

DAIRY COWS AS BIO-INDICATORS OF *ANAPLASMA PHAGOCYTOPHILUM* AGENT OF TICK-BORNE FEVER IN FRANCE

Guy Joncour¹, Christophe Brard², Bruno Courtay³, Jean-François Labbe⁴

¹Groupe Vétérinaire de Callac, Callac, France
Callac.Veto@wanadoo.fr

²Clinique Vétérinaire du Marcadieu, Nay, France

³Merial, Villeurbanne, France

⁴Groupe Vétérinaire de Broons, Broons, France

1. INTRODUCTION

Tick-borne fever in cattle, was identified for the first time in France in 1991 (Argenté *et al.* 1992) and defined as an under-diagnosed infectious disease, possibly inoculated, non-contagious from mammal to mammal. It is seen as an ubiquitous, immunosuppressive disease, with a high seroprevalence rate among adult animals. This disease can be acute or subclinical, with sporadic outbreaks during the periods of ticks activity, sources of infection. A solid knowledge of semiology is essential for veterinary practitioners: from 1998 to November 2005 identified sources of infection rose from 2 to more than 700. Last but not least, Tick-borne fever is an emergent zoonosis. It may be associated with other dual infections, such as babesiosis, Lyme borreliosis or Q fever (Joncour *et al.* 2003). Due to a rickettsia-like bacteria, *Anaplasma phagocytophilum*, a compulsory intracellular blood-cells “parasite”, Tick-borne fever seems to be an emergent disease in Brittany and in France, based on the number of sources of infection and clinical cases on cows. The first outbreak, identified by Collin (1991) (Argenté *et al.* 1992) was followed, in 1998, by Joncour (Joncour *et al.* 2000), veterinary practitioner in the Côtes d’Armor region. In this second one, there was a 100% acute form prevalence (35/35) in one year: “BSE-stamping out” occurred two years ago in this dairy farm. A 70 days diagnostic delay, in spring 1998, convinced us to use reliable, cheap and efficient diagnostic tools: these came after clinical examination and a good knowledge not only of the biology of ticks, and of other sometimes poorly identified potential vectors, but also of the epidemiology of vectorial diseases often associated to dual infections (George, 1999, 2002; Joncour *et al.* 2003; Liz *et al.* 2000) (co-infections) and inducing other medical disorders. Our results are unpretentious, although we have ambitious goals.

A better information for vets, but also for GPs, epidemiologists, agronomists, ethologists, entomologists, zoologists and other biologists, sanitary services (for animals and humans) but also for dairy farmers, mainly and Roe deer hunters resulted in more than 700.

We have another rickettsial Tick-borne although un-zoonotic disease: bovine anaplasmosis (Joncour *et al.* 2003), due to *Anaplasma marginale*, whose “micro-biotope” is red cells. Named by farmers “white babesiosis”, it is also an Arthropod-borne disease. We better keep the previous denomination, Tick-borne fever. However human doctors call it now, human anaplasmosis.

France is divided in 96 administrative departments, local regions or subdivisions. Brittany includes 4 of them, 5 at historical level (Loire-Atlantique). Our fieldwork extends on Côtes d’Armor, Finistère, Ille-et-Vilaine and Morbihan departments (Figure 1). It is associated, with “Pays-de-Loire” federation (233 members), through the Loire-Atlantique Veterinary society. It shares the same goals and works with the same board. In this study, epidemiological and environmental committees worked together.

Figure 1. Tick-borne fever prevalence agent, in France, from 1991 to November 2005 (GTV study results)

We notice a fast widespread geographical occurrence enlargement for the French departments, from 1998 to late November 2005. Our information pressure and works towards our colleagues seem to have been efficient. An emergent pathology definition is: “Health disorders whose incidence has increased over the last 20 years, or threatens to increase soon”. Not easy to say whether Tick-borne fever is -or not- an emergent disease. Literature alone cannot answer this question. Efficient diagnosis means largely better *A. phagocytophilum* (and ticks) occurrence data, both qualitative and quantitative. But we know that meteorological disturbances and planet warming up are a potential change factor for ticks and other *Arthropodae*. We think that we have contributed, during these last seven years -and still go on- to prove that Tick-borne fever and *A. phagocytophilum* presence in proper biotopes is not an “only Breton sanitary speciality”. This national map of departmental prevalence is probably the same as *I. ricinus* one’s (Joncour *et al.* 2003; L’Hostis, 1994). Easy to compare to babesiosis one’s: 74 departments, at least, “shelter” *A. phagocytophilum*, from 1998 to November 2005, in France. This survey illustrates, too, the bio-indicator species (or individual) concept: as previously mentioned, in Marne (51) and Hautes-Pyrénées (65), Roe deer and izard (*Rupicapra rupicapra pyrenaïca*) show that. There, no human, equine or bovine case has been identified up to now. Up today in Meuse (55) and Bas-Rhin (67), Eastern part of France, man seems to be a “marker”, a bio-indicator, for herds, at least: George identified the first two cases on patients (George, 1999, 2002). In 2003, Brouqui (Remy, 2003) diagnosed an atypical pneumonitis induced by the bacteria, in the other. Animals, there, seem to be healthy. In the 58 other French departments, where bovine cases were discovered, the “cow is really able to be a bio-indicator for mankind” (Joncour *et al.* 2003).

Sources of infection on cattle were identified in France up to November 2005. We highlighted our findings through broad information means, mainly toward vets, trying to involve rural doctors. Our first goal is to decrease the economical loss of the agriculture and, accordingly, it’s a veterinary objective, an objective of animal health. Therefore we must propose relevant sanitary guidelines and actions. Information is the most important. Another goal is public health and safety. An important part of our field study is also to make the national health districts, agricultural (animal health, safety and food head office at agriculture ministry), “health and social” departments (National Institution for Sanitary Surveillance) and local sanitary agents aware of the presence of *A. phagocytophilum* in France.

2. DIAGNOSIS

We refer to our field experience and works, from 1999 to 2005. Our tools: human means, first, our regional field network (16 practices/100 vets), Swiss (Pfister, Liz, Pusterla, Lütz) and other European or Northern American scientists, vets or not, the advisory commission: the first named and Degeilh, L’Hostis, Davoust, Vassallo. Information tools brought to them and elsewhere by means for training-information: vet specialities wholesalers and professional press, the breeders, the hunters and other wildlife samples collectors, finally, our 3 trainees. Financial funding came from territorial authorities, Brittany’s regional county council, Côtes d’Armor (22) and Loire-Atlantique (44); local councils, animal health companies (Merial, Schering Plough, Virbac, Boehringer Ingelheim, Intervet, Bayer, Ceva), our society (PCR cost limited investigations, despite pooling the swabs). Our other tools where clinical diagnosis knowledge. Epidemiology is the main, about Tick-borne fever early diagnosis. *Ixodes ricinus* is the main vector and a good carrier-reservoir (Chevalier, 2002; George, 1999; Joncour *et al.* 2003; Liz, 1994; Stuen *et al.* 1998; Wilson *et al.* 1964; Woldehiwet, 1993). Tick-borne fever is, as ticks lifecycle, consequently biphasic on cattle: spring mainly and autumn. Very ubiquitous, as his vector-and-reservoir, Tick-borne fever is monitored on horses, cows, goats, sheep (Stuen *et al.* 1998; Wilson *et al.* 1964) and pets as cat or dog (Joncour *et al.* 2003). Also on wild ungulate (Joncour *et al.* 2003; Liz J, 1994), Foxes, Bats (Simpson, personal communication) and even llamas (Joncour *et al.* 2003). In the fields

(Joncour *et al.* 2003), only acute stages are diagnosed. Easier for anademic form than for sporadic ones. In a dairy herd agalaxy is the first sign (Argenté *et al.* 1992; Joncour *et al.* 2000; Pusterla *et al.* 1997). The respiratory disorders associated with a high rectal temperature can class this syndrome among “Summer Flu-like -cough- Syndrome” with inter-specific semiological convergences (George, 1999, 2002; Joncour *et al.* 2003) on cows, it includes too dictyaucolosis and the unexceptional Q Fever respiratory form Lars (Joncour *et al.* 2003).

