



## Update of the Resist project: An *in vitro* test for the assessment of ticks resistance to acaricide soon available in the Caribbean !

### EDITO

By Jennifer PRADEL (veterinary epidemiologist, CIRAD Guadeloupe) chair of the Ticks & tick-borne Diseases (T&TBD) working group of CaribVET

Already 1.5 year that the project started! Despite ambitious objectives and a number of challenges (material, financial, human resources, regulatory, administrative and several other organizational constraints), participants in the project managed to organize themselves to find alternatives, always with a view to improving ticks and tick-borne diseases control in the Caribbean, for the benefits of the farmers and of the cattle and small ruminant industries in the Caribbean.

Most important achievements so far include the ***in vitro* test for ticks resistance assessment** which is in its final validation phase at CIRAD Animal health laboratory (Guadeloupe) and which will soon be used for first testings; **finalized protocol** for the field evaluation of acaricide efficiency; the **conclusion of the feasibility studies** dealing with the pilot projects in Martinique and Nevis; organization of several **sensitization campaigns for farmers on ticks resistance** in Martinique; several exchanges with other regions experiencing similar issues and the finalization, testing and validation of the **CaribVET survey on Tick-borne diseases status and acaricide use in the region** by the Ticks and Tick-borne diseases working group! Other activities involving the veterinary services of Barbados, St. Vincent and the Grenadines, Ste. Lucia, Dominica, Antigua, the US virgin Islands, Guadeloupe and Martinique dealing more with **surveillance activities** of Tropical Bont Tick and tick-borne diseases, also started recently. They will be detailed in the 3rd issue of the "Resist" newsletter.

**The 1st year of the project required significant amount of preparatory work and energy** for the conduct of those activities most of which are new to the Caribbean and to the persons involved. This highlights the importance of **sharing experience and lessons-learned** with other regions, especially with New Caledonia who has been working on the issue of resistance for more than 10 years (see picture & legend below), and collaborating with experts, and other disciplines, always in close **interaction with decision makers, representatives of animal industries and farmers**. This was especially key for the implementation of the new *in vitro* test for the assessment of tick resistance (p.2) and for the feasibility of the pilot project of Martinique - aiming at studying the use of an anti-tick vaccine against Cattle tick as an alternative tick control strategy, and of Nevis - aiming at studying the use of natural predators (chickens or guinea fowls) for the control of the Tropical Bont Tick.

All activities of the project will be reviewed during the **2nd Meeting of the Resist project / 4th Meeting of the CaribVET T&TBD WG**, which will be held in Barbados, 18-20th November 2014. (More information on the meeting: [caribvet.net](http://caribvet.net))

Enjoy your reading, and warm thank to project partners & for their motivation & excellent input to the project & newsletter!



Credit: CIRAD  
Visit of Thomas Hue from the IAC (Institute of Agronomy of New Caledonia) at the research center INRA-CIRAD in Guadeloupe, 1-3 Oct. 2014  
Left to right: C. Marie Magdeleine, M. Mahieu, S. Depraz, T. Hue, M. Naves and J. Pradel.

# Resist Newsletter

## Second Edition

November 2014

### SUMMARY



Members of CIRAD CMAEE and collaborators (left to right : Nidia Cangi (University of Maputo), Nathalie Vachieri, Candice Sant (UWI) and Adela Chavez) attended the **12th Biennial Conference of the Society for Tropical Veterinary Medicine (STVM) and the VIII International Conference on Ticks and Tick-borne Pathogens (TTP-8)**, held from 24 to 29 August 2014 in Cape Town, South Africa.  
More information : <http://www.savetcon.co.za/TTP8/index.php> ;  
<http://www.caribvet.net/en/meeting/internationalandregionalmeetings> ;  
<http://epigenesis.avia-gis.com/node/354>

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## Event : The Larval Tarsal Test: an innovative and promising test to assess acaricide resistance in the Caribbean region

