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BABESIA MAJOR : ABOMASAL TRANSMURAL POTENTIAL DIFFERENCE, AND ANTRODUODENAL MOTILITY CHANGES ASSOCIATED WITH EXPERIMENTAL INFECTION IN CALF

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Résumé

L'effet sur la sphère gastroduodénale d'une infestation parasitaire par *Babesia major* a été envisagé par la mesure de la différence de potentiel transmural, du pH et des concentrations ioniques au niveau de l'abomasum chez trois veaux. En outre, les variations de la motricité gastroduodénale ont été appréciées par électromyographie et mécanographie. Dès le deuxième jour suivant l'injection intraveineuse de 8×10^9 *Babesia major*, une chute significative du potentiel transmural abomasal est observée. Cette dernière est concomitante de l'augmentation de la concentration des ions Na^+ et K^+ dans le contenu abomasal. Les modifications motrices (augmentation de la fréquence des complexes myoélectriques duodénaux, ralentissement du rythme électrique de base antral) apparaissent au cours du 5^e jour après l'infestation, i.e. lorsque les premiers signes cliniques deviennent manifestes. Les modifications sécrétoires et motrices disparaissent dès que cesse l'hyperthermie et donc lorsque la parasitémie décroît. Les modifications du potentiel transmural comme les variations dans les concentrations ioniques au niveau de l'abomasum peuvent être considérées comme des signes précoces de l'infestation à *Babesia major*.

Bovine babesiosis, which was first recognized in France at the end of the 19th century, is caused by a hemoprotozoa of the genus *Babesia*. The first species involved in this pathology, *Babesia divergens* (Euzéby and Rancien 1966), is considered to be the most pathogenic. It is the main agent of bovine babesiosis in France. A second species, *Babesia major*, present in French livestock, has been described in Sergent's work in 1945 (Sergent *et al* 1957). *Babesia major* was isolated from calves in 1985 in Southern France (Lot) (Dorchies *et al* personal communication).

The clinical manifestations of bovine babesiosis, due to *Babesia divergens*, are characterized by fever (40 ± 0.3 °C), hemolytic anemia associated with hemoglobinuria and jaundice. Alteration in digestive function i.e. anorexia and diarrhoea, are often observed.

As mechanisms of these disorders are still poorly understood, this study was conducted to elucidate the activity of *Babesia major* on the motor and

secretory functions of the digestive tract. The antroduodenal area was chosen as a test region for its regulatory role in the control of the gastric emptying as well as its ability to initiate the basic motor activity of the intestine in ovine species (Ruckebusch and Bueno 1977).

Materials and Methods

Animals

Three calves of the French Friesian breed, 2 months-old and weighing 50-70 kg were used. They were kept until the onset of the experimental period in a tick and *Babesia free* environment. Hay and water were delivered *ad libitum* with two meals per day (9:00 and 17:00 h). The animals had free access to a salt block.

Motility recordings

Pairs of insulated nickel-chrome wire electrodes were implanted under halothane anaesthesia on the antimesenteric border of the antrum (10 and 5 cm anterior to the pylorus) and on the proximal duodenum (2 cm and 10 cm posterior to the pylorus). A strain-gauge transducer was sutured onto the serosal side of the antrum (5 cm anterior to the pylorus). The tip of a polyethylene tubing (4 mm in diameter) inserted through the wall of the greater curvature

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of the abomasum was positioned inside the lumen of the mid-antrum. Finally, a silastic tube (1.5 cm in diameter) was fixed to the abomasum in order to sample the abomasal juice.

Recording of myoelectrical activity started on the 8th day after the above-mentioned electrode implantation using a modified electroencephalograph machine (Minihuit, Alvar, Paris) with a time constant of 0.1 second. The electrical activity of the duodenum was summed each 20 seconds and plotted on a multichannel potentiometric recorder. The spike activity occurred in periods lasting 40 to 50 min on the duodenal bulb. These periods included a phase of irregular spike activity bursts (ISA) (50 min), followed by a short phase (2-3 min) of intense and regular spike activity (RSA) and the quiescence or no spike activity (NSA) (10 min). The mechanical activity of the antrum was also recorded potentiometrically via a Wheatstone bridge.

