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Original article

Residual foot-and-mouth disease virus antibodies in French cattle and sheep six years after the vaccination ban

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Abstract – A serological survey was carried out on French cattle to establish a reference pattern of residual vaccine antibodies and non-specific reactions against the foot-and-mouth disease virus 6 years after the ban on vaccination and in the absence of any foot-and-mouth disease outbreak. Most of the multi-vaccinated cattle still displayed high titres of antibodies and up to 50% of those which had received a single injection still had antibodies. Non-specific reactors were also recorded among animals born during and after 1991. Most of them displayed low titres close to the threshold. Sheep were also tested and, as for cattle, 4.6% of non-specific reactors were recorded, with titres close to the threshold for two-thirds of them. As part of these animals have been resampled and retested, sera revealed negative confirming that these animals are true non-specific reactors. Serological testing as a mean of FMD control should take these facts into account.

foot-and-mouth disease / serology / vaccination / cattle / sheep

Résumé – Persistance des anticorps contre le virus de la fièvre aphteuse dans le cheptel bovin et ovin français six années après l'arrêt de la vaccination. Une enquête sérologique a été entreprise afin de déterminer hors de tout contexte d'épidémie de fièvre aphteuse, le profil de réponse sérologique du cheptel vis-à-vis du virus de la fièvre aphteuse six années après l'arrêt de la vaccination. Cette enquête a permis également de déterminer l'importance des réactions non spécifiques chez les bovins nés après l'arrêt de la vaccination ainsi que chez les ovins. La majorité des bovins nés avant 1989 étaient réagissants et présentaient encore de forts taux d'anticorps. Parmi les animaux n'ayant reçu qu'une seule injection de vaccin, 50 % étaient encore positifs. Des réactions non spécifiques ont également été observées chez les animaux nés en et après 1991, la majorité de ces réactions se situant près du seuil de positivité. Parmi les ovins testés, 4,6 % de réactions non spécifiques ont été relevées dont les 2/3 étaient aussi proches du seuil de positivité. Des prélèvements ont pu être refaits sur une partie de

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ces animaux et se sont révélés négatifs, confirmant le caractère non spécifique de ces réactions. L'utilisation de la sérologie comme outil de contrôle doit tenir compte de ces réalités.

fièvre aphteuse / sérologie / vaccination / bovin / ovin

1. INTRODUCTION

Foot-and-mouth disease (FMD) continues to be regarded as the most contagious and economically devastating disease of livestock. While clinical disease is confirmed by virus isolation and typing, serological surveillance in most European countries has been hampered by routine vaccination. The possibility to distinguish vaccinated from infected animals appeared too recently.

Since the 1991 ban on annual vaccination against FMD in the European Community (EC), serological investigations have become part of a device to insure that after an FMD outbreak, any contaminated animal will be detected and slaughtered. In fact, following the request made by the EC, this was applied in Italy in 1993, in Greece in 1994 and 1996, and in the Balkan countries in 1996 after outbreaks due to O and A serotypes.

In order to be able to draw conclusions from such a work, it is necessary to establish what could be a reference serological pattern of residual antibodies and non-specific reactions in French cattle in the absence of any FMD outbreak. So, a national serological survey was undertaken on 3 840 head of cattle and 2 780 sheep, six years after the ban on vaccination.

2. MATERIALS AND METHODS

2.1. Animals

Blood samples were collected during 1997 from 3 840 cattle and 2 780 sheep distributed respectively among 108 herds and 83 flocks chosen in eight areas of France: Hautes-Alpes, Isère, Maine et Loire, Pyrénées-Atlantiques, Cantal, Meurthe et Moselle, Nord and Côtes d'Armor. The herds and flocks were chosen at random and were representative of the different kinds of breeding systems, i.e. for milk or meat producing cattle: 50% of the animals belonged to meat producing breeds (mainly Charolais, Aquitaine Blond and Salers cattle), 25% to dualpurpose breeds (Montbéliard, crossed Holstein, Abondance) and the last 25% to dairy breeds (Holstein, Breton Black Pied cattle (Bretonne pie noire)).