By Stéphanie DEPRAZ (project manager, CIRAD Guadeloupe)

*The Larval Tarsal Test (LTT) was developed in Switzerland by Novartis in 2011, in order to evaluate the resistance status of cattle ticks, Rhipicephalus (Boophilus) microplus, to acaricides. It is currently being implemented in the CIRAD animal health laboratory in Guadeloupe, as part of the "ResisT" project, with a view to assess the situation of Cattle tick resistance to acaricides in the French Antilles, and in the Caribbean region.*

The control of cattle ticks, *Rhipicephalus (Boophilus) microplus*, in the world is essentially based on chemicals. However resistance to acaricides is reported worldwide in many tropical/subtropical countries, leading sometimes to very difficult situations when farmers cannot use any acaricide to control tick populations. It is therefore essential to assess the resistance status of ticks in order to help farmers to choose the best control strategy.

At the moment, different tests are available to evaluate the resistance profile of ticks, with two of them more frequently used and recommended by the Food and Agriculture Organisation of the United Nations (FAO): the Larval Packet Test (LPT) and the Adult Immersion Test (AIT) (FAO, 2004). In 2011, a new bioassay to determine the resistance of tick populations has been developed: the Larval Tarsal Test (LTT) (Lovis et al, 2011). It is performed in microplates pre-treated with acaricides, in which eggs are distributed and larval mortality is evaluated after the hatching.

**Principle of the test:** engorged females are collected on cattle and kept in controlled conditions until they lay eggs. The test is performed in multi-well plates, in which acaricidal compounds diluted in a solvent (DMSO, dimethyl sulfoxide) are distributed at different concentrations. Plates are placed for 1 h in a sample concentrator to complete DMSO evaporation. Eggs are individualized and distributed in the wells (about 50 eggs in each well, using a curette). Then, plates are incubated, in an environmental chamber to allow hatching and larvae growth. And about two weeks after the hatching, larval mortality is evaluated by counting dead or surviving larvae using a stereomicroscope.

We evaluate the mortality rates according to the acaricide concentrations and they are compared to mortality rates obtained with a susceptible reference strain population. Lethal concentrations at respectively 50% (LC<sub>50</sub>) and 90% (LC<sub>90</sub>) mortality rates, and the corresponding resistance ratios (RR) (RR<sub>50</sub> and RR<sub>90</sub>) are calculated.

**Interpretation of the test:** Populations are considered to be **susceptible** to a compound when the RR<sub>50</sub> is smaller or equal to 4, **moderately resistant** for  $4 < RR_{50} \leq 10$  and **highly resistant** for RR<sub>50</sub> greater than 10. (Lovis et al, 2011; Lovis et al, 2013)

The LTT offers many advantages to evaluate precisely the resistance:

- A large number of acaricides and doses can be tested (12 concentrations of 5 compounds in one plate), allowing to obtain a full dose-response mortality curve.
- It allows to identify emerging acaricide resistance (to anticipate the development of high resistance).
- It requires a low number of engorged females ticks (as the eggs are used).
- Results obtained with *R. sanguineus* showed that the LTT could also be applied to evaluate the resistance profile of other tick species.

Continued on next page



## Update on "ResisT" activities

### • In Guadeloupe :

- **Implementation of the LTT (Larval Tarsal Test) in CIRAD laboratory** : After the validation of several key steps of the test (i.e. egg laying and hatching in controlled conditions, eggs distribution, DMSO evaporation, ...), trials are in progress to test the 96-well plates preparation and conservation, with cattle ticks strains collected in the field, in Guadeloupe and Martinique.

### • In Martinique :

- **Feasibility study on the use of an anti-tick vaccine, as an alternative control strategy of Cattle ticks**: Despite all the efforts made, we were not able to obtain the documentation on production process of the anti-tick vaccine (GAVAC®) which was needed to be authorized to import the vaccine in Martinique (submitted to EU regulation). Therefore, it was decided to suspend the pilot study and to change strategy and to rather focus the evaluation of ticks resistance situation in Martinique using the LTT, and to evaluate in the field, with the farmers, the efficacy of the acaricides they use in parallel. Hopefully, we will be able to test alternative ticks control strategies later.

