, which has been in use since 2005 (note that short-action ivermectin is used at 200 µg/kg of body weight and the pharmacology of LAA with ivermectin is 630 µg/kg of body weight), and (2) a commercial pour-on formulation of fluazuron, which has been used since 2008. These LAA are applied only by GDSA technicians as part of official management plans. These products are not accessible over the counter for producers. Research on the strategic application of LAA in New Caledonia is needed because their improper use can select for resistant populations of *Rhipicephalus* spp. cattle ticks, as has been documented elsewhere (Rodríguez-Vivas et al. 2018; Reck et al. 2014). Thus, the integrated use of LAA with other approaches like pasture management can enhance biosecurity, for example, against *R. australis*, by mitigating the risk of moving cattle infested with acaricide-resistant (amitraz-resistant) ticks within and between farms (Miraballes et al. 2019).

This study reports the analysis of field observations registered by cattle producers during and after treatments with LAA. Efficacy data were considered according to the circumstances under which the LAA were applied. Although resistance to macrocyclic lactone products used for their endectocidal activity has not been reported in New Caledonia (Petermann et al. 2016), the information reported herein provides an empirical basis for reduced and more effective use of these antiparasitics, which could delay the development of resistance not only to ticks but to gastrointestinal nematodes (Alegría-López et al. 2015). Resistance to fluazuron among *R. australis* populations remains to be assessed in New Caledonia.

Materials and methods

Study area

New Caledonia is a French island in the South Pacific Ocean with an oceanic tropical climate (Petermann et al. 2016). The cattle population consists of ≈80,000 animals, which are raised under extensive conditions by ≈650 producers (Hüe 2019). Due to poor soil quality, the average stocking rate is ≈0.5 head per hectare. The hot and wet season is usually from late January to April, followed by a cold season from May to August, with possible rain. The dry season is from September to December. Producers monitored in this study are located on the West Coast, where annual rainfall ranges from 600 to 1200 mm. Daily mean temperatures are 27–28 °C during the hot season and 20–22 °C during the cold season.

Tick and pasture management

The method to control *R. australis* through herd and pasture management was developed by the Agronomic Institute of New Caledonia (IAC) in 2015 as described by Hüe and Fontfreyde (2019). This approach was transferred from IAC to the technicians of GDSA in 2017. At the end of 2019, 33 producers were monitored by the staff of this service. This method requires maintaining a calendar of herd movements among paddocks, as well as detection of on-host ticks and tick treatments within each paddock. Considering the duration of off-host tick development, this allows reliable estimations of when and where tick larvae were present in paddocks. It was thus possible to predict which paddocks would be infested by tick larvae. With this prediction, a LAA treatment was planned to maximize the collection and killing of larvae by treated cattle. The ivermectin (IVM) injectable product (IVOMEK GOLD®, Boehringer Ingelheim Animal Health) and the commercial fluazuron (FLZ) pour-on formulation (Acatak®, Elanco) were used per label instructions.

The policy in New Caledonia is to use LAA as infrequently as possible, and never more than two times per year. When LAA were first used in New Caledonia, their lethal activity on tick larvae was expected to be 6 weeks (Citroni et al. 1999; Ferlat 2004). Once the LAA treatment was completed, the planning of rotations was established by a technician in consultation with the producer to rotate treated cattle among the infested paddocks during the expected period of LAA efficacy. Initially, advice for post-LAA treatment rotations was not always given or was given orally and unevenly followed. However, since 2018, this advice has been transmitted in writing by technicians directly to the producer. After the subsequent tick treatment, technicians collected information about actual herd rotations to determine whether the advised rotation plan had been followed. From January 2015 to December 2019, 85 LAA treatments were recorded on 39 herds present in 21 farms (Table 1). These 85 treatments included the 29 for which rotation plan was written and the 56 for which rotation plan was not specified or specified orally. The choice of the product applied depended on several factors. For example, the long withholding period of the FLZ-LAA on calves was considered when lactating cows were treated. Therefore, this product was avoided if producers planned to slaughter calves in the next 4 months. Furthermore, alternating use between FLZ-LAA and IVM-LAA was considered to delay the development of resistance. If rain was forecasted, injection of IVM-LAA was preferred rather than a pour-on application of FLZ-LAA.

Indicators of treatment efficacy

The first indicator was the minimal delay between the LAA treatment and the following tick treatment with amitraz.

