Epidémiol. santé anim., 1997, 31-32

RETROSPECTIVE EPIDEMIOLOGICAL SURVEY ON FALSE POSITIVE SEROLOGICAL REACTIONS IN BOVINE BRUCELLOSIS IN SAÔNE-ET-LOIRE (FRANCE)

Pouillot R.[1], Lescoat Ph.[2], Garin-Bastuji B.[3], Repiquet D.[4], Terrier P.[5], Gerbier G.[1], Bénet J-J.[2], Sanaa M.[2]

Cette étude décrit le phénomène des réactions sérologiques faussement positives (RSFP) observées dans le cadre de la prophylaxie de la Brucellose bovine dans le département français le plus touché, la Saône-et-Loire. Avant 1990 existait un faible taux de RSFP (taux de prévalence annuel cheptel de l'ordre de 0,3%), compatible avec la spécificité couramment admise des tests sérologiques employés. Le phénomène a explosé durant la campagne de prophylaxie 1990-91 (6%), a atteint un pic en 1992-93 (12%), pour décroître par la suite jusqu'à la dernière campagne étudiée (4% en 1995-96). 25% des cheptels ont été atteints au moins une fois. Dans la grande majorité des cas, une ou deux RSFP seulement étaient observées par cheptel.

A l'échelle du troupeau, 4 facteurs de risque présumés ont pu être mis en évidence : (1) l'apparition d'au moins une RSFP dans le troupeau était fortement et positivement liée au nombre d'animaux testés; (2) au cours d'une campagne de prophylaxie donnée, la prévalence diminuait de Décembre à Août; (3) une RSFP mise en évidence lors d'une précédente campagne augmentait le risque d'apparition pour une année donnée; et (4) la présence d'au moins un caprin dans l'exploitation était statistiquement liée à une plus forte probabilité de RSFP. A l'échelle individuelle, les jeunes animaux semblaient préférentiellement touchés, alors que le sexe ou la race ne semblaient pas être un facteur de risque présumé.

Cette étude épidémiologique renforce l'absence de lien entre les RSFP et la Brucellose. L'hypothèse d'un agent causal largement distribué lié à une faible susceptibilité individuelle est envisagée.

Since 1990, unusual high rates of positive reactions has been observed in bovine Brucellosis serological tests in the EU (Belgium, France, Great-Britain, Italy and The Netherlands) and in New-Zealand. Since no epidemiological, clinical or bacteriological link with *Brucella* infection has been observed, these reactions were considered as false positive serological reactions (FPSR). No practical serological test can nowadays discriminate these FPSR from real Brucellosis. FPSR might reveal an humoral immune response to the infection of cross-reacting bacteria, sharing epitopes with the smooth lypopolysacharide of *Brucella*. Amongst these, *Yersinia enterocolitica* O:9 has been frequently put forward, as it has been recently isolated on several occasions from cattle and other ruminants in areas where cases of FPSR had been reported. The aim of this study was first to describe the phenomenon in a highly affected region of France, and second to evaluate the linkages between some individual or herd factors with the occurrence of FPSR.

MATERIALS AND METHODS

The study population was composed of the whole screened cattle population from « Saône-et-Loire », an East-Centre French area. Due to the very low level of Brucellosis observed and the absence of vaccination in this area since 1984, FPSR were easily differentiated from positive reactions due to *Brucella* infection.

A database was built from routinely collected files of the local veterinary services and the farmer adviser services. An animal was classified FPSR if it had shown at least one positive serological result (Rose-Bengal or complement fixation test) at the screening test, in a herd which has been confirmed as free of Brucellosis on the basis of a specific epidemiological investigation.

The approach was exhaustive for the studied population. Therefore, in order to evaluate the linkages between FPSR and some individual or herd factors, differences between raw data would have been theoretically sufficient to discriminate sub-populations without any statistical test. Nevertheless, as several factors were studied, confusion bias might have occurred. In order (*i*) to evaluate meaningful sources of variation and (*ii*) to evaluate and discard interactions between these factors, categorical data modelling analyses were performed using the SAS CATMOD procedure (SAS Institute Inc, 1989). If interactions between two factors appeared to be significant, analyses were performed on each sub-population. Data were analysed within each annual screening campaign to avoid a possible repeated measurement effect for a given herd or animal.

RESULTS

Animal and herd prevalences of the FPSR were very low in 1988-89 and 1989-90 screening campaigns. The FPSR phenomenon burst out in 1990-91, reached a peak in 1992-93, and then decreased till 1995-96 (Table I).

75.7 % of the herds present from the beginning to the end of the survey have never been touched. Amongst the affected herds, 69% were concerned only once, 22% twice, 7% three times and 2% more than three times.