- **Field evaluation of acaricide efficacy by farmers**: a new and easy protocol is being finalized by GDSM & CIRAD.

- **A trainee to assist with the project!** A student in master degree in animal health & epidemiology (Manon Hamon), supervised by GDSM and CIRAD, will work on this project from January 2015 for a 6 month period. Objective is to determine and characterize the level of resistance of Cattle ticks in Martinique, in relation to tick control strategies and herd management practices. Preliminary results will be presented at the 10th Steering Committee of CaribVET (April 2015, Guadeloupe).

### • In Nevis :

- **Feasibility study on the use of chickens on Tropical Bont Tick (TBT) populations, as an alternative TBT control strategy** : A mission was organized in June, involving the local team (P. Bartlette and staff of Vet. services), R. Pegram, S. Depraz, and D. Pleydell (modeler at CIRAD). The general context of ruminants breeding and TBT surveillance and TBT infestation levels were taken into consideration to assess feasibility of the scientific study, and how the preliminary protocol which was developed could be implemented in Nevis. A number of constraints would prevent evidence of significant impacts of chickens on the time frame of the project: generally low level of TBT populations, difficulty to establish close contact between ruminants and chickens considering cattle management practices and absence of local facilities, lack of data, etc. To fill those gaps, it was recommended to update surveillance form, use a database and finalize analysis on risk factors of TBT infestation (initiated during the VEP project). It was also suggested to assess costs of the current tick control strategy in Nevis.

### • Caribbean survey on TBT, TBDs and Acaricides

- The questionnaire has been finalized by the members of the members of the T&TBD WG of CaribVET during a virtual meeting, in July 29th and final layout modifications were made in Oct. after the questionnaire has been sent for testing and validation to 2 CVOs (Curaçao and Sint. Eustatius) in August. The questionnaire will be circulated to the steering committee of CaribVET in November.

### • Regional passive surveillance of Heartwater:

- A protocol has been circulated to the participating countries (Ste. Lucia, Dominica, Martinique, Antigua, Barbados). Blood / ticks collected on suspect animals will be sent to CIRAD for molecular diagnostic. Surveillance will start in November 2014. Objective is to update heartwater situation in the Caribbean.



La Collectivité au service du pays

It has also some constraints - some of which could be avoided with modifications in the protocol:

- The test is quite long as results are obtained about 6 weeks after tick collection (same duration as the LPT).
- It requires specific equipment, which could limit its implementation in a small laboratory. Alternative and simpler equipment has already been proposed (Lovis & al, 2013), but the evaporation of DMSO, a toxic solvent still requires expensive and specific infrastructure.

The LTT already allowed demonstrating the presence of acaricide resistance in cattle ticks in Brazil, Argentina, South Africa and Australia (Cutullé et al, 2012; Lovis et al, 2013). LTT resulted in resistance ratios comparable to those obtained with the FAO-recommended LPT.

Within the frame the ResiT project, which aims to assess cattle tick resistance to acaricides in the Caribbean Region, it was decided to implement the LTT in CIRAD animal health laboratory in Guadeloupe as there was no known laboratory testing ticks resistance in the lesser Antilles. It was also suggested to start studying resistance in the French Antilles, where farmers, farmer associations and veterinarians reported increasing problems of acaricides efficiency, suggesting potential resistance problems during recent years.