Table 1 Characteristics of the 21 New Caledonian farms and 39 herds on which 85 LAA treatments were recorded from January 2015 to December 2019

	No. herds per farm ^a	Surface area per herd (ha) ^b	No. parcels per herd ^c	Stocking rate ^d
Mean	2.1	186.8	5.5	0.7
Maximum	8	769.0	13	2.4
Minimum	1	8.5	2	0.2
Median	1.0	111.0	5.0	0.6

^aHerd represents a group of cattle that rotate through pastures together; two herds do not rotate in the same pastures

^bSurface area (hectares) used per herd

^cNumber of fenced pastures through which cattle can rotate

^dNumber of animal per hectare

Breeders were free to decide when they wanted to apply this following treatment. There was no threshold of infestation recommended to treat, and treatments were usually done when adult ticks were detected. The average delay between the LAA treatment and the following tick treatment was then analyzed considering several factors that can influence the reinfestation of animals. These factors included which product was used, whether technical advice was followed by producers, and whether producers observed adult ticks the day of the LAA treatment.

The last indicator was related to the length of grazing time needed to collect a maximum number of larvae in a plot, which depended on the stocking rate. This approach aimed to know how long a herd treated with a LAA had to stay in a paddock to decrease larval infestation low enough to avoid reinfestation of animals when cattle were moved back into the paddock. Assessment of this indicator required that a paddock was pastured during the period of activity of the LAA, and pastured once more by the herd just after the end of the expected efficacy of the treatment. If cattle were treated 2.5 to 3 weeks after their second stay in the pastured paddock, the stocking rate and/or the length of stay was considered insufficient to collect enough larvae during the first stay. If animals were not treated, the stocking rate and the length of stay in the paddock under LAA activity were considered sufficient.

Statistical analysis

Because the data were not normally distributed and the samples were small, a nonparametric test, Mann–Whitney *U* test, was used to compare (1) the minimal periods between a LAA treatment and the following tick treatments with FLZ-LAA and IVM-LAA; (2) the effect of adaptive herd rotation on the period between LAA application and the following tick treatment; (3) the effect of presence of ticks the day of LAA treatment on the period between LAA application and

the following tick treatment; and (4) the effect of stocking rate on length of stay required to collect larval ticks under LAA. The stocking rate was calculated as the ratio between the total number of animals and the surface area of the paddock. To compare the different situations, the length of stay per paddock under LAA activity was divided by seven to obtain a “week equivalent.” A stocking rate equivalent of a week of pasture was calculated with the following formula: stocking rate per week = (number of cattle/surface in ha)/(length of stay in days/7). This value was then aggregated into 4 classes: > 3, > 4, > 5, > 6 head/ha/week. Depending on whether or not a treatment was necessary for a herd staying in a certain paddock, the estimation of pasture infestation for cattle to be exposed to larvae described above was considered as efficient or not. Percentage of efficiency and inefficiency for each class was then calculated. All tests were performed under the $\alpha = 0.05$ significance threshold.

Results

Minimal period between LAA treatment and the following tick treatment

Among the total 85 treatments, 38 treatments with FLZ-LAA and 47 treatments with IVM-LAA were registered. Minimal delays between a treatment with FLZ-LAA and IVM-LAA and the following tick treatment were 6 weeks and 7 weeks, respectively (Fig. 1). Since the development of *R. australis* on cattle from larva to adult requires ~ 3 weeks, it was assumed that cattle were infested by larvae 3 weeks earlier. Thus, the minimal periods of larval efficacy between a treatment with FLZ-LAA and IVM-LAA and the following larval infestation were estimated at 3 weeks and 4 weeks, respectively. However, except for one treatment observed within 6 weeks after an FLZ-LAA application, 11% and 8%

of the following tick treatments occurred 8 and 9 weeks, respectively, after LAA application. After an injection of IVM-LAA, 11%, 9%, and 19% of the following tick treatments occurred within 7 weeks, 8 weeks, and 9 weeks, respectively. An observation of particular biological relevance was that within 9 weeks after initial LAA treatment, follow-up treatment was required for 21% of the cases after FLZ-LAA pour-on application and for 38% of the cases after prior subcutaneous injection of IVM-LAA. However, the periods between treatment and the following tick treatment within these 9 weeks were not significantly different between the LAA tested (Mann–Whitney *U* test, $U = 55$, z -score = 0.4539, $P = 0.3264$).