Before 1990-91, only one animal was affected in almost all FPSR herds. From 1990-91 to 1995-96, the number of affected cattle per FPSR herd remained low: less then 3 animals were affected in more than 80 % of the case herds. The average number of FPSR animals per FPSR herd was the highest in 1992-93, *ie* at the peak of the phenomenon. The most affected herds were the largest. For instance, in 1992-93, 95% of the herds with more

1 Unité Epidémiologie, CNEVA-Alfort, BP67, 94703 Maisons-Alfort cedex, France
2 Laboratoire d'Etude et de Gestion de la Santé Animale, ENVA, 94704 Maisons-Alfort cedex, France
3 Laboratoire de Référence OIE pour la Brucellose, CNEVA-Alfort, 94703 Maisons-Alfort cedex, France
4 Direction des Services Vétérinaires, 267 rue des Epinoches, 71000 Mâcon; 5 Etablissement Départemental de l'Elevage, 52 rue du 18 Mars 1962, 71000 Mâcon, France

than 2 FPSR animals included more than 70 tested animals, whereas this herd size class accounted for only 40% of the affected herds.

Table I
Herd and animal prevalences of FPSR in Saône et Loire

Screening campaign	Herd prevalence (%)		Individual prevalence (%)	
1988-89	29	(0.45)	30	(0.0136)
1989-90	21	(0.28)	24	(0.0086)
1990-91	480	(5.90)	793	(0.2362)
1991-92	468	(5.65)	657	(0.1836)
1992-93	1040	(12.19)	1860	(0.5046)
1993-94	755	(9.27)	1148	(0.3067)
1994-95	659	(8.44)	893	(0.2325)
1995-96	295	(3.91)	359	(0.0906)

The table II shows odds-ratios of some significant studied factors calculated from the results of the SAS/CATMOD procedure at the herd level. Herd prevalence of FPSR increased obviously with the screening operation size, whatever the studied campaign and the production system. Prevalence was lower in summer, reached a peak during the winter, and then decreased slowly. In 1994-95, two different shapes were observed depending on the production system. Whereas this trend was confirmed in beef herds, this factor appeared to be weakened and differently distributed in dairy herds. A previous FPSR increased the risk of appearance of a FPSR in a given campaign. For two campaigns (1992-93 and 1993-94), the presence of at least one goat was related to a higher screening operation prevalence of FPSR.

Table II
Odds-ratio of the herd prevalence of FPSR associated with significant factors

Factor	Class	Screening campaign				
		1992-93	1993-94	1994-95		1995-96
				Dairy	Beef	
Number of animals tested	1 to 15	1*	1*	1*	1*	1*
	16 to 40	4.1	4.2	2.7	1.6	1.5
	41 to 70	6.1	6.2	7.5	3.5	2.2
	more than 70	8.7	8.0	12.0	5.6	3.6
Sampling date	March to November	1*	1*	1*	1*	1*
	1 to 15 December	2.6	2.1	0.8	2.8	2.5
	15 to 31 December	2.9	1.8	1.7	2.9	2.3
	1 to 15 January	2.8	1.5	1.7	3.1	2.4
	15 to 31 January	2.0	1.1	1.5	2.3	1.9
	February	1.6	0.8	0.5	1.3	1.5
Presence of goats	Yes **	1.3	1.3			
Previous FPSR	Yes **	2.2	2.4	1.6	2.1	2.9

* 1: contrasted with this class; ** : contrasted with « No ».

The table III shows odds-ratios of some significant studied factors at the individual level. The herd size, the screening date, and the age of the animal were significant for beef cattle population. For dairy cattle and, in 1995-96, for the whole studied population, the age and the screening date were the only isolated sources of variation. Old animals (more than 9 years old) were much less affected, whatever the breed and the campaign. The risk factor « sampling date » was consistent with the one observed at the herd level. This result was less obvious in dairy cattle. Number of sera tested in the herd was only significant for beef cattle in 1994-95. Furthermore, changes in the herd size definition cancelled this effect (results not shown).

DISCUSSION

Analyses were performed only on concerned animals, *ie* tested ones, which accounted for *ca.* 60% of the entire cattle population: animals younger than one year and fattening herds were excluded. As no clinical effect associated with the FPSR have been recorded until now, it was not possible to evaluate the FPSR prevalence in the non serologically tested population. A specific study should be performed in a more representative sample of the whole population. The studied sample was nevertheless exhaustive for the tested population during the period. This approach strengthened the analyses, but results could not be extrapolated to other populations in France or in other countries. Risk factors underlined in this work should then, until further studies, be considered as « assumed risk factors ».