Secretion parameters

The potential difference (PD) across antral mucosa and serosa was measured according to the method described by Davenport *et al* (1964) between the saline-filled tube implanted in the antrum and a second tube (15 mm in external diameter) filled with Agar-KCl mixture and fixed subcutaneously in the midthoracic region. These electrolyte bridges were connected to a millivolt-meter (Metrohm, Basel) by means of two beakers filled with 3 M KCl solution and two balanced calomel half-cell electrodes (Ingold). The PD was recorded potentiometrically after low-pass filtering (0.02 Hz, 6 dB) and its mean value at 5-min intervals was calculated by means of a desk-top computer in order to obtain a condensed graph of the 24-h recording as well as the daily parameters of the PD.

Three times a day (8:00, 12:00, 19:00 h), during ISA phase, a sample of gastric juice was retrieved. Acidity and pH of the sample were measured immediately. The remaining gastric juice was frozen. At the end of the experimenta-

tion, Na⁺-K⁺ concentrations were calculated by atomic absorption and Cl⁻ concentration was obtained with a chlorimeter.

Infection

Control data were obtained eight days after surgery from the motor and secretory components of the antroduodenal area. Infection with *Babesia major* was established on 14th day after surgery with a standard dose of 8×10^9 parasites injected intravenously. *Babesia major* was previously collected in heparine from a 5-month old calf, a known carrier of *Babesia major*. Beginning the first day after infection, rectal temperature was recorded and blood was sampled from the caudal vein every morning. Parasitemia level was determined according to the method of Brocklesby *et al* (1973) at the end of the experiment, from daily specimens.

Statistics

All the results were presented as mean \pm SD. Statistical significance was tested using one and two-way analysis of variance.

Results

1. Course of the disease

The course of the disease in the three calves was quite similar (incubation period defined by rise in body temperature : 5 ± 1 days). The three calves presented a milder form of the disease, *i.e.* mild anemia and absence of hemoglobinuria or jaundice. The maximum temperature recorded was 39.5 ± 0.9 °C. However, parasitemia was very moderate (fig 1).

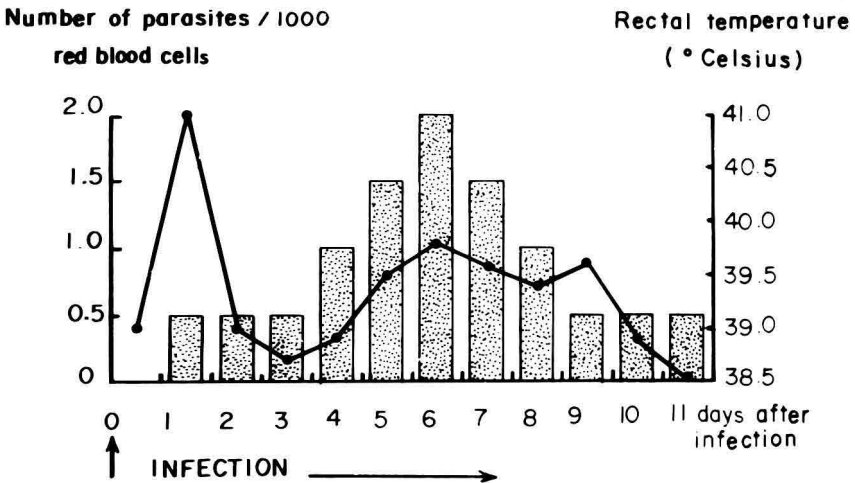


Fig 1. - Time course of the body temperature (●) and parasitemia (▨) after an infection with a standard intravenous dose of 8×10^9 *Babesia major* at day 0 (↑).

2. Antroduodenal motility changes

Minor variations in the antral motor activity were present after infection. Phases of irregularities in the antral slow-wave frequency (dysrhythmia), occurred at random at the - 5 cm site, 5 days and 6 days after infection (fig 2). This pattern was infrequent during the control period (1 episode in 5 days) as well as after the 6th day post infection. Periods of dysrhythmia were especially frequent immediately before the initiation of a regular activity phase onto the proximal duodenum ($10 \pm$

3 phases at day 5 and 13 ± 5 phases at day 6 after infection). No significant modifications were present on the mean level of antral mechanical activity within this period.