Effect of adaptive herd rotation on period between LAA application and the following tick treatment

Beginning in 2018, written technical advice with recommended pasture rotations was provided to producers and compared afterward with the effective rotations for 29 LAA treatments. Advice was followed for 18 (62.1%) of these treatments. Some producers did not follow the advice because it did not correspond to their expectations or because the forage resources in the pastures did not allow them to rotate the animals. Or, conversely, some producers did not want to remove animals from a parcel with unutilized forage resources just to follow the recommendations. The average period between the LAA application and the following tick treatment was 11.0 ± 5.3 weeks when advice was not followed and 17.7 ± 12.6 when advice was followed (Fig. 2). Following recommendations for the rotations after a LAA application significantly increased the period before the following tick treatment (Mann–Whitney *U* test, $U = 51.5$, z -score = 2.1125, $P = 0.0174$). This technical advice was critical to extend the average period until the next treatment

Fig. 1 Distribution of minimal period between initial and the following tick treatment with commercial long-acting acaricides consisting of an ivermectin injectable formulation (IVM-LAA) or a pour-on fluzuron formulation (FLZ-LAA)

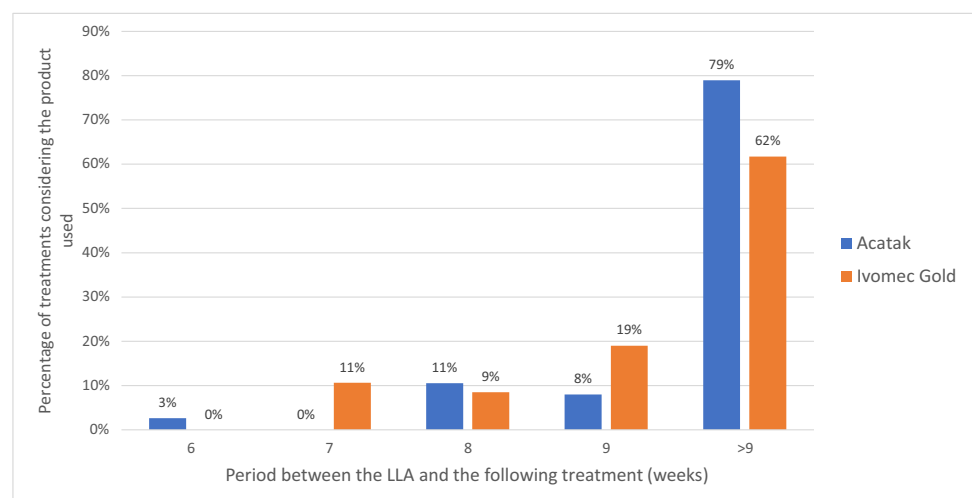
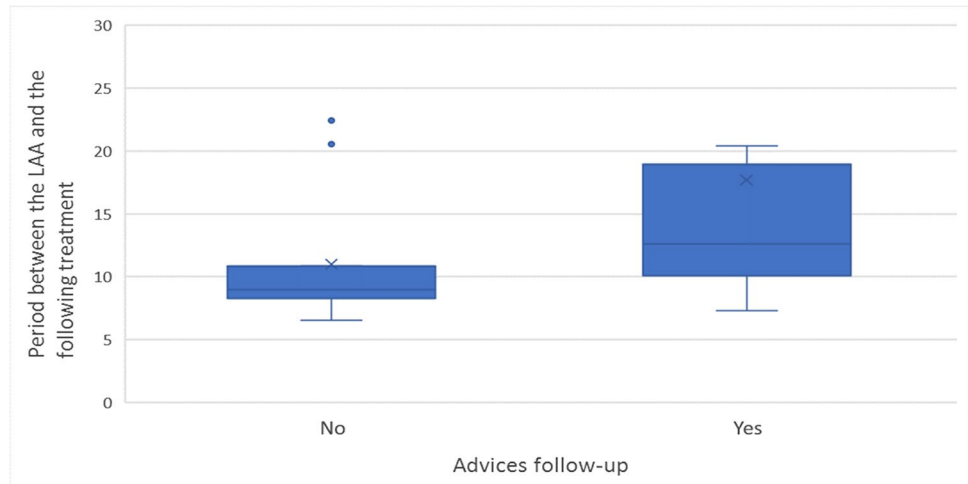