Locomotion disorders such as oedema on distal limb parts (“enlarged pastern disease”), with a herd incidence from 0 to 10%, are significant when present in a herd located in a tick biotope. Reproduction disorders (abortions, embryonic mortality, return to heat) are uneasy to prove. But we did. Haematological disorders (cells and biochemical parameters) (Achard’s paper and SNGTV 2004 inquiry) are not very often investigated in a rural practice. The drop of the white cells blood line induces immuno-depression (Joncour *et al.* 2003). The acute stage will sometimes be associated with recurrent fevers (ovine) (Chevalier, 2002), a milk production decrease, often complete agalaxia (Joncour *et al.* 2000), hard breathing, a possible susceptibility to viral (RS virus, ...) (Joncour *et al.* 2000), bacterial or “other” parasitic diseases (Joncour *et al.* 2003). Complications are pyohaemia due to ticks with many abscesses with (*Staphylococcus aureus*) (Joncour *et al.* 2000), dermatitis on cow udder skin (Kaufmann, personal communication), other various induced and (or) associated (dual infections) pathologies (Joncour *et al.* 2003) (Summer-flu-like syndrom, Lyme borreliosis, ocular listeriosis/uveitis, babesiosis, Q Fever).

A conspiracy. A careful examination of the skin is a complementary important tool, trying to find in appropriate places of the body (towards the udder-sides, groin and umbilicus, armpits, dewlap) gorged fixed females ticks, or, at least, fixing scars. This also helps to diagnose. The chronic stage is mainly represented by asymptomatic carriers (not easy to identify).

These represent the “submarine part of the iceberg” and identified by specific serology (immuno-fluorescent antibody assay (IFAt). It identifies the “markers” of a prior *A. phagocytophilum* infection.). A “reservoir role”? Is there a potential transfusion risk for human health and public security (Joncour *et al.* 2004)?

One individual will classically be sick during five to ten days, but it sometimes goes on for a whole year in a herd. Recurrences may be seen from time to time, at the right seasons (other strains, more or less pathogen) (Joncour, 2000, 2003), up to eight years after the first cases were noticed. Natural immunity seems rather strong, despite few relapses were observed. In prime-infection, adults are more affected than young heifers, in risky plots (as for babesioses) (Joncour, 2000, 2003).

2.1 Experimental definite diagnosis: given by the laboratory

Blood smear cyto-haematological examination, MGG or RAL coloured show *morulae*, G Ig serology (IFAt/threshold: 1/80), especially useful for the prevalence study in sources of infection (and kinetic studies) and DNA agent high-lightning with a specific PCR probe on blood on EDTA were used. IfAt helps an *a posteriori* diagnosis, if one “missed” the clinical -acute- stage: positive possible response from D21 and until D120. Un-visible forms are only highlighted by IFAt positivities disclose an important “back-ground noise” about antibody response, proving a past, actual or future “infectious activity” on cattle and on their sympatric: the iceberg effect.

Table I summarizes some characteristics. These various elements helped the veterinary experts with the diagnosis. It is made possible by fieldwork and especially by taking into account invaluable bibliographical elements (Scotland, Sweden, Norway, USA, Switzerland, Spain): Tick-borne fever has been known, even by cow breeders, for longer time in these countries (Öberland, Switzerland). Some of these are on the borders of France. These first

implements were widely spread by the means of veterinary wholesalers (Alcyon-Centravet-Coveto) and professional press. Our other implements were: Côtes d'Armor (22), local veterinarian tests laboratory (LDA22), the 16 field-vets practices, three trainees, on one hand, financial means necessary on the other. As a prime restrictive factor: private means, and from 2000, public assistance permitted to balance the ~ 90 000 Euro budget.

Table I. **Diagnosis means**

Methods	Biological support	Sensibility	Specificity	Efficiency range
Clinical examination	Animal + environment	+/-*	+/-	D0** - D10
Cytohaematology	Blood on EDTA or heparin	+/-*	+++	D0 - D4
IFAt serology	Sera, dry tube	+/-	+++	D21 - D120
PCR	Slide, sera/plasma, organs, ticks	++++	++++	D0 - D4 (D10 ?)

* Sensibility differs from laboratory biologist's and clinician's practice (from 10 to 80%)

**D0 = the breeder report's day

2.2 URGTV Bretagne Epidemiological field investigations

Good epidemiological knowledge allows ensuring a reasoned disease management, by maintaining a balance "host-parasite-environment" in the affected farms (L'Hostis, 1994). The study went from 2000 to 2001, biological analyses being finalized up to 2003, and then to 2005.

Towards the end of 2001, 20 "active" outbreaks (with clinic cases confirmed by LDA22) were identified in Brittany. In February 2002, out of 80 known national herds affected seven also experienced a "stamping out" related to a prior BSE occurrence. When we had 120 active sites.

2.2.1 Objects

We believe that involving different disciplines is surely the main tool to fight zoonoses. The scientific advisory commission (1999), to which we associated experts, LDA22 fellow-members, National Veterinary School professors, general practitioners, epidemiologists specialized on vectorial diseases from Veterinary National Agencies and Medical (Rennes University) Schools had to control, first, whether Tick-borne fever was an emerging pathology, in Western France. Then in the whole country: up to November 2005, we detected 58 "infected" departments (Figure 1), using the classical four diagnosis tools (Table I). Improve our epidemiological knowledge for our western herd and biotopes, monitoring 21 dairy farms in the five Breton subdivisions. Evaluate the infection impact, its consequences on livestock productions, where we include the point "reproduction", i.e. consequences on embryos and especially on the foetus ("abortion" point). An immunity breakdown or hyperthermia consequence. Test the small reservoirs (ticks, *I. ricinus*, and micro-mammals, such as field-voles) and the larger ones (biotopes, herds and sympatric fauna in these focuses). Since 2000, the geographical areas with *A. phagocytophilum* presence have moreover been in departments where, for the moment, no case has been identified on cow: serologies (IFAt) on ranching Red deer (*Cervus elaphus*) and mainly on free Roe deer (*Capreolus capreolus*), wounded by cars or hunted. Giving a print on and through these sympatric and territorial ungulates. Good reservoir or not? Some surveys say no: only temporarily competent for the bacteria (Chevalier, 2002; Joncour *et al.* 2003; Stuen *et al.* 1998, 2001). Marne (51) department is "positive", only taking into account on 67% IFAt positive results (40/60), on Roe deer (Joncour *et al.* 2003). In 2004, two Roe deer are PCR positive on spleen in Loir-et-Cher (41) (National Game Hunting and Wildlife administration sanitary service data) (Figure 1). There, now, we have identified sick cows (Jallu, 2005).

