Epidémiol. santé anim., 1997, 31-32

PREVENTION, ERADICATION AND MOLECULAR EPIDEMIOLOGY OF BOVINE TUBERCULOSIS (*MYCOBACTERIUM BOVIS*) IN BELGIUM

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La réémergence de la tuberculose bovine (Mycobacterium bovis) en province de Liège en 1995, nous a permis d'évaluer les différents outils de diagnostic de la tuberculose bovine disponibles à l'heure actuelle: tuberculination (simple ou comparative), test IFN-γ (Iddex, France), sérologie Antigène A60 (Anda Biologicals, France). Au cours de l'hiver 1995-1996, un dépistage intensif a été réalisé dans 1500 fermes. Dix fermes ont été suivies en parallèle par les différents tests. Près de 15% des troupeaux présentaient des animaux réagissant au test de tuberculination simple, alors que le taux de prévalence réel de la tuberculose dans la région considérée est de 3%. Lors du dépistage, le test IFN-γ montre une meilleure spécificité que le test de tuberculination simple. Cependant, quel que soit le test utilisé, un ou plusieurs contrôles sont nécessaires afin de préciser le statut sanitaire du troupeau.Le statut sanitaire du troupeau peut être défini en une seule intervention si le test IFN-γ et la tuberculination simple sont associés et leur résultat interprété en série. Lors de l'épuration, au sein d'un troupeau, le test IFN-γ détecte des animaux infectés, présentant une tuberculination négative mais porteurs de lésions de tuberculose. Le test sérologique (Antigène A60) réalisé après adsorption des sérums avec l'antigène extrait de M. vaccae, présente une sensibilité très faible (20% des animaux classés positifs par le test IFN-γ et la tuberculination sont classés positifs en sérologie). Les souches de M. bovis isolées d'animaux infectés ont été analysées par la technique RLFP (Restriction Length Fragment Polymorphism) basée sur la séquence d'insertion IS 6110. Toutes ces souches présentent un profil unique à 9 bandes. Ceci suggère une origine unique et récente à la réémergence de tuberculose bovine dans cette région. Nos résultats ainsi que les données actuelles de la littérature suggèrent une origine "exotique" à cette réémergence de tuberculose bovine

INTRODUCTION

The implementation of bovine tuberculosis (TB) control and eradication programs in the EU member States after World War II, was due to commercial interests and, to a lesser extend, to public health concern. At that time, among 2.2 million Belgian cattle, almost 20 % were found infected. By 1960, 397 000 tuberculin reactors were slaughtered, and tuberculosis was no more considered to be an animal nor a human health problem. In 1971, according to the favorable epidemiological situation, the tuberculin test was performed every third year in cattle herds and on trade, e.g. purchased animals. In 1992, only 0,025 % of the cattle herds were not declared "officially free of tuberculosis". The surveillance program was then restricted to tuberculin tests at trade and postmortem TB diagnosis at slaughter.

In this favorable context, an increasing number of TB lesions were reported during Fall 1995 through meat inspection at the slaughterhouse in Liège. Back- and forward-tracing of cattle movements has led to the designation of a "high-risk area" in the 20th veterinary district (city of Huy surroundings) where a (re)emergence of TB has been documented.

The aim of this study was:

1° To perform an intensive screening (single tuberculin test) in a "high-risk area" (1500 herds) to assess the TB prevalence rate

2° To compare the IFN-γ assay to the single tuberculin test, as a screening method in 10 herds selected by back- and forward-tracing

3° To compare the IFN-γ assay to the comparative tuberculin test in herds where (single) tuberculin reactors were detected during the intensive screening

4° To compare the IFN-γ assay to the tuberculin test in a herd depopulation scheme

5° To conduct molecular epidemiological studies on the *Mycobacterium bovis* isolates by analysis of the IS*6110* RFLP profiles

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RESULTS AND DISCUSSION

Table I
Comparison of the tuberculin test and the IFN-γ assay at screening and at the first control