Fig. 2 Period measured in weeks between LAA application and the following treatment when considering or not the technical advice provided



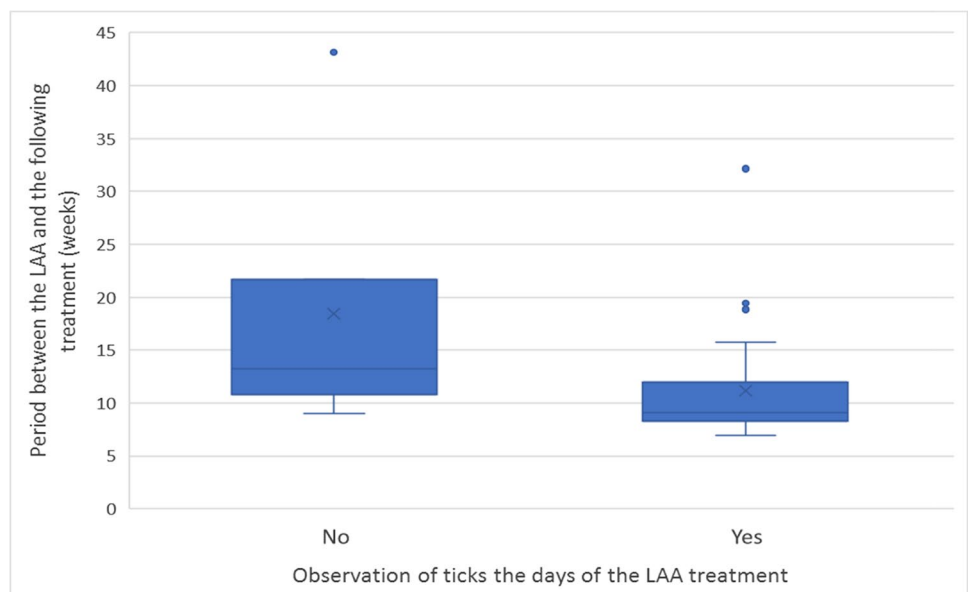
by more than 6 weeks. This approach helps to predict when and which paddocks are likely to be infested by tick larvae. Thus, strategic LAA treatment can be applied when the largest number of paddocks is infested. The aim of this strategy is to adapt rotations after LAA application to collect and kill as many larvae as possible during the period of LAA efficacy. If a herd has not grazed in an infested paddock during the period of LAA efficacy, tick larvae will be present and cattle that come into the paddock soon after the end of the efficacy period will have to be retreated because they are parasitized rapidly.

Effect of tick detection the day of LAA treatment on period between LAA application and the following tick treatment

Of the 34 LAA treatments for which information on the presence or absence of *R. australis* on cattle the day of the application was recorded, no ticks were reported for 7 treatments and ticks were present for 27 treatments. The period between LAA treatment and the following tick treatment was 11.2 ± 5.3 weeks if ticks were observed and 18.5 ± 11.7 weeks if no ticks were observed (Fig. 3). The difference between the two periods was statistically significant (Mann–Whitney *U* test, $U = 37.5$, z -score = -2.4064 , $P = 0.0080$).

The distribution of cases according to tick observation the day of the LAA treatment and compliance with the

Fig. 3 Period measured in weeks between LAA application and the following treatment considering tick detection the day of LAA treatment



recommended rotation schedule is presented in Table 2 when data were available in both cases. Among these 34 LAA treatments, recommendations concerning pasture rotations were followed for 21 cases (61.8%). For these 21 situations, the period between LAA treatment and the following treatment was 11.4 ± 5.9 weeks, whereas it reached 21.3 ± 14.9 weeks when ticks were not observed the day of the LAA treatment and advice was followed.

Based on the 34 LAA treatments for which information on the presence or absence of *R. australis* on cattle the day of the LAA was recorded.

This suggests that farmers who understand the principle of tick management through rotations and correctly anticipate the date of the LAA are also the most likely to follow the recommendations after LAA treatment.