Then, tend to identify the occurrence and risk factors and propose some disease prevention elements adapted to this affection which is not as rare as one could have thought: our main vet objective in farm productive animals group pathology is obviously to contribute to reduce the economic losses of the stockbreeders. Animal health has, often, a public health facet (Chen *et al.* 1994; Davoust *et al.* 1999; George, 1999; Joncour, 2004, Petrovec *et al.* 1997). Its study is, too, one of our aims.

3. METHODS

Sera were analysed (IFAt) at LDA22.

3.1 Regional prevalence study in identified sites/farmings (“focuses” and pilot sites)

In spring 2002, blood sampling was carried out on 20 animals in 11 “clinical infected farms”, in March, before turn-out of young stock. These same 20 females were tested again, in October. Between July 15th and August 15th, in 10 contiguous, directly adjacent to these 11 “infected” dairy farms, 10 young heifers, from 6 to 20 months old, were blood tested. This group had from none to one previous summer grazing season. These farms, selected by our trainees’ field inquiries and scientific leading committee, are used as control/pilot -or “sentry”- sites. For these two paired breeding sites, plots management and neighbouring “wild” biotopes are very similar.

3.2 Reproduction incidence point

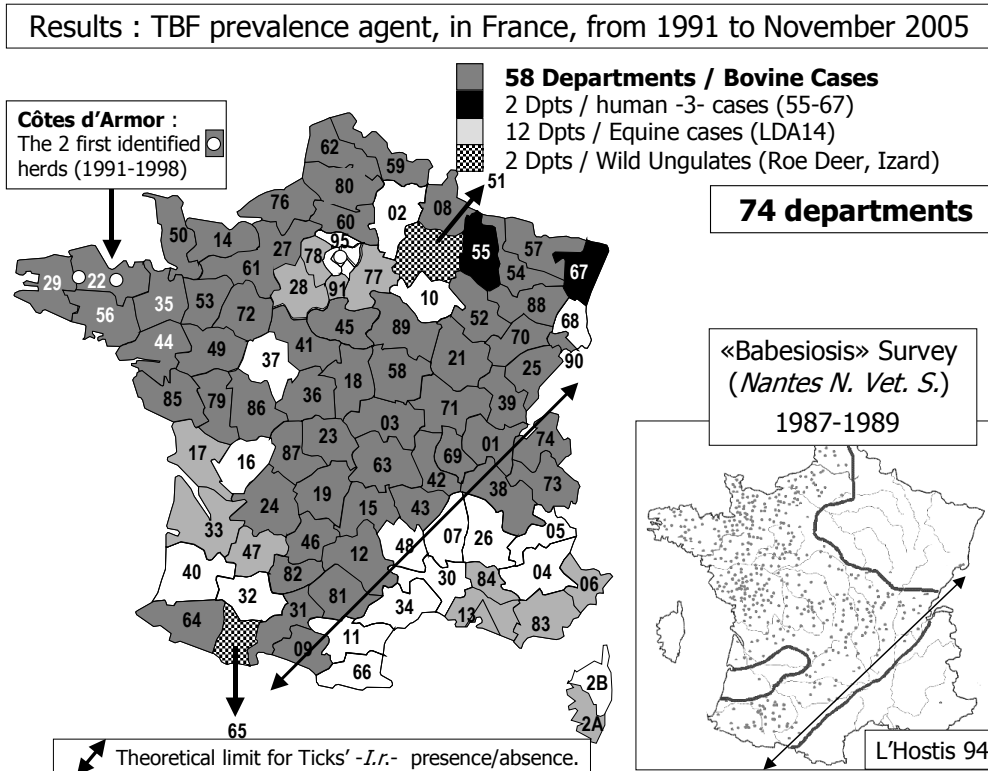
As early as 1961, in Scotland, and without many efficient diagnosis tools, Foggie & Wilson demonstrated the aetiological role of this bacteria in cows abortion epidemics (Wilson *et al.* 1964). On ewes and goats, much more studies have been realized (Chevalier, 2002; Joncour *et al.* 2003). When sporadic and early, even late, abortions, the causative reason is much more difficult to identify. At low cost. With veterinary practitioners’ help, the local farmer association for sanitary protection (GDS22) has set a diagnostic protocol which is systematically applied when three or more un-brucellic enzootic abortions occur in a herd. Serological (brucellosis, leptospiroses, neosporosis, IBR, MDV, Q Fever) and bacteriological (salmonellosis on mixed faeces) investigations are applied on the miscarrying cows group and on up to seven other reference females. Three of these kind of abortive sites (focuses), in my practice, were chosen on biotopical and sanitary criterions, on December 2001. 27 sera were analysed (IFAt).

Moreover, in July 2003, a survey was carried out in seven selected herds, in Côtes d’Armor, answering these criteria of inclusion (Vassallo): spring abortions, blood samples done at least 15 days after and before 90 days after abortion (Joncour *et al.* 2003; Liz, 1994).

The “biotope effect” was: “at least one Q fever seropositivity per herd, as potential dual infection and potential tick presence”). 66 IFAt (26 miscarrying cows and 40 pilot-reference ones) were done, blood taking carried out between mid May and mid June, 2003.

3.3 National TBF prevalence agent, 1991 to Nov. 2005. GTV Study results. (Fig. I).

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But we know well that meteorological disturbances and the Planet warming up are able to be a good change factor for Ticks and other *Arthropodae*. We think that we have contributed, during these seven last years -and we go on- to prove that TBF and *A. phagocytophilum* presence in proper biotopes is not an “only Breton sanitary speciality”. This National map of Departmental prevalence is probably the same as *I. ricinus* one's [8,10]. Easy to compare to Babesiosis one's : **74** Departments, at least, “shelter” *A. phagocytophilum*, from 1998 to November 2005, in France. This inquiry illustrate, too, the bio-indicator species (or individual) concept : as previously mentioned, in Marne (51) and Hautes-Pyrénées (65), Roe Deer and Izard (*Rupicapra rupicapra pyrenaïca*) show that. There, no human, equine or bovine case has been identified up to now. Up today. In Meuse (55) and Bas-Rhin LR (67), eastern part of France, Man seems to be a “**marker**”, a bio-indicator, for herds, at least : GP J-C. George identified the first two cases on patients, in 1999 [5,6]. In 2003, P. Brouqui [16] diagnosed an atypical pneumonitis induced by the bacteria, in the other. Animals, there, seem to be healthy. And Ticks [6] ? In the **58** other French Departments, where bovine cases were discovered, the “cow is really able to be a bio-indicator for Mankind”[8].

3.4 Blood samples on wild species

Blood, spleen and ticks were collected, *post-mortem* mainly, between November 10th, 2002 and February 28th, 2003, from “big reservoirs”: 103 Roe deer, 31 Red deer in “ranching”, 31 Red foxes (*Vulpes vulpes*) and six boars (*Sus scrofa*). Among 143 sera, 148 blood samples, 92 deep frozen spleens and 19 groups of ticks on Roe deer, only 99 specific serologies and 63 PCR on spleen were done. Only 85 sera and 53 spleens (Roe deer), from our practice, were monitored. The “small reservoirs”, mainly 132 European Woodmice (*Apodemus sylvaticus*) were trapped in four infected spots, between October 24th and November 17th, 2002. 132 EDTA bloods, 132 spleens and pooled ticks from 56 parasited mice were stocked. PCR test was applied on blood and spleen, pooled by five.