Implementation of the test required first to acquire the necessary equipment, slightly adapt the protocol to local constraints and get used to the technique - from handling/maintaining ticks to plates reading. The validation of the LTT in the CIRAD Guadeloupe is in progress and is reaching final phase. Once validated, LTT will be used to assess Cattle Tick resistance in Martinique, where this phenomena is strongly suspected by field surveillance practitioners, especially in some farms where none of the authorized acaricides work anymore, however it was never confirmed *in vitro*. The long term objective is to provide diagnostic tool for the partners of CaribVET, and to help other labs in the Caribbean to implement the test, if appeared to be relevant to the country. If the test was satisfactory in the Caribbean conditions, it could be considered to modify the protocol in order to reduce cost of the equipment (use alternative less toxic solvent, test anaerobic atmosphere to store the plates rather than N2 atmosphere, ... ).

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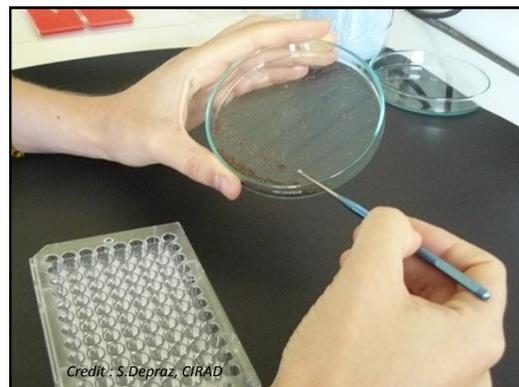
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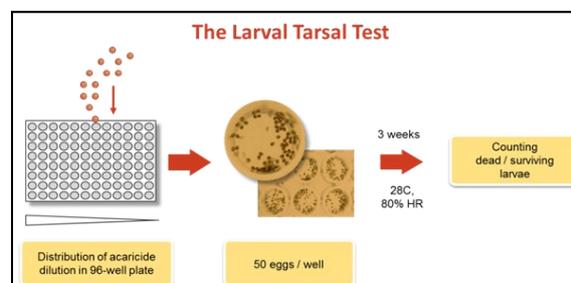
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Picture : Cattle tick egg distribution in a 96 well-plate, with a curette.



Schema: The different steps of the Larval Tarsal Test (@L. Lovis).



Picture: Cattle tick larvae, in a 96 well- plate. After hatching, larvae come in contact with acaricide at a given dilution. After about two weeks of exposure, mortality of the larvae is assessed in each well using a stereomicroscope.

## Similar project conducted in the region

### Research Project for Integrated Control of the Southern Cattle Fever Tick in Puerto Rico

By Robert J. Miller, Felix Guerrero, Matthew Messenger, Fred Soltero, Jose Urdaz, Carmen Oliver-Canabal, Myrna Comas-Pagan, and Adalberto Perez de Leon

Puerto Rico (PR) is infested with the southern cattle fever tick (CFT), *Rhipicephalus (Boophilus) microplus*, which is considered the most economically important external parasite of livestock worldwide. A research coalition involving the livestock industry in PR, the PR Department of Agriculture (PR-DA), and the United States Department of Agriculture (USDA) has been established to develop an integrated CFT control program.

The project consists of five phases that are expected to be completed in thirty-six months, i.e. three years. Four farms have been selected to have equal representation of the main dairy farming areas of Puerto Rico. One location is a beef production farm located in the southwest.

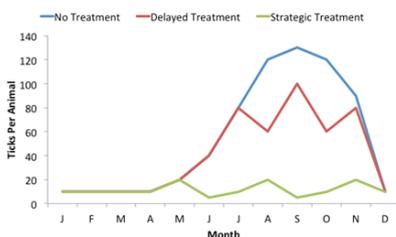
This approach addresses food safety and environmental health concerns with the ecological impact, and residue levels of synthetic acaricides in cattle products like milk. The project contemplates the implementation of good acaricide management practices through the acknowledgement of parasite economic thresholds prior to treatment and the use of novel pesticide formulations containing natural products, which are labeled for use in, and around lactating cows. Through this project, dairy and beef cattle producers in PR will have access to an integrated tick control program allowing them to manage, in a sustainable manner, the economic impact of the CFT on their operations as a result of the concerted efforts taking place between the animal health industry, and federal and state regulatory agencies.