The frequency of migrating myoelectric complex (MMC) was fairly constant during the control period, as well as during the first days (days 1, 2, 3, 4) after infection with a value of 46 ± 15 min. A significant increase in the MMC frequency existed at days 5 and 6 after infection ($P < 0.01$), and vanished afterwards (fig 3). This decrease in the MMC interval was associated with an increase in

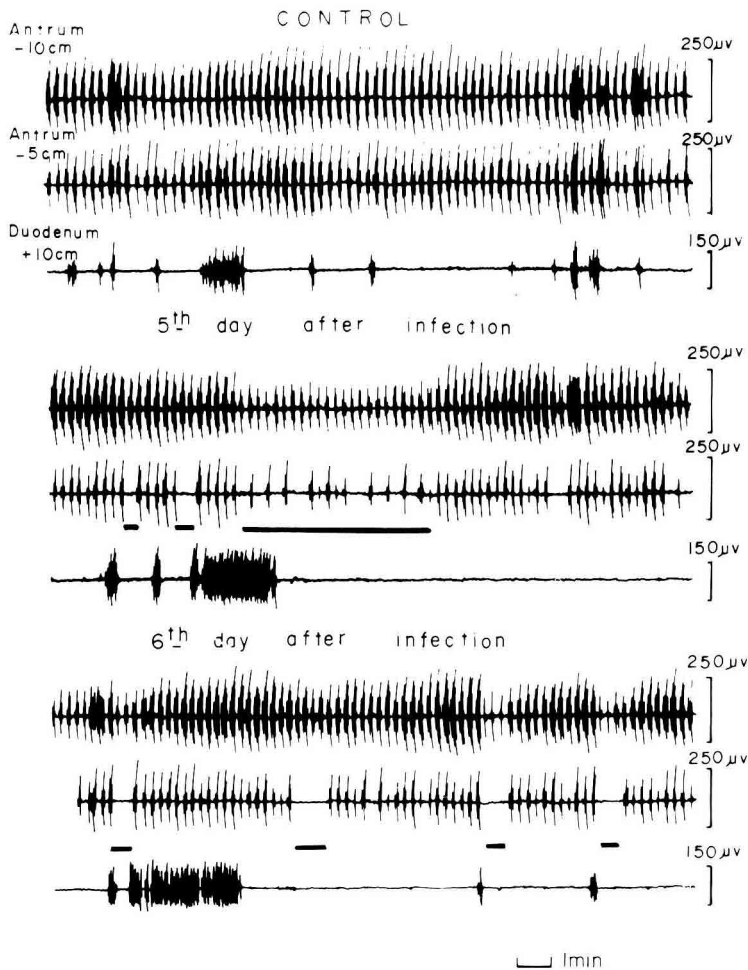


Fig 2. — Direct trace recording of the antroduodenal electrical activity. Note on the 5th and 6th days after infestation, the frequent phases of irregular antral slow waves (dysrhythmia ; →).

the duration of the no spike activity phase (14 ± 3 min vs 7 ± 4 min, $P < 0.05$).

3. Antral PD changes

The antral PD presented low amplitude-low frequency (< 2 mV), oscillations unrelated with either antral or duodenal motor activity. The mean PD value before infection was -20 ± 2 mV. This value was significantly lowered (-24 ± 3 mV ; $P < 0.01$) on the 2nd day after infection. However, the lower PD value (-35 ± 5 mV) was recorded on the

6th day after infection (fig 4). The PD control values were recorded within the 12th day after infection. Maximal amplitude in PD fluctuation unrelated to motility events, were observed on the 6th day after infection, i.e. when the PD reached its lower value (fig 5).

4. Abomasal fluid pH changes

The abomasal fluid pH increased significantly ($P < 0.05$) after infection with a peak on the 5th day. No changes occurred in the acidity or in the chlo-