3.5 Ectoparasites

Nine larvae, 1,233 nymphs, 74 females and 85 males free-living, and 24 engorged females *I. ricinus* -1,425- were collected, mainly flagging vegetation, in the 21 study areas. 176 PCR were done. 151 on ticks pooled from each site (from 3 to 19 per spot), taking into account fed, unfed, free-living or fixated parasites and stage. Bloodsucking *Dipterae* -399- (mainly *Stomoxys g.* and *Tabanus g.*) were collected, in August 2002 in two “infected” herds apparently “tick-free”, using a domestic vacuum-cleaner. 5 *Tabanidae* and 16 pooled heads of *Stomoxidae* (cleavage very easy after deep freezing and shaking) were tested on, to try to appreciate a potential mechanical transmission (iatrogenic way) (Joncour *et al.* 2003; Woldehiwet, 1993). In one herd, two engorged ticks, previously fixed on sick cows, “PCR-pos” in blood, were “PCR- neg”. On June 2005, after an 8 years remission, three rather old dairy cows were severely struck down.

4. RESULTS

About Western region prevalence in the 21 monitored herds, all the 50 youngest heifers never released on pastures before, are seronegative at the first test. 35%, from 213 sera sampled in spring are positive in the 11 “clinical” herds. 51% of the 123 tested again in autumn (in only 8 sites). Only 6/10 herds in the “bio-indicator” group, were tested at a proper time: 64.2% are positive (43/67) in summer.

About the reproduction incidence point, we suspected, from the outset of this study (1999), on the one hand *A. phagocytophilum* as an abortive agent, on the other hand, an important infra-clinical positive seroprevalence being: the “iceberg effect”, undetectable only through the “clinic”. 29.63% (8/27) of blood samples collected in winter 2001 in three herds with autumnal abortions are positive. They are in suitable ticks biotopes, but a “major” abortive aetiology was demonstrated in each case (*Neospora*, MDV, salmonellosis). This shows that a “background noise” does exist, and immunity breakdown is also one of the correlated factors. No clinical signs. In March 2004 we identified a sporadic Lyme borreliosis case in one, on a newly bought heifer (IFAt 1/800). Only 5.75% (5/66) are positive in the 7 dairy herds tested. However one herd was really an *A. phagocytophilum* abortive site (4 IFAt positive on 5 aborted cows, 4 late and 1 early, also highly seropositive to Q fever agent, in a good biotope).

About biological analyses on wildlife, on Roe deer, 74.12% (Joncour *et al.* 2003) (63/85) usable bloods collected on the sympatric populations of the areas studied are positive by IFAt, in winter 2002-2003. When we compare to a national sample (400 sera), 75% are positive, too (1999-2003) (Joncour *et al.* 2003). 2/3 to 3/4 positive seroprevalence for *A. phagocytophilum* antibodies is a good approximation, on Roe deer in winter, at least in our Western country. Among 12 sera and 8 spleens tested on Red fox, only one blood was seropositive. The homologous spleens were not analysed (Joncour *et al.* 2003). All the woodmice blood and spleens were negative (Joncour *et al.* 2003). All the ticks collected in the 21 study areas and mainly in “risky spots”, on cows or flagging, also PCR negative (176).

5. DISCUSSION

5.1 Information means and data conveyance

The quick study area increase, to the other Breton departments was helped through wide news and braining information toward vets: in early Spring 2002, we had at least, one “clinical” herd per Breton subdivision. Only possible when Tick-borne fever emergencies are diagnosed, on acute stage.

Mainly sending diagnosis cards through wholesalers and on internet website. Through these means, we decreased the “undiagnosed” feature of this widespread pathology, even out of the Western study area.

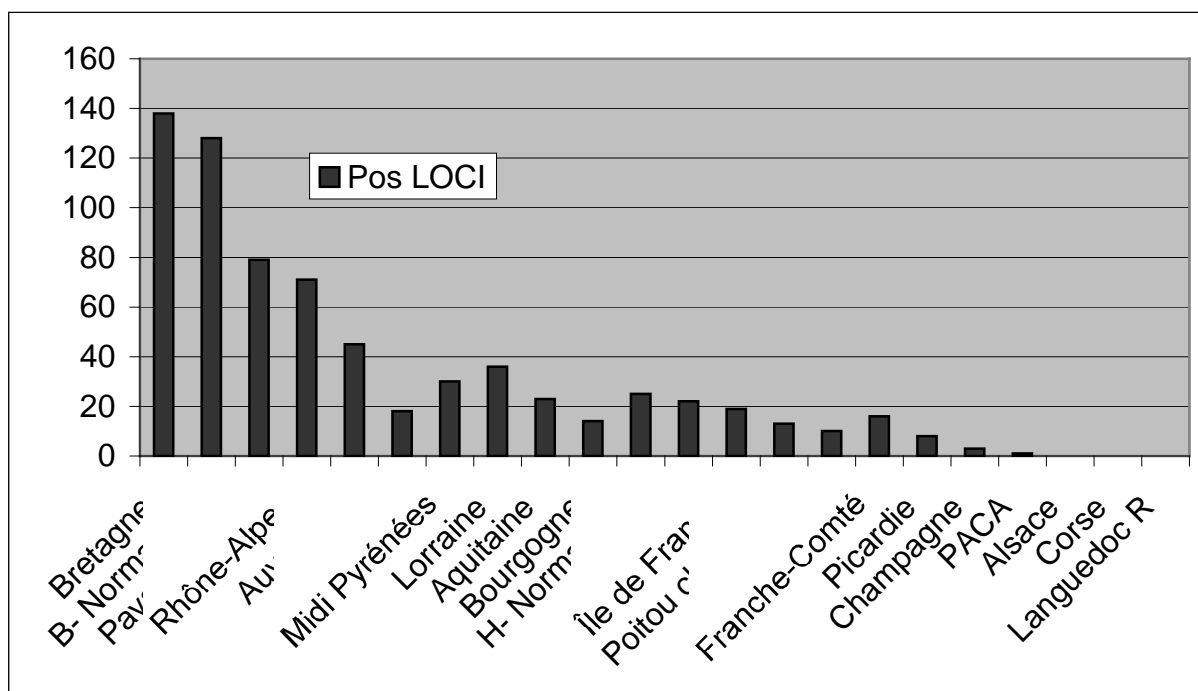


Figure 2. Tick-borne fever infection focuses in 22 French Regions

Sources: LDA22, LDA14 and LDA79 results (Feb. 2002 - Nov. 2005).

Cows, mainly, plus 138 horses [LDA14] (> 700 loci)