\* USDA is an equal opportunity provider and employer.

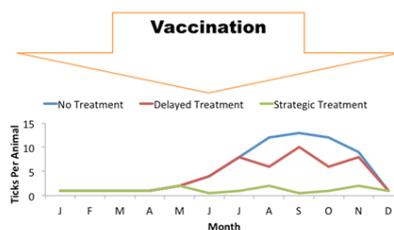


**Pictures :** Collecting blood bovine samples and scratching dairy cattle for epidemiological survey of ticks for epidemiological survey of tick-borne diseases in candidate farm for Integrated CFT Control project in Puerto Rico.

#### Pesticide Treatment Strategies



#### Vaccination Places Downward Pressure Population Regardless of Treatment Strategy



Contact : Robert J. Miller , USDA ARS Cattle Fever Tick Research Laboratory : [robert.miller@ars.usda.gov](mailto:robert.miller@ars.usda.gov)

Links : [http://www.ars.usda.gov/research/projects/projects.htm?ACCN\\_NO=426553](http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=426553)

[http://www.ars.usda.gov/research/projects/projects.htm?ACCN\\_NO=426922](http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=426922)

#### Project Duration: 36 months (3 years)

Phase 1: 0-6 months

Phase 2: 4-10 months

Phase 3: 6-20 months

Phase 4: 22-28 months

Phase 5: 30-36 months

**Phase 1.** Epidemiological assessment of CFT infestations, CFT-borne diseases, and acaricide resistance in dairy farms of Puerto Rico. Epidemiological surveys will be conducted to identify and enroll five farms for study. Parameters to be assessed in the herd and premises include, but are not limited to: georeference and animal census; prevalence of CFT infestation, bovine babesiosis, and anaplasmosis; acaricide susceptibility testing of CFT populations; mongoose density and status of CFT infestation.

**Phase 2.** Laboratory and field-testing for efficacy against CFT of commercially available technologies with tick control claims in the label that can be used in dairy cattle. Existing and emerging technologies are commercially available that can be used in dairy cattle and have tick control claims in the label, but efficacy against CFT, or CFT from Puerto Rico has not been documented.

**Phase 3.** Research and development of anti-CFT vaccine for dairy cattle in Puerto Rico. The anti-CFT vaccine program at the KBUSLIRL (Knippling-Bushland U.S. Livestock Insects Research Laboratory ) involves a partnership with an animal health company interested in developing the next generation of anti-tick vaccines, including one that could be used to protect dairy cattle in Puerto Rico against CFT infestation.

**Phase 4.** Pilot field testing of integrated CFT control program. Pilot testing of one to two protocols integrating various technologies will be conducted on the dairy farms enrolled in the project. This information will be used to establish a standard protocol for integrated CFT control in dairy farms of Puerto Rico.

**Phase 5.** Partnership with stakeholder groups for deployment of integrated CFT control program in Puerto Rico dairy farms. Plans will be developed in collaboration with stakeholder groups to coordinate the implementation of the standard protocol. It is expected that the protocol will consist of technologies that can be rotated so CFT populations can be managed in a sustainable manner to avoid pitfalls like the development of treatment insensitivity.

## Partners and collaborators corner



**Belkis Corona Gonzalez**, microbiologist at CENSA (Cuba) and member of the Ticks and TBD working group of CaribVET.

I obtained my DVM in 2004 in CENSA. I am member of the Ticks and TBD Working group of CaribVet since 2011. I am working with tick borne disease, *Anaplasma marginale*, *Babesia bovis*, and *Babesia bigemina*, specifically in the diagnostic using molecular techniques and in the molecular characterization of different isolates. My PhD focuses on *msp5* gene and MSP5 recombinant protein of a Cuban isolate of *Anaplasma marginale* for the bovine anaplasmosis detection and I published over 30 scientific papers related to haemoparasites thematic. I'm very happy to be part of the CaribVET network because I can work with researchers with knowledge's in the epidemiology and the surveillance programs for ticks and ticks borne diseases.