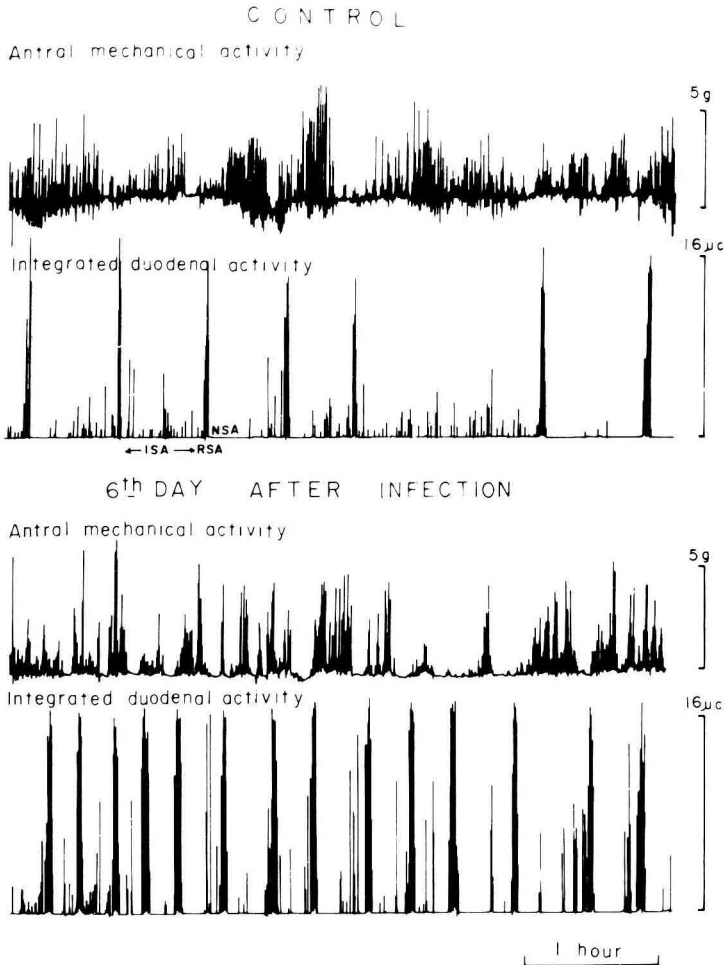


Fig 3. – Increase of the MMC frequency on the duodenum on the 6th day after infestation. The amplitude of antral mechanical activity was unaffected by infection.

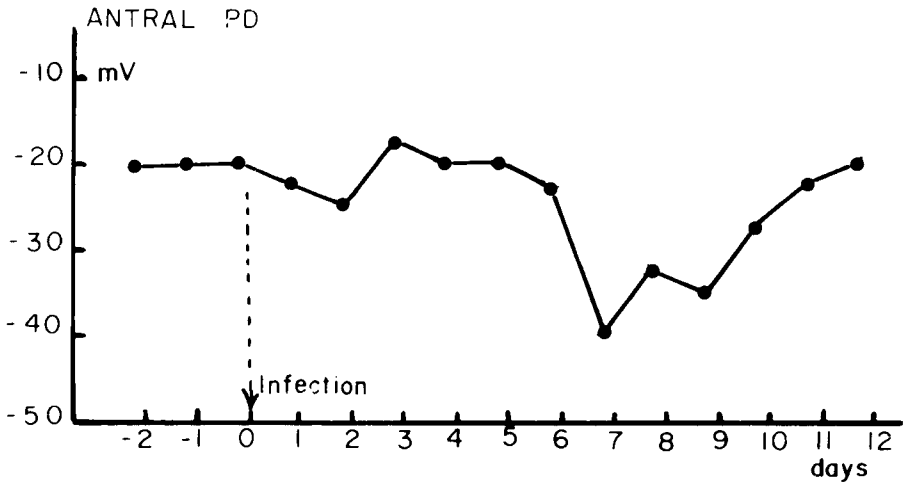


Fig 4. – Antral PD evolution over the experimental period. Two drops in PD were present but the larger one was observed on the 6th and 7th days after infection. The PD recovered its normal value after 12 days.

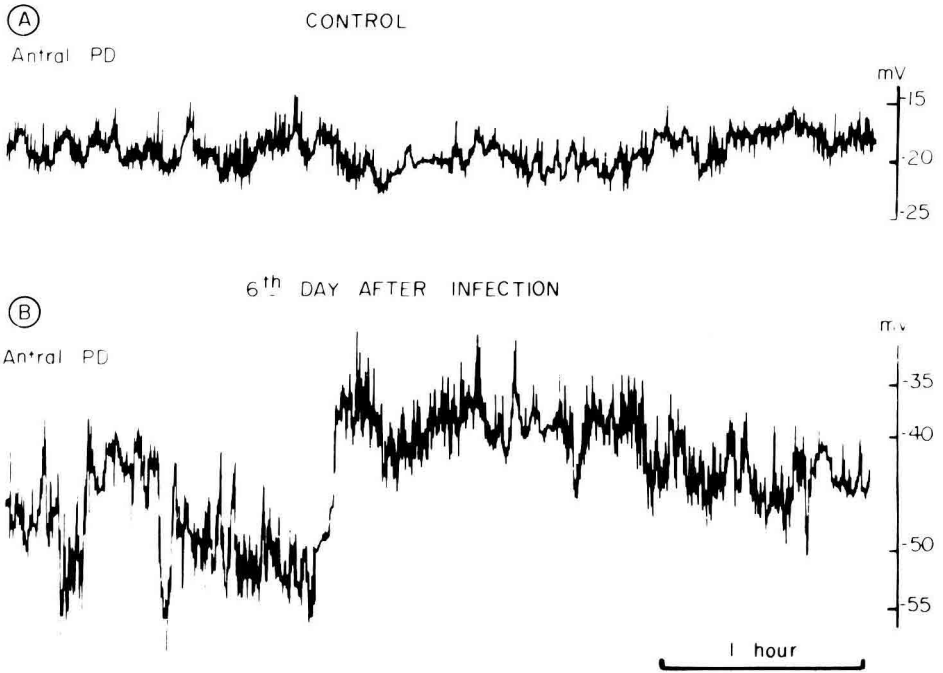


Fig 5. – Antral PD recorded after low-pass filtration. Note on tracing B, the level of the baseline and the high amplitude-low frequency fluctuation of the PD value which were absent from the control (A) tracing.