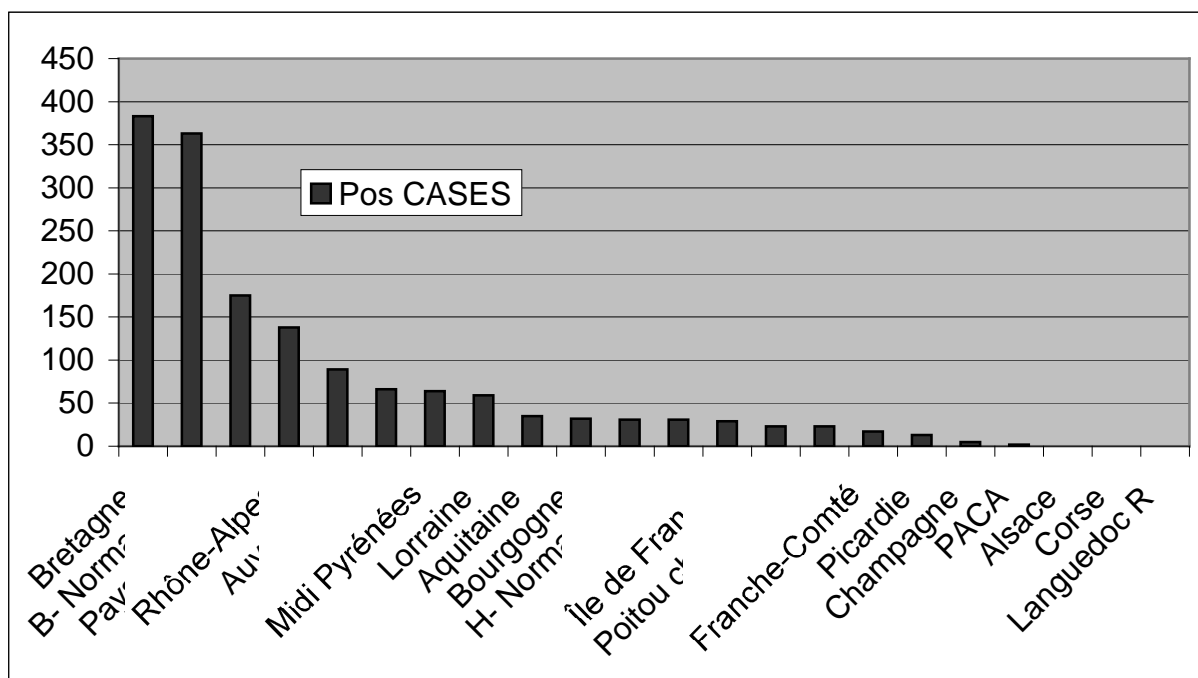


Figure 3. 1,578 Tick-borne fever cases, diagnosed by cyto-haematology (40), IFA (1450) or PCR (88)

Figures 2 and 3 show the diagnosis pressure in the 3 Western regions: 921 “positive” cases (~ 58%). This does not demonstrate that Tick-borne fever is localized in these western districts... This activity improved our attempt to prove, show, discover and meet with European practitioners, spread news and learn from others (vets, but also doctors and other biologists), even in this World Buiatric Congress.

5.2 Prevalence enquiries

The short life time of G Ig antibodies allows many reverse seroconversions, throughout a year (Joncour *et al.* 2003). However, our results, through this 21 sites study, even incomplete and coming from a medium size sera samples would be useful for sanitary prevention: seroprevalence on the same group (n =10) of cattle “touching” this bacteria grows from 35% to 51%, from spring to autumn. The average prevalence in the "reference" sites (n = 11) is 64% in summer. This group, juvenile “healthy” heifers, we think so, has the advantage of a primo-infection useful to a natural providing immunity (Joncour *et al.* 2003). Precisely when they produce only “meat and bones”. No embryos, nor milk (Joncour *et al.* 2003). In Brittany, with a mild wet oceanic climate, heifers (12 to 24 months) and dairy cows use to graze all year round, at least in broad daylight for the last ones. Then, they may be infected by ticks at every season.

We proposed other prevalence tests suitable in (or after) some sanitary events: one of our four dairy herds, before BSE “stamping out” in August 2001, was located in a valley not far away from two active Tick-borne fever focuses. It appeared convenient to us, according to the assets, to control the herd, before departure, by specific *A. phagocytophilum* serology. The 20 heifers and young cows were negative. Introduced animals (merging or translocation) may be “naïve” in front of the local pathogen agent. Therefore they are very susceptible and receptive to it. This explains the high disease incidence on newly recombined herds, after sanitary policy procedures (on the 120 first western outbreaks, 12 were previously BSE ones).

In anademic well identified areas, when bringing in pregnant or milking adult cows, vets must know and assess the risks due to a potential primo-infection (Joncour *et al.* 2003). The purchaser will wrongly blame the seller.

About reproduction point, a Scottish team (Wilson *et al.* 1964) showed brightly the abortive capacity in the last third of pregnancy. Abortion is possible, too, before 6 months. What we know well: hyperthermia is “embryotoxic” (Joncour *et al.* 2003). Crossing of the placental barrier was highlighted (Pusterla *et al.* 1997). On an aborting sick cow, we found only “PCR positive” on blood of the calving cow and up to the cotyledon attachments. Nothing in foetus (Joncour, Argenti, Brouqui, in preparation).

But, when sporadic, abortions may, too, have a Tick-borne fever aetiology. Not easy to prove, then: the only biological test, according to french sanitary rules is brucellic tests (bacteriology on afterbirth and serology). This aetiology will have, later and also elsewhere, to be considered with attention. And taking into account sanitary policy based now, on the annual sanitary inquiry, for each cattle unit: late Tick-borne fever abortions are no longer a costarmoricain dairy cows feature.

5.3 Wildlife biological samples - “Reservoir” species

This vet fieldwork is not a wildlife pathology investigation: our main aim is domestic animals and Public Health related to zoonoses. But better infection knowledge of the potential natural pathogen carriers, for vector-borne diseases and their serological status of the wild harbouring species is necessary to well understand the eco-pathogeny. Then treatments and control. About serology, in a previous study (1999-2002), 75% (230/306) Roe deer sera were positive. No reason that out of the whole study area, this prevalence will be equivalent, at least, in central Brittany. Winter, the hunting season, is a bad season for ticks. And for all places where ticks and Roe deer are sympatric, in France, at least. We use to examine some Roe deer from the coastal zone, heavily infested by ticks all around the year: it would be interesting to analyse RD sera, from there and from elsewhere, at each season.

Nevertheless, Roe deer is a very good “restaurant”, all year long, for *Ixodes ricinus*. On Red deer (“ranching”) blood samples -little ones-, seroprevalence was from 11 to 60% (Joncour *et al.* 2003). This type of breeding can be very useful and easy to investigate for a prevalence investigation on a broader and even national scale (sanitary controls on these meat -and antlers- producers do exist).

PCR associated to IFAt test: Liz (1994) on Helvetian Roe deer blood samples (1988-1989), found 78.2% seropositive sera (104/133). In a second study (2002) (Liz *et al.* 2000), on 103 of these sera, 18.4% are PCR positive (PCR-pos). He notes a “non-obligatory correlation” between seropositivity and a positive PCR result. On the other hand, he shows that 82.4% of the positive PCR sera are seropositive and only 26.2% of seropositive sera are PCR positive, too. For our part, among the 85 monitored Roe deers, we sampled 53 spleens on which PCR test was paired with serology: 85% (17/20) of the “spleen PCR-pos” are also seropositive. And 44.7% of the seropositive are also “spleen PCR-pos”. This compared to Liz’s study seems to indicate, here, a higher positivity in spleen (44.7 vs 26.2%). Three seronegative animals, among the 20, were “spleen PCR-pos”. But, low samples. As for other bacterial and parasitic infections, spleen seems to be an important host-organ harbouring for a long time. These pathogenic agents (Chevalier, 2002; Joncour *et al.* 2003; Stuen *et al.* 2001) were also used in many challenge studies, also surgical splenectomy, to prove a persistent infection. Mainly Roe deer, even an epidemiological dead end (Joncour *et al.* 2003), is a good study model: adults are proper hosts for ticks, territorial-living in a small vital area (from 1 to 2 km²), sedentary, sympatric with cows and widespread in France, elsewhere. Red fox is, too, as a canine species, receptive to this bacterial infection. Our short results (one seropositive for 12 available sera = 8.33%) cannot allow any conclusion. This prosperous predator and scavenger lives very often close to dairy herds: it is even seen in the cowsheds and calving rooms, but it is more an host for *Pholeoixodes hexagonus*, a cavernicolous tick. Studies on this species and its epidemiological place go on (but not as for neosporosis...).