Stocking rate effect on length of stay required for cattle treated with LAA to be exposed to *R. australis* larvae in infested pasture

There was a tendency (Mann–Whitney *U* test, $U = 16$, z -score = 1.2374, $P = 0.1075$) for higher stocking rates to have a more efficacious outcome than lower stocking rates based on the 15 LAA treatments (Table 3). Rotations post-LAA treatments were planned to collect larvae in all paddocks considered infested during the period of acaricide efficacy. Rotations and lengths of stay per paddock were defined considering the number and the size of the paddocks. Higher weekly stocking rates resulted in more efficient collection of larvae. A stocking rate of 6 animals per hectare per week under our conditions enhanced the collection of

larvae. Efficiency (percentage of cattle with ticks) falls to 86% with 5 animals per hectare per week. A higher stocking rate requires herds to remain longer in paddocks. However, if several paddocks are infested, sufficiently long stays are not always possible given the expected 6 weeks of initial LAA efficacy. Since the usual stocking rate of around 2 ha per animal is relatively low in New Caledonia, sufficiently long stays in paddocks are problematic. Furthermore, among the 13 situations where the stocking rate was less than 6 animals/ha/week, the collection of larvae was nevertheless efficient to avoid a reinfestation of cattle in 7 cases (54%). This percentage decreased to 38% in the 8 cases when the stocking rate was less than 5 animals/ha/week. But, it increased to 50% in the 6 situations where the stocking rate was less than 4 animals/ha/weeks. The stocking rate of 5 cattle/ha/week appears to be a reasonable compromise between animal production considerations restricting stocking rate and length of stay considerations allowing sufficient collection of larval ticks by herds treated with LAA.

Efficacy represents the percentage of times animals did not need to be treated when re-grazing to a previously infested plot. Numbers in parentheses denote number of cases.

Discussion

A major finding of this study is that the actual time between treatments when the two LAA were tested under field conditions was 6 to 8 weeks. When they were first used in the Caledonian tick control program, the FLZ-LAA and IVM-LAA commercial formulations were expected to help manage population of *R. australis* larvae for 6 weeks. This means that tick detection by producers and tick treatments should not occur before 9 weeks after the previous LAA treatment, considering the ~3-week period for larvae to reach the adult stage on infested cattle. Results reported here for the LAA evaluated also contrast with previous findings in that the period of efficacy was expected to be longer for FLZ-LAA than for IVM-LAA. Whereas the label claim for the IVM-LAA product tested mentioned a control period against species of *Rhipicephalus* (*Boophilus*) ticks affecting cattle of up to 75 days (i.e., 11 weeks), other studies reported a

Table 2 Distribution of cases according to tick observation the day of the LAA treatment and compliance with the recommended rotation schedule

Recommended rotation schedule followed	Ticks observed the day of the LAA treatment		
	No	Yes	Total
No	3	21	24
Yes	4	6	10
Total	7	27	34

Table 3 Impact of weekly stocking rate on *R. australis* larvae collection after a LAA treatment in an infested pasture

Stocking rate per week greater than	Efficient when the stocking rate was greater than	Stocking rate per week less than	Efficient although the stocking rate was less than
3 (14)	57% (8)	3 (1)	100% (1)
4 (9)	67% (6)	4 (6)	50% (3)
5 (7)	86% (6)	5 (8)	38% (3)
6 (2)	100% (2)	6 (13)	54% (7)

shorter control period. Davey et al. (2010) reported control of *R. microplus* larvae during 14 days after an injection of this IVM-LAA, and larvae released on cattle for artificial infestation at 28-d posttreatment produced considerably larger engorged ticks than those released at 14-d posttreatment. Gomes et al. (2015) reported 80% efficacy after 4 weeks of treatment with the same IVM-LAA product, which decreased to 62.0% at 5 weeks posttreatment. By comparison, Bull et al. (1996) observed that practically no adults were recorded up to 12 weeks after a treatment with the FLZ-LAA pour-on applied at 1.5 mg/kg of bodyweight (BW). Mendonça (2010) reported a residual acaricidal effect superior to 90% during 13 weeks posttreatment with FLZ-LAA 2.5 mg/kg BW, while Benavides et al. (2017) observed semi-engorged females 8 weeks after treatment with FLZ-LAA. A possible reason for the lower persistence effect of LAA in some cases (on some farms) might be that the spread of these chemicals within cattle is fat-dependent, so a shorter time of efficacy could be related to poor body conditions in the dry season. Another possible explanation might be that the date of the following treatment depends on the rhythm of cattle observations by the producer. Thus, the producer can apply this following tick treatment as soon as he observes the first ticks if he is every day in the herds or it could occur some days later if he just observes herds once or twice a week. However, due to the small number of observations, it was not possible to rigorously explore the impact of the season or rhythm of observation.