**Philippe Pelonde**, director of the GDSM\* (Sanitary Defense Association in Martinique) since 2011 and member of the Ticks and TBD working group of CaribVET.

My professional career is quite atypical. After studies on electronic engineering, I decided to come back to my first passion, agriculture, and I joined the GDSM as a technician during its creation in 1994.

The GDSM is in charge of the administration of public control plans against diseases in livestock farming, which are not listed by the French government. In 2003, I went in France to complete my agricultural training to become engineer in agronomy (ENITA of Clermont-Ferrand). I came back to the GDS of Martinique as technical manager and then as director. I'm interested, above all, to improve the sanitary status of livestock in Martinique, to help the livestock breeders to be profitable and also respectful of the environment.

\*GDSM—Groupement de Défense Sanitaire de Martinique.

## Calendar

## ResisT project activities :

## November 2014:

- Validation of all steps of the LTT test in the CIRAD laboratory (Guadeloupe) and preparation of scripts for future data analyses using packages of R software.
- Selection of farmers who will participate in the field study (Martinique) to assess ticks resistance to acaricides. **Ticks collection and samples shipments** to Guadeloupe are organized to identify gaps and test the whole process.
- Mission in Martinique: meeting with the selected farmers to explain the project and to start **the ticks collection and perform first diagnostic tests** at CIRAD.
- Nevis Pilot projet: Draft concluding **remarks on the feasibility study** on the use of chicken as alternative control strategy for TBT control.
- Circulation of the regional survey on TBT, TBDs and Acaricides in the region, to all the CVOs through CaribVET.

## December 2014 :

- Validation of the field study protocol in Martinique "observation of acaricide usage and efficacy".
- Tick resistance field survey in Martinique: Ticks shipment to Guadeloupe to start the characterization of the resistance with the test.
- Importation of engorged females from susceptible reference strains from Cattle Fever Tick Research Laboratory (CFTRL) of the USDA (see article p 2) and run of LTT with these strains to obtain reference curves.
- Preparation of training workshop to strengthen surveillance capacities in Nevis (data management and analysis; work on database for TBT surveillance in Nevis).
- Update of surveillance activities: First blood samples for Heartwater passive surveillance should be submitted to CIRAD.

## International meeting:

- T&TBD working group meeting will be held in November 18th - 20th, in Barbados.
- Steering Committee Meeting of CaribVET and Steering Committee Meeting of Epigenesis will be held jointly in April 2015, in Guadeloupe.

## Publication / communication :

- Interview on NTV8 (Local TV in Nevis): S. Depraz, R.Pegram and D. Pleydell presented the "ResisT" project and more especially the pilot study in Nevis. The interview presented the interest to find alternatives strategies to acaricides for the tick control in Nevis, and to follow the Tropical Bont Tick surveillance. <https://www.facebook.com/photo.php?v=884968928183707> (at 8min43 sec)

## Livestock farmer corner

### CODEM : Cooperative of bovine farmers in Martinique

By Stéphanie Depraz, according to an interview of Marie-Claude Blume (CODEM Director)

Created since 1984, the CODEM (cooperative of bovine farmers in Martinique) works to improve bovine livestock farming in Martinique. It includes 125 members (beef farmers) and the main objective is to structure the bovine sector at different levels, from to the production to the commercialization.

It's structured with an administration council with nine members, and the president is André Prosper, and 7 employees.

The main activities are:

- A technical support for the adherents, to offer them advices about herd management, but also to elaborate investment projects, to participate to training workshops...
- A management of bovine sales and purchase and activities with a traceability control.
- A meat cutting workshop qualified CEE. CODEM distributes beef to about sixty clients, in supermarkets, butcher's shops and also directly to restaurants.
- Furnish department, especially with a sale of seeds for pastures.