None PCR carried out on spleens and blood of the micro-mammalian populations was positive in our study. Some parameters can explain these negative results: more than sample conservation, the PCR probe temporarily ineffectiveness. Matsumoto seems to confirm it (Matsumoto *et al.* 2005). Liz however proved that these micro-mammals are a good reservoir (Liz *et al.* 2000).

Ixodes ricinus is the main biological vector. And reservoir, moreover (bacterial survival and division, growing). Many other arthropods, insects mainly and specially dipterous can be iatrogenic agents (Joncour *et al.* 2003; Woldehiwet, 1993). This is well known for bovine anaplasmosis due to *A. marginale* (Joncour *et al.* 2003), widespread in France, usually on sporadic form. “Risky” biotopes and plots for babesiosis (*B. divergens*), bovine anaplasmosis, Q Fever (*C. burnetii*) or Lyme disease (*Borellia g.*) are good potential reservoirs for Tick-borne fever agent, too. Other vectors potentially involved in Tick-borne fever epidemiology would also be an assumption. Not confirmed, but probable for many rural French veterinary practitioners, these last seven years. This emerging disease on cows living on plots -and in larger areas- where ticks *I. ricinus* have never been found suggests the existence of other biting vectors. And other reservoirs (Woldehiwet, 1993).

Only nymphs and unfed adult females are able to infect cattle. For man, nymphs are the usual, accidental vectors for these Tick-borne diseases. No and evidence on our 1,425 ticks, although we had privileged the “risky” plots, in 21 places. We think that our PCR process, for vectors and tissues (except blood and RD spleen), failed. However, DNA extracts from 156 tick pools and from 18 bloods and 17 spleens of these European Wood-mice were applied for DNA extraction and PCR at the National Rickettsial Reference Unit of Marseille -Pr. Raoult- (Matsumoto *et al.* 2005). 113 samples and one of 17 spleens were positive for PCR for *Anaplasma/Ehrlichia* 16S rRNA gene, and 2 of 17 blood samples (rodents) were positive. Sequencing of 16S rRNA gene, among randomly chosen positive tick samples applied for 16S

rRNA PCR, two were related to *A. phagocytophilum* group and one was 100% similar to *Ehrlichia* sp. HI-2000. In this study, Matsumoto demonstrated *Ehrlichia* sp. presence in *I. ricinus* for the first time, isolated, before, on *Ixodes ovatus*. The distribution of *I. ricinus* ticks infected with *A. phagocytophilum*, and the presence of small rodent infected with *A. phagocytophilum* in Brittany was also proved. Infected ticks were detected in 6 of 17 places throughout Brittany. But the prevalence rate of ticks infected was unknown: the samples used in this study contained those from pooled ticks.

5.4 Tick-borne fever control

The reference curative treatment is Oxytetracyclin 10% (3 days), associated to possible symptomatic therapies. The companies do not mention this indication on the data sheets (Joncour *et al.* 2003).

The agent is transmitted by biological bloodsucker carriers, very resistant in their environment. So, the control is not easy (Joncour *et al.* 2003) for biological vectors. Tick-borne fever agent is really ubiquitous. Wild various reservoirs do exist. Moreover, it is an “environmental disease”: biotopes are reservoirs, too. About disease prevention, offensive and defensive measures aim to limit the ectoparasitic pressure without totally cancelling it. This ground-report (Joncour *et al.* 2003) seems to show the existence of natural primary infection immunity. As the effect of a primo-infection, inducing a premonition immunity with rather strong and efficient protection.

Environmental measures management resolutions can also be proposed (Joncour *et al.* 2003): a reasonable up-keeping of herbaceous formations on hedges and the use of the wire fences can efficiently reduce heavy ticks infestations, in our region at least. And consequently decrease contaminations (primary or not) or spread them, with less pressure, through a longer period: rational and moderate slopes pruning, drawing back the electric wire fence at some distance from slopes and fences. And mechanical weeding under it! Forsaking these plots accused to be good reservoirs to waste lands or fallow fields or alternative farming can be a good alternative: they can be included in rotations of crops (fodder maize and others). But, in a better way, in our opinion, it is mostly better to keep them for un-pregnant heifers, for the reason already mentioned. An ecological strategy!

Diffusion of information contribute too, to control Tick-borne fever : the 2003 International Congress, in Brittany, took place on the site of “ISPAIA-Zoopôle Développement Research Center”, Ploufragan (22). We released our first conclusions upon that under-diagnosed Tick-borne fever/human anaplasmosis, veterinary and human aspects. Among the 120 people taking part in the debates, from five nationalities (Swedish, Swiss, Italian, French and... Breton). Physicians, National Veterinary Schools professors, vets from the local veterinary laboratories, from public safety and social regional agency, from sanitary policy services, practitioners, agronomists and professional vet press agents showed their interest for this broad theme. It enabled also fruitful contacts with other European sanitary structures. And to be aware of the importance of requests from veterinary practitioners about zoonotic *Arbo*-bacterioses.

Seventeen evening meetings of information, all around France (one in EIO end of January 2004), about “Summer Flu Syndrom”, enabled the veterinary practitioners (60%: 225/374 and 7 human practitioners) to decrease the field diagnosis delay and increased the number of “infected” departments. This tour was helpfully enabled by logistic support of Merial, main sponsor, from the beginning. In September 2004 we participated to a round table about Tick-borne disease in the “Entretiens de Bichat” Congress. Mainly for general practitioners.

A Sardinian Congress (May 2004), on the emerging Tick-borne disease/human anaplasmosis), ESCMID meeting, in Logroño (Portugal, June 2005) and TTP5, in Neuchâtel (Switzerland,

September 2005). International Congresses usefully completed our contacts, meeting famous European and American searchers. We hope some more useful encounters, in WBC Nice 2006, this year, enthusiasts.

5.5 Prospectives

Some findings and topics have been assessed. Many others must go on. Mainly on sensitive species/hosts, vectors and reservoirs, aetio-pathogeny. Epidemiological studies at a very advanced level are thus limited in methodology and conclusions according to the cycle of the vectors, inducing (or not) an external parasitism of the wild species-hosts, seasonal variations in the clinical cases occurrence on dairy cattle, mainly in spring and secondary in autumn. We would have to improve our national qualitative prevalence: wildlife and especially, Roe deer will be an efficient tool. A good bio-indicator for cows. And consequently for humans too. In France, a survey started during the last hunting season (2005-2006) in 22 departments where no signs of *A. phagocytophilum* presence were seen on cows, horses. The two where acute stages have been diagnosed on man, were monitored, too. Ranching wild species such as Fallow deer (*Dama dama*) or Red deer (Joncour *et al.* 2003) are also tested. In Highlands and mountainous departments, 26 with biotopes at an altitude between 700 and 1500 metres, other *Artiodactylae* -except boar will be tested. *Sus scrofa* is taken into account, for Corsica island, where no Roe deer and ... ticks on it. *ricinus* in France (Estreda-Peña, 2005).

Four sera (IFAt), spleens and ticks (PCR) will be analysed per department. Hunting season is the best period to collect biological samples on hunted mammals and store them. On other hand, winter is not a very suitable season to collect ticks on these hosts (Joncour *et al.* 2003).