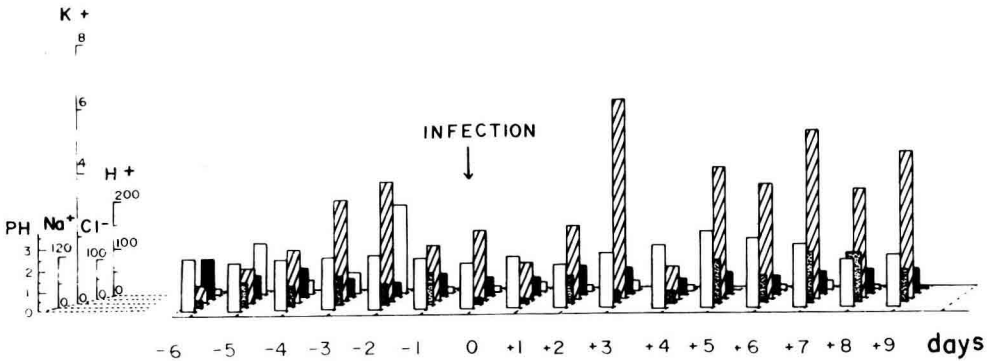


Fig 6. — Changes occurring in acidity, pH, sodium, potassium and chloride concentration in the abomasal juice before and after *Babesia* infection.

ride concentration of the abomasal contents. Conversely, the sodium and potassium concentrations of the abomasal contents steadily increased ($P < 0.05$) on the 2nd day (fig 6).

Discussion

Parasitemia and fever peaked on the 5th day after infection. These were associated with major changes in antral PD and antroduodenal motility pattern. As suspected, gastric dysrhythmia was observed during fever, but was not associated with a decrease in the gastric mechanical motor activity. Converse to the minor motility changes observed at the antral level, the duodenal MMC pattern was heavily modified. Indeed, a significant increase in the MMC frequency occurred during the 5th and 6th days after infection. This faster rhythm was associated with an enhanced duration of RSA-NSA phase and as a consequence a reduced duration of ISA phase. Hyperthermia is known to significantly reduce, then abolish the MMC pattern in the ruminant small intestine. The increased MMC frequency reported in this study, was another aspect of a minute rhythm already described during diarrhoea episodes (Bueno and Fioramonti 1980).

Diarrhoea observed in clinical cases of *Babesia*

infection was absent in our work. However, early myoelectrical signs of diarrhoea were observed on the duodenum. The major decrease in antral PD value at days 5 and 6 was the most significant parameter observed at the onset of the first clinical sign of *Babesia* infection.

The decrease in antral PD value was also important in detecting early signs of babesiosis. On the 2nd day after infection, a low but significant decrease in PD value was observed. This phenomenon was associated with changes in sodium and potassium concentrations within the abomasal fluid.

We conclude that antral PD value and Na^+K^+ concentrations within the abomasal contents could be considered as early signs of babesiosis, whereas motility changes, which occurred during the period of clinical babesiosis, were late signs. Future work needs to be performed to correlate the variations in the ionic concentration occurring within the abomasal contents with those occurring within the blood.

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Abstract

Transmural potential difference, pH and ionic concentrations of the abomasal contents were measured in three calves. Antroduodenal motor patterns were recorded using electrodes and strain-gauge transducers fixed on the antrum and the duodenum. The calves were infected with 8×10^9 *Babesia major* intravenously and a significant drop in transmural potential difference, concomitant with a steady increase in Na^+ and K^+ concentrations of the abomasal contents occurred within 2 days. The onset of fever on the 5th day after infection was associated with an increased frequency of duodenal migrating myoelectric complexes and

episodes of bradygastria on the distal antrum. These changes disappeared at the end of fever. We conclude that transmural potential difference changes and $\text{Na}^+\text{-K}^+$ concentrations in the abomasal fluid could reflect changes in the ionic permeability of the gastric wall. These effects may represent early signs of *Babesia* infection.

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