The epidemiological pattern observed confirmed the lack of relationship between FPSR and Brucellosis. Within a herd, FPSR is sporadic. This distribution could be observed in latent Brucellosis, even though it is little likely in this region almost Brucellosis-free since years. Without any slaughter procedure, the intra-herd rate of infection should increase between months and years, either with an exponential epizootic or a slow enzootic shape.

The FPSR prevalences observed before the 1990-91 screening campaign could be explained by the commonly accepted specificity of the RBT. After 1990-91, FPSR cases might be the addition of these usual FPSR, which are negligible, and new FPSR connected with (a) new cause(s). All along this survey, high structural changes of the studied population were observed, dominated by the fast concentration in beef and milk production

connected with the EU Common Agricultural Policy. It ensues from these profound structural changes new agricultural management practices, which could have led to a higher or a new exposition to a possible infectious agent. Few hypotheses could explain the fading of the FPSR prevalences observed all along the four last screening campaigns. In first approach, it could result from a lower pressure of the infectious agent or from a higher resistance of the animals after a first contact with it. This latter hypothesis could explain the higher prevalence rate observed in young adult animals, which was not recorded the year of emergence of the phenomenon.

Table III
Odds-ratio of the animal prevalence of FPSR associated with significant factors

Factor	Class	Screening campaign		
		1994-95		1995-96
		Dairy	Beef	
Number of animals tested	1 to 15		1.6	
	16 to 40		1*	
	41 to 70		1.7	
	more than 70		1.6	
Sampling date	March to November	2.9	1.5	1*
	1 to 15 December	1.7	2.6	4.3
	16 to 31 December	3.4	2.3	6.2
	1 to 15 January	3.8	2.5	5.5
	15 to 31 January	3.3	1.9	3.9
	February	1*	1*	5.0
Age	2 years old	3.3	4.2	2.9
	3 years old	5.9	2.7	2.6
	4 years old	2.7	2.9	2.3
	5 to 9 years old	3.6	2.4	2.1
	more than 9 years old	1*	1*	1*

* 1: contrasted with this class.

Within a screening campaign, FPSR prevalence was higher at the onset of the winter. It has to be emphasised that, in connection with the production system, 90% of the beef animals are tested during this season when they are kept in stables. The decrease of the FPSR prevalence observed all along the season could be explained either by a single transient serological response to a causal agent present in the cowshed, or by the end of the contact outside with this causal agent. As FPSR prevalence was not high in summer, this latter hypothesis would assume a stimulation of the immune reaction following the coming back in the cowshed. Decrease observed during winter could be explained by the delay between this stress and the date of the screening test.
Goat could act as a reservoir of the FPSR causal agent. Few cases of goat FPSR have been reported, but were not connected up to now with cattle FPSR. Further studies should be conducted to investigate this hypothesis.
At the individual level, age was the most significant source of variations. This could be linked either to different management practices according to the age group and the production system, which could lead to different exposures with the causal agent, and/or to different individual susceptibilities *eg* immunological response.
Some individual or herd factors were never found significant, whereas statistical tests were very powerful. For instance, whereas the phenomenon was classically described as a beef herd issue, it was generally observed in this area at the same rate in both dairy and beef herds. This surprising observation could be reported because the dairy herds were tested from blood sample in this area, while Ring-Test is generally used. At the individual level, no meaningful effect were found between male and female, or between herd size classes as well. No clear « assumed risk factors » were finally isolated in this study, except one: within a herd, the more animals were tested, the more FPSR herds were detected, while the probability of a given animal to show FPSR was not influenced by the size of its herd. One hypothesis could be put forward: a large distribution of the causal agent, with a low individual sensitivity to the infection. A lot of herds could have been infected, but very few animals were detected as positive. Therefore, the main explaining factor to detect a FPSR herd was the number of tested animals. The individual probability to be either infected, or detected positive nevertheless did vary with time and area. Assumed causal agent could therefore be widely distributed all over the studied area, with a density varying with time or area.
Further studies should be performed to confirm the isolated risk factors and to search for others, particularly at the agricultural management level. Nevertheless, difficulties might be encountered to define case and control herds and/or animals according to this survey results. As *Yersinia enterocolitica* O:9 infection appears to be the most likely causal agent, another way would be to investigate the phenomenon using a test more specific to this bacterium which is still to be developed.

ACKNOWLEDGMENTS
We are very grateful to François Pouilly and Gilles Laborie, from the « Direction des Services Vétérinaires de Saône-et-Loire », Isabelle Vivier, from the « Etablissement Départemental de l'Elevage », and Georges Mollard, from the « Direction Départementale de l'Agriculture » for their collaboration. This work was supported by the FAIR 134 EU project.