This study also identified areas to advance integrated *R. australis* management practices in New Caledonia. Our results confirmed that producers must practice biosurveillance for infestations before the end of the expected initial period of efficacy to enhance coordination with tick program technicians to adapt the application of LAA (Wang et al. 2020). Technical advice to producers also needs to be redefined taking these new data into account. The short period we observed between LAA application and the following tick treatment underscores the potential for the development of acaricide resistance and other factors that could affect overall efficacy. No resistance to IVM has been detected thus far through the macrocyclic lactone bioassays conducted routinely at the IAC laboratory (Hüe 2019). However, it has been documented that the use of IVM-LAA to treat gastrointestinal infections is a predisposing factor selecting for IVM resistance in tick populations infesting cattle (Alegria-López et al. 2015). This also underlines the need to adapt a bioassay to assess resistance to fluzuron in New Caledonia because even though its use on cattle is limited to a maximum of once per year and usually it is applied only once every 2 years, cattle ticks can become resistant to this chemical acaricide (Reck et al. 2014).

Our results highlight the importance of pasture monitoring before and after a LAA treatment to know when and

which paddocks are infested by tick larvae. Because substantial numbers of engorged ticks are known to drop off cattle early in the morning (Hitchcock 1955; Wharton 1970), detecting infested cattle on the day of LAA application indicates some ticks already fell into the paddock. In New Caledonia, the period between dropping-off of engorged ticks and the hatching of eggs varies from 6 weeks during the hot season to 10 weeks during winter (Desquesnes and Vignon 1987). As observed in this study under natural conditions, the efficacy of LAA treatment is less than 6 weeks. If engorged ticks have fallen into the paddock the day of LAA treatment, the acaricide will not be effective when eggs hatch and larvae become present in the paddock. Therefore, animals will be reinfested as soon as they pasture in these newly infested paddocks and will need to be treated once more within a few weeks after LAA application. By contrast, if no engorged ticks are observed the day of LAA treatment, assuming the paddock where animals come from is free of ticks and that posttreatment rotations have been correctly planned, this increases the likelihood of efficient collection of larvae by grazing cattle from the other paddocks. Knowing this and considering the population dynamics of *R. australis* in New Caledonia, if tick detection is reported by the producer, then program technicians can recommend to treat first with a short-acting acaricidal product, for example, an amitraz spray or macrocyclic lactone pour-on (Hüe and Fontfreyde 2019). Doing this delays LAA treatment as long as possible and avoids the application of these products to cattle when ticks were not detected infesting them.

This study helped optimize the use of LAA for integrated *R. australis* management by combining information on the use of pastures under New Caledonia conditions (Hüe and Fontfreyde 2019). Since our results were based on the observations of producers, we acknowledge the potential bias. Nonetheless, our results confirmed that the period of efficacy against larvae for the LAA tested in this study can be less than 6 weeks. Importantly, collaborating with cattle producers, we demonstrated the importance of using LAA not only retroactively to treat tick infestations, but also pro-actively during strategic periods before infestation to reduce larval populations in pasture. This decreased the overall number of treatments required to manage *R. australis* populations. Pro-active use of LAA is only possible if producers maintain a calendar noting their herd rotations and tick observations to know when and where larvae were present in paddocks to make informed decisions that anticipate future tick infestations.

Information generated through this study can be used to develop guidance concerning stocking rates to ensure efficient larval collection in pasture after LAA treatment. The stocking rate of 5 cattle/ha/week recommended in this study is also consistent with Sutherst et al. (1977) who concluded that a stocking rate of 5 cattle per hectare picked up between

50 and 85% of the tick larvae in a week compared with 30 to 70% with two animals per hectare.

All of these results are directly relevant to extending science-based evidence to producers for adaptive integrated tick management. This approach can now be consolidated with other livestock husbandry studies to advance cattle breeding under changing agroecological conditions.

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Author contribution TH conceived and designed research, wrote original draft, and revised manuscript. TH and CF conducted experiments and collected data. HHW and WEG analyzed data and revised manuscript. PDT and APdL reviewed the manuscript. All authors read and approved the manuscript.

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Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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Consent for publication Not applicable.

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