In each herd, all bovine that were more than one year old were sampled. The number of herds tested was determined according to the mean size of herds in a given area, so that at least 500 animals were tested in each area. In sheep flock, most of the sampled animals (80 to 90% depending on the flock) were born after 1991.

The objective was to be able to detect a seroprevalence of 0.6% at least with a 95% level of confidence.

2.2. Serological testing

The viral seroneutralisation test (VNT) was adopted as described in the Office International des Epizooties (OIE) manual [12] using the IBRS^2 cell line – Sera were first assayed against FMD virus type O1 (Aullendorf). The tests were performed against O1 type for economical reasons and because this serotype had a higher prevalence in the past years in this area. Doubtful and positive sera determined according to the OIE threshold, and providing enough material was available, were then tested against type A5 (Allier) and C1 (Noville) FMD viruses. A

titre of 1.5 (reciprocal log10 of the serum dilution) and above was considered as protective according to Frenkel cited in [5, 10].

2.3. Results expression

Neutralising antibody titres of sera from cattle in all herds were grouped according to the year of birth since the number of individual vaccinations is faithfully related to the age of the animals. Cattle more than four months of age were routinely vaccinated with a trivalent (A – O – C) vaccine including aluminium hydroxide as adjuvant, during the winter every year until April 1991, the beginning of the ban in France. For animals born in 1989 and in 1990, the number of vaccinations may have been different according to the season of birth. So, the results were also grouped depending on whether the animals were born in the first or second half of these two years. Since most animals born in 1991 were born during the second half of the year, they were grouped with animals born after 1991, i.e. with animals which had never received vaccines as the ban has been properly implemented.

3. RESULTS

3.1. Bovine sera

The seroneutralising titres obtained against the type O FMD virus are summarised in Table I.

Among animals born in 1988 and before, 84% still had FMD specific antibodies and for 77% of them, at a protective level. Meanwhile, animals born in 1988 and before still constituted 24% of the herd for meat producing cattle and at least 5% for dairy herds.

Among animals born in the first half of 1990 which likely received one vaccination,

Table I. Dispersal of bovine viral neutralisation test (VNT) titres against type O FMD virus according to the year of birth (data are expressed as number of animals and corresponding percentage).

VNT titres (reciprocal log10)	Year of birth					
	1988 and before (*n* = 548)	1989 first half (*n* = 140)	1989 second half (*n* = 69)	1990 first half (*n* = 146)	1990 second half (*n* = 86)	1991 and after (*n* = 2851)
Less than 1.2	89 (16.3)[a]	45 (32)	31 (45.5)	73 (50)	69 (80)	2689 (94)
1.2–1.34	37 (6.8)	20 (14)	8 (12)	22 (15)	6 (7)	109 (4)
1.5–1.7	69 (12.6)	22 (16)	14 (20)	29 (20)	8 (9)	38 (1.4)
1.8–1.95	49 (9)	9 (6.4)	5 (7)	7 (5)	2 (2)	6 (0.2)
2.11–2.25	65 (11)	13 (9.2)	2 (3)	6 (4)	0 (0)	1 (0.03)
2.40–2.55	76 (14)	16 (11.4)	6 (7)	5 (3.4)	1 (1)	6 (0.2)
2.7–2.8	163 (30)	15 (10.7)	3 (4.4)	4 (2.7)	0 (0)	2 (0.07)
Number of positive animals (%)	459 (84)	95 (68)	38 (55)	73 (50)	17 (20)	162 (6)
Percentage of the age group in the total sample	14	3.6	1.8	3.8	2.2	74

[a] Number (%) of reactors.

Table II. Reactivity of positive bovines to 1, 2 or 3 serotypes of FMD virus whether they were born before or after the vaccination ban.

Animals born before 1991 (n = 562)			Animals born after 1991 (n = 125)		
Positive to 3 types (O, A, C)	Positive to 2 types (O, A or O, C)	Positive to 1 type (O1)	Positive to 3 types (O, A, C)	Positive to 2 types (O, A or O, C)	Positive to 1 Type (O1)
434 (77)[a]	82 (15)	46 (8)	14 (11)	18 (14)	93 (74)

[a] Number (%) of reactors.