These data would be added to our 5,750 ones, from LDA-22, LDA-14 and LDA-79 results (mid-February 2000 to the end of November 2005), i.e. (179 doubtful), 3,970 “negative” and, moreover, the 1,578 “positive” (from cows, mainly and, then, from horses), by cyto-haematology, IFAt or PCR (Figures 2 and 3). This would be useful, for “measuring the risk by ticks. Through improvement of a high-resolution model to map the habitat suitability of *I. ricinus* in France (Estrada-Peña, 2005).

On equidae, specialized French veterinary networks are studying the “Piroplasmosis-like Syndrome” (i.e. “Babesia-like Syndrom”), including equine anaplasmosis/Tick-borne fever. Possible prevalence survey would be very useful, too, on sheep and goats, too (named as “good reservoirs”) ((Joncour *et al.* 2003; Stuen *et al.* 1998, 2001), especially where *I. ricinus* and chlamydophilosis, Q. Fever or eperythrozoonosis are present, and where sheep on the move (Alp and Pyrenean Mountains). About the human constituent, during our regional survey (Brittany), we tried to inform rural vets and their colleagues, general practitioners, especially in “Lyme disease endemic spots”, and health and safety administrations to be “sensitized”. Prevalence investigations on, first, 250 blood sampling (IFAt) were proposed, for populations who run a risk and on sera from Lyme borreliosis western patients “+ +”. Without any reply. In a second way, try to pair, at national scale, serological surveys associated to Lyme disease or arboviroses as central European tick encephalitis (TECE) or West-Nile fever studies. Moreover, a cheap diagnostic tool has been tested (our trainee: Achard), through a national haematological investigation (cytology and biochemistry). Acute stages, clinically diagnosed confirmed on 38 dairy cows (98 “neg”) by cyto-haematology or PCR, have been studied, from France, in 2004-2005. Severe but temporary thrombocytopenia and leucopenia are a good presumptive evidence. Increase of hepatic parameters on people, too. These last do not seem to be obvious on cattle... One aim was to better field diagnosis on cows. Another, was to compare semiology (hepatic enzymology) with human parameters: these were investigated, at three occasions (D0, D7 and D30) and for the last two, on 3 to 5 “pilot” commensal cows. The study was stopped at the end of November 2005. Some of these cows died, on acute stage: it appeared important to try to identify the lethal -bad- strains of *A. phagocytophilum* by DNA sequencing on spleen, mainly. That would be interesting, too, in

terms of compared infectiology. About bloodsuckers as *I. ricinus*, a genuine national and widespread sample survey would be an emergency. Not really done, still in France. A wide multi-centric network is going on, now, coordinated in CNRS/IRD Montpellier (Groupe Tique REID). One of his aims is a ticks cartography. We suppose that iatrogenic biological conveyance of the pathogen by some dipterous (*Tabanidae* or *Stomoxidae/S. calcitrans*), is possible (Joncour, 2003 ; Woldehiwet, 1993).

6. CONCLUSION

From two Tick-borne fever sources in 1998, identified in Brittany, more than 700 are currently known, all over France (2005). This study resulting from field data (1999-2005) proved the importance of a background noise highlighted by positive serologies (IFAt) on many species in suitable Western biotopes. Elsewhere, too. We showed a really widespread national presence area, in focuses, proved abortions. Seroprevalence is significantly more important in young animals tested in control bovine “healthy” herds than in infected bordering ones. Our work concludes that it is essential to achieve a providing premonition immunity by first voluntary infection, in order to avoid problems during gestation or lactation: this can be done putting first, in spring, un-pregnant heifers in plots known “at risk”. Tick-borne fever is a “disease of the translocation”, by the introduction of “naïve” animals into a “reservoir”. Ten percent of the 120 first sources identified in the context of the “stamping out” for BSE are not a result of the chance. It is unrealistic to want to fight against ticks, vectors and other reservoirs agents (environment and wildlife). Largely underestimated and under-diagnosed in France, the lack of knowledge by general practitioners of human anaplasmosis is comparable to that noticed among veterinary practitioners for Tick-borne fever. This collective study shows the need for joint multidisciplinary teams and networks (veterinary and human practitioners, agronomists, zoologists and entomologists, too) in order to make known and to prevent these types of “biotopes pathologies”. It highlights the effectiveness of the informal network for monitoring epidemiology between country vet. practitioners (SNGTV), in spite of the difficulties of work in veterinary rural practices. Roe deer may be a bio-indicator species for the cow. And cows, potentially for mankind, when acute stages. The man-veterinary and doctor- must be a sentinel for these shared pathologies, the Tic-borne diseases/VBDs. Here, compared clinic shows numerous semiological identities, one of them is Summer Flu-like Syndrome (but headache is hard to identify on cattle). Classified by the sanitary authorities as a “minor zoonosis”, another goal is public health and safety, by convincing them of the presence and impact of *A. phagocytophilum* in France. In the same time, as often, animals would be useful tools, bio-indicators for public health and safety.

7. SUMMARY

Bovine Tick-borne fever is too often under-diagnosed in Brittany as in the whole country, especially in its sporadic or sub-acute stage. On the other hand, the acute disease can be suspected when it is associated with infectious pneumopathies in tick infested pastures. This multispecific “flu-like syndrome” regularly associated with persistent temperature and mild prostration is, mainly, a “dairy cow pathology”. Locomotion signs (e.g. “cold” oedema on distal limb parts) are pathognomonic, when present. The financial impacts can be high, if the diagnosis is late. An epidemiological survey in Côtes d’Armor, which was initiated in 1999 by a rural veterinary practitioners group (URGTV Bretagne) was extended in 2001 to the whole of Brittany. The programme was extended from 2002 to June 2005 to the rest of France, through the SNGTV network. Undiagnosed in France before 1991 and widely unknown up to 1998, *Anaplasma phagocytophilum* and its effects have now been identified in different mammalian hosts in 74 of 96 departments, including 58 cows, 12 horses, 1 Roe deer, 1 lizard and 2 humans (November 2005). More than 700 sites of bovine infection have been identified, affecting 1 to 40 cows in each site.

A large network of practitioners, a good knowledge of the clinical diagnosis confirmed by laboratory or an *a posteriori* diagnosis, associated to private and public support, allowed us to improve our knowledge of the disease. Our first aim is to reduce the economical loss to agriculture and, accordingly to improve animal's health. Therefore we must inform and propose relevant sanitary directives and actions and share broadly our new experience and knowledge. Classified by the French sanitary authorities as a "minor zoonosis", another goal is to improve public health by convincing the authorities of the presence and impact of *A. phagocytophilum* in France. In the same time, as often, cows -and other animals, domestic or wild- can be useful biological tools, bio-indicators for public health and safety.

8. KEY WORDS

Anaplasma phagocytophilum, Tick-borne fever, dairy cows, bio-indicators, ticks, vectors, reservoirs, biotopes, epidemiology, human anaplasmosis, zoonoses.