Herd	Nbr	Screening					Nbr	1st control (6 weeks after the screening)						
		sIDR		IFN-γ		Agreement		cIDR			IFN-γ		Agreement	Bact
		Reactors	Pos	*Myc spp*	*Myc bov*			PPD av	PPD bov	Pos	*Myc spp*	*Myc bov*		*Myc bov*
Mar	69	0	0	0	0									
Lec	85	0	0	4	0									
Fle	128	3	1	31	0		40	14	3	0	2	0		
Kem	97	3	1	3	0		62	8	0	0	4	0		
Por	135	1	6	7	5	4	61	14	6	4	4	3*	3	+
Pre	134	7	3	3	2	0	107	4	6	3	3	0	0	-
Laf	62	1	0	10	4	0	40	3	0	0	nt	nt		
Mah	86	0	2	10	3	0	55	14	0	0	3	0		
Sme	70	2	0	1	1	0	40	0	0	0	0	0		
Her	97	6	3	22	2	0	54	34	13	0	1	0		

*: 1 animal cIDR positive, not tested by IFN-γ
nt: not tested

Table II
Comparison of the tuberculin test and the IFN-γ assay in a depopulation scheme in an infected herd

Nbr	Screening		1st control				2th control			3th control	
	sIDR	IFN-γ	cIDR		IFN-γ	Lesion	sIDR	IFN-γ	Lesion	sIDR	IFN-γ
		bov / av	PPD av	PPD bov	bov / av			bov / av			bov / av
8853	3	-	-	-	-		-	-		-	-
8846	-	3.71	slaughtered			+					
5054	6	3.65	5	6	2.89	+					
5079	8	-	4	6	1.79	-					
6405	-	-	3	2	-		1	-		-	-
5071	1	-	-	2	-		2	-	-	-	-
5062	5	3.06	3	7	2.76	-					
7187	21	7.99	-	16	nd	nd					
5413	15	ni	4	8	ni	-					
5088	10	5.62	2	8	3.9	+					
5114	-	-	-	-	-		3	-		-	-
5121	-	-	-	-	-		3	-		2	-
5074	-	-	0	1	-		5	-	-		
5116	-	-	-	-	-		15	>2.3	nd		
4374	-	-	-	-	-		19	2.03	-		
4372	-	-	3,5	0	-		9	2.81	+		
4352	-	-	-	-	-		20	2.63	nd		

Table III
Comparison of the tuberculin test and the IFN-γ assay in a "Reactor" herd

Nbr	Screening		1st control				2th control	
	sIDR	IFN-γ bov / av	cIDR PPD av	cIDR PPD bov	IFN-γ bov / av	Lesion	IDRs	IFN-γ bov / av
9350	-	9.33	-	-		-		
8468	-	5.43	-	-		-		
6271	1.5	0.95	-	-			-	0.62
9281	1.5	-	2	8.5	-	-		
6275	5	-	-	-			-	-
9277	1.5	-	-	-			-	-
9282	2	-	4	>16	0.72	-		
9280	3	-	-	-			-	-
9348	3	-	-	-			-	-
9279	-	-	0	8.5	-	-		
9269	-	-	1	3	ni		3	-
8287	-	-	2	2.5	-			
8490	-	-	1.5	4	-		2	-
4990	-	-	-	-	-		-	2.3
8482	-	-	-	-	-		-	0.93
6271	1.5	0.95	-	-	0.44		-	0.62

sIDR, cIDR: skin thickness in mm; IFN-γ : OD PPD bov / OD PPD av; nd: not done; ni: not interpretable

Reactors were detected in 15 % of the herds screened in the risk area by the sIDR, although the real prevalence rate was found to be 3 % in the considered area. Similar aspecific prevalence rates have been documented in the UK and in Ireland. In this context, the IFN-γ assay appears to be more specific than the sIDR. Nevertheless, more than one intervention is needed before the herd status can be define, whatever the test used. The status could be define after one intervention if the sIDR and the IFN-γ assay were performed at the same time and results were interpreted in parallel. In a depopulation scheme, one animal classified sIDR negative, IFN-γ assay positive was slaughtered and was found infected. In "Reactor herds", the poor degree of concordance between the tuberculin test and the IFN-γ assay suggested the absence of TB.
The TB iELISA (A60 antigen, preadsorption with *M. vaccae*) showed a lack of sensibility: only 20 % of the animals originating from infected herds and classified positive by both the IDR and the IFN-γ assay, were detected.
All *M.bovis* isolates in these TB outbreaks were found to carry 9 copies of the Insertion Sequence IS*6110*, which is a characteristic feature of isolates originating from animals other than cattle (1). Our results are consistent with one *M. bovis* strain being implicated in these TB outbreaks. No direct epidemiological link with TB in deer or badger could be evidenced, so far, in Belgium. However, the question of the wildlife-to-bovine transmission should be addressed (2).

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