50% still had FMD specific antibodies, but for most of them, below the protective level. No conclusion could be drawn from animals born in the second half of this year since some of them may have received no vaccine. No significant difference was observed between breeds (data not shown).

Among animals born in 1991 and after, 6% of the animals showed non-specific reaction. Out of these 162 non specific reactors (representing 6% of the non-vaccinated animals), 109 displayed titres close to the threshold value (log VNT 1.2) and should be considered as doubtful. Another 1.4% (38 animals) showed titres that exceeded the threshold by one dilution (log VNT 1.5–1.7). Fifteen animals had higher titers and most of them reacted to the three serotypes. Sixty-six of these 162 non-specific reactors were able to be resampled and retested. All of them, including 3 sera with high titers, were negative. Therefore, the percentage of non-specific reactors (6%) should be considered more approximative than definitive.

Table II shows the reactivity to type A and C of some (but not all previously tested) animals found doubtful or positive to type O depending on whether they were born before or after the ban of vaccination. Most vaccinated animals (77%) still had antibodies to the 3 serotypes of FMD virus. No difference could be detected between titres against each serotype. On the contrary, most positive animals born during and after 1991 only reacted to one serotype (74%).

3.2. Ovine sera

The serological results obtained on sheep sera are shown in Table III. Out of 2 781 animals tested, 129 (4.6%) reacted positively out of which 106 animals (3.8%) had VNT titres equal to the threshold or one dilution above.

Among these reactors, 80% reacted only to one serotype, 14% to 2 serotypes and 6% to three serotypes. Forty four animals have

Table III. Ovine viral neutralisation test titres against type O FMD virus.

	Viral neutralisation titres (reciprocal log10)						
	Less than 1.2	1.2–1.34	1.5–1.7	1.8–1.9	2.11–2.25	2.4–2.55	2.7–2.8
Number of animals (%)	2652 (95.4)	72 (2.6)	34 (1.2)	8 (0.3)	6 (0.2)	6 (0.2)	3 (0.1)

been resampled and retested and 37 became negative but 7 were still positive.

4. DISCUSSION

As previously described by Fish et al. [7], Terpstra et al. [14] and more recently by Dekker and Terpstra [5] who tested Dutch cattle in 1996, this survey also demonstrates that FMD antibodies can still persist for several years at high levels and without the need for booster vaccination.

A permanent booster effect from unrelated pathogens possessing common T-helper lymphocyte epitopes like enteroviruses has been suggested by Amadori et al. [2] who described a more sustained FMD antibody response in cattle multivaccinated with enterovirus or Leptospira vaccines. This antibody response was also correlated with a specific FMD cellular immune response involving CD4 T cells. Other mechanisms like the persistence of antigen presenting cells may be involved in the maintenance of an FMD antibody response, as demonstrated in mice [15].

The aim of our study was also to estimate the number of non-specific reactors.

Non-specific FMD virus inhibitory factors have long been recognised in cattle and porcine sera [1, 3, 4, 13]. Their presence is still a problem in serological tests when used for import and export control serology ([8], reviewed in [6]) and to ensure that a country is free of any FMD virus after an outbreak [11]. Although the liquid phase blocking ELISA was reported to be less proned to non specific reactions than VNT [9], in practice, the VNT is still recommended as a confirmatory test [12] and Haas found the same ratio of non-specific reactors (4%) using ELISA or VNT [8].

This percentage is not so different from our results. However, 67% of them (i.e. 4% of the total number of animals) display VNT titers equal to the threshold and are better considered as doubtful than really non-specific reactors. Retesting these doubtful and false positive animals dramatically reduced the percentage of non-specific reactors. This suggests that stress factors were probably involved for those animals with higher non-specific titres.

With the end of obligatory vaccination, serology against FMD is a new tool, quite recently developed in field conditions. The first results obtained in Europe around outbreaks (Greece, Balkan countries) or without an outbreak (this study) have shown some difficulties in the interpretation of the results. The question of the tests and threshold is still an important issue to be solved even with the arrival of the non-structural protein antibody tests.

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