9. RESUME

L'ehrlichiose granulocytaire bovine est, encore de nos jours, une pathologie sous diagnostiquée, en Bretagne et au niveau national, surtout dans les formes sporadiques ou en phase sub-aiguë. On peut suspecter la forme clinique aiguë lors d'affections pulmonaires en pâtures infestées de tiques. Ce "syndrome respiratoire/grippal d'été" d'étiologie souvent multifactorielle et associé à une hyperthermie tenace et à de l'asthénie, est d'abord une pathologie des vaches laitières. Les signes locomoteurs (œdème froid des articulations distales/paturons) sont pathognomoniques, quand ils sont présents. L'impact économique peut être élevé si le diagnostic est tardif. Une étude épidémiologique dans les Côtes d'Armor, initiée en 1999 par une association de praticiens (URGTV Bretagne) a été étendue, dès 2001 à toute la Bretagne. Ce programme s'est généralisé, de 2002 à fin 2005 au reste de la France, dans le cadre du réseau de la SNGTV. Inconnue en France avant 1991 et très largement ignorée jusqu'en 1998, l'agent pathogène *Anaplasma phagocytophilum* tout comme ses effets, sont maintenant bien identifiés chez divers mammifères-hôtes dans 74 Départements sur 96, dont 58 à cas bovins, 12 à chevaux, 1 à chevreuil, 1 à isard et 2 à cas humains, à novembre 2005. Plus de 700 foyers d'infection chez les bovins ont été localisés, comprenant de 1 à 40 individus.

Nos progrès dans la connaissance de cette affection ont été permis grâce à un réseau étendu de praticiens, une bonne connaissance de la clinique confirmée par des outils de laboratoire, parfois *a posteriori*, associés à des aides privées et publiques.

Notre objectif principal est de réduire les pertes économiques de nos éleveurs. En parallèle, d'améliorer la santé animale. Dans ce but, nous devons faire circuler l'information et proposer des axes et stratégies d'ordre sanitaire tout en exportant notre expérience et acquis. L'ehrlichiose granulocytaire à *Anaplasma phagocytophilum* est classée par les instances sanitaires françaises parmi les "zoonoses mineures". Parmi nos objectifs, l'amélioration de la santé publique en est un autre en tentant de convaincre ces Institutions sanitaires du statut et de l'impact potentiel de cet agent en France. Par la même occasion, comme bien souvent, les vaches et les autres animaux dits inférieurs, domestiques ou sauvages, peuvent constituer des outils biologiques efficaces, des bio-indicateurs/marqueurs, en santé publique.

10. MOTS CLES

Anaplasma phagocytophilum, ehrlichiose granulocytaire bovine, vaches laitières, bio-indicateurs, tiques, vecteurs, réservoirs, biotopes, épidémiologie, anaplasmosse humaine, zoonoses.

11. ZUSAMMENFASSUNG

Das Weidefieber der Rinder in der Bretagne und in ganz Frankreich ist zu oft unterdiagnostiziert, besonders wenn es sporadisch auftritt oder subakut verläuft. Andererseits kann die akute Erkrankung vermutet werden, wenn sie mit erregerbedingten Lungensymptomen und Zeckenbefall der Weiden assoziiert ist. Dieses unspezifische grippeähnliche Syndrom ist regelmäßig mit persistierenden Temperaturen und leichter Erschöpfung verbunden. Ein Befall des Bewegungsapparats (i. a. ein Ödem der unteren Extremitäten) ist pathognomonisch, sofern vorhanden. Die ökonomische Bedeutung kann hoch sein, wenn die Diagnose zu spät kommt. Eine epidemiologische Untersuchung, die 1999 von einer veterinärmedizinischen Vereinigung (URGTV Bretagne) in Côtes d'Armor begonnen wurde, wurde 2001 auf die ganze Bretagne ausgedehnt. Von 2002 bis Juni 2005 wurde der Rest Frankreichs durch das SNGTV Netzwerk miteinbezogen. Nicht diagnostiziert vor 1991 in Frankreich und weitgehend unbekannt bis 1998, wurde *Anaplasma phagocytophilum* nun bei verschiedenen Säugetierspezies in 74 von 96 Departements gefunden, darunter 58 Kühe, 12 Pferde, 1 Reh, 1 Gämse und 2 Menschen (November 2005). Mehr als 700 Gebiete mit Infektionen von Rindern wurden identifiziert, wobei zwischen 1 und 40 Kühe betroffen waren.

Ein weites Netzwerk von Veterinärmedizinern, eine gute Kenntnis der klinischen Diagnose, eine labormedizinische Bestätigung und private und öffentliche Unterstützung haben uns erlaubt, die Kenntnis der Erkrankung zu verbessern. Unser erstes Ziel ist die Reduktion des wirtschaftlichen Verlusts und die Verbesserung der Tiergesundheit. Deshalb wollen wir Informationen und relevante Richtlinien verbreiten und unsere Erfahrung und unser Wissen zur Verfügung stellen. Da das Weidefieber von den französischen Gesundheitsbehörden als untergeordnete Zoonose klassifiziert wird, ist ein weiteres Ziel, die Behörden von der Bedeutung von *Anaplasma phagocytophilum* in Frankreich zu überzeugen. Kühe und andere Haus- und Wildtiere können als Indikatoren für die öffentliche Gesundheit und Sicherheit dienen.

12. SCHLÜSSELWÖRTER

Anaplasma phagocytophilum, Weidefieber, Milchkühe, Indikatoren, Zecken, Vektoren, Reservoir, Biotope, Epidemiologie, humane Anaplasmosis, Zoonosen.

13. RESÚMEN

La fiebre bovina por garrapatas es a menudo poco diagnosticada tanto en Bretaña como en el país completo, especialmente en su estadio sub-agudo, esporádico. Por otra parte, se puede sospechar de la enfermedad aguda cuando se asocia con neumopatías infecciosas en pastos infestados por garrapatas. Estos "síndrome gripal" multiespecífico, regularmente asociado con fiebre persistente y con postración suave, es, principalmente, una patología de vacas de leche. Los signos locomotivos (edema frío en la porción distal de las patas) son patognomónicos cuando aparecen. Los impactos económicos pueden ser altos, si el diagnóstico es tardío. Un estudio epidemiológico en Côtes d'Armor, que se inició en 1999 por una sociedad de veterinarios rurales (URGTV Bretagne) se extendió en 2001 a toda la Bretaña. El programa se extendió entre 2002 a Junio de 2005 al resto de Francia, a través de la red SNGTV. Sin diagnosticar en Francia antes de 1991 y casi desconocida hasta 1998, *Anaplasma phagocytophilum* y sus efectos han sido diagnosticados en diferentes mamíferos en 74 de los 96 Departamentos, incluyendo 58 vacas, 1 gamo, 1 reptil y 2 humanos (Noviembre de 2005). Se han identificado más de 700 sitios de la infección bovina, que afectaban a entre 1 y 40 vacas de cada sitio.

Una amplia red de veterinarios, un buen conocimiento del diagnóstico clínico confirmado por el laboratorio a posteriori, asociado con el apoyo público o privado, no ha permitido mejorar el conocimiento sobre la enfermedad. Nuestro primer objetivo es reducir las pérdidas

económicas en la agricultura y mejorar la salud animal. Por ello, debemos informar y proponer a las autoridades sanitarias relevantes, acciones y directives, compartiendo nuestra experiencia y conocimiento. Clasificada por las autoridades francesas sanitarias como una “zoonosis menor”, otro objetivo es mejorar la salud pública convenciendo a las autoridades sobre la presencia y el impacto de *Anaplasma phagocytophilum* en Francia. Al mismo tiempo, otros animales, domésticos o silvestres pueden ser adecuados útiles, bio-indicadores para la salud pública.

14. PALABRAS-CLAVES

Anaplasma phagocytophilum, fiebre por garrapatas, vacas lecheras, bio-indicadores, garrapatas, vectores, reservorios, biotopos, epidemiología, anaplasmosis humana, zoonosis.

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