One of the objectives of the cooperative is to work on improved herd reproduction management in Martinique. In this goal, the CODEM works to the production of European breed bulls born in Martinique, to improve the meat capacities of local breeds, meanwhile keeping the rustic aspect and local environment adaptation (climatic conditions, pathogens...). At the moment, the work of selection is done by CODEM, in the context of the departmental genetic program in meat cattle.

CODEM works with various local partners (UEBB = Union of Brahman cattle farmers ; Agricultural chamber, the food-processing research Center in Martinique) and it want to improve exchanges with the Caribbean region, especially with importation and exportation of cattle to other islands.

Contact : Marie-Claude BLUME, CODEM director : [direction@codem.pro](mailto:direction@codem.pro)



**Picture 1:** Cattle of the CODEM, presented during the 1st Agricultural show in Martinique



**Picture 2:** Exchanges between CODEM members and the former prefect of Martinique, during the Agricultural show.

### Focus on Tick issues (interview of Marie-Claude Blume, CODEM Director)

**- Is the « tick and tick-borne diseases » a priority issue for CODEM and the bovine breeding sector in Martinique?**

Of course. The presence of ticks in the island is the main sanitary issue for our farmers. Twenty years ago, we had many problems of dermatophilosis [associated with TBT infestation and high susceptibility of some Brahman breeds, Ed] in Martinique. Now [after genetic selection of animals, reforms of susceptible animals and continuous TBT surveillance and control, Ed], farmers report mostly anaplasmosis cases, even in Brahman farms, though considered to be naturally more resistant.

**- Which acaricides are currently used by your members? Do they report problems of acaricides efficacy?**

The acaricides most commonly used are Tactic (Amitraz), Bayticol (Flumethrin) and Butox (Deltamethrin). In recent years, we observed that these molecules seem to be less effective than in the past, at least in some farms. For example, some farmers report that the Cattle Ticks (*Rhipicephalus (Boophilus) microplus*), stay on animals even after Amitraz treatment. So, we are very interested to get more information about the current situation in Martinique and tick resistance, because we are aware that the chemical control is not a long-term solution.

**- Do you communicate on tick issues in your cooperative? More generally, how do you organize the communication in CODEM ?**

At the moment the sanitary activities decreased in the cooperative, because most were transferred to the GDSM (Sanitary defense association in Martinique) since the reorganization of the cattle sector 3 years ago. Otherwise, in general, we communicate with our members during farmers meeting or through technical training, when we try to invite different technical organisations to share knowledge on specific issues.

# Entertain yourself!

By Stéphanie Depraz (ResisT project manager, CIRAD Guadeloupe)

## QUIZZ on the LTT test

The first person sending good answers will receive a CaribVET gift! So let's go!

- 1/ What is the translation of LTT?
- 2/ How long does last a LTT test, from the collection of ticks on the animals to the results?
- 3/ Which tick stage in contact with acaricides is evaluated with the LTT?
- 4/ What is the quantitative data obtained with the LTT which allows calculating the Resistance Ratios?
- 5/ Cite two others in vitro tests used worldwide to assess tick resistance, and cite the tick stage needed for each test.

Send your answers to [stephanie.depraz@cirad.fr](mailto:stephanie.depraz@cirad.fr)

### Answer of the previous crossword

										<sup>1</sup> C		<sup>2</sup> H
		<sup>3</sup> D	O	M	I	N	I	C	A			E
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<sup>4</sup> C	<sup>5</sup> I	R	A	D			<sup>6</sup> N			I		R
	I						E		B			T
	C		<sup>7</sup> C					V	V			W
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				T				S		<sup>9</sup> T	B	T
<sup>10</sup> C				T								E
<sup>11</sup> A	M	B	L	Y	O	M	M	A				R
P				E								

- For any suggestion or comments on the Newsletter, or if you want to be included in the ResisT newsletter mailing list, please write to [caribinfo@caribvet.net](mailto:caribinfo@caribvet.net)
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