

# Short Communication

## Sensitivity of $\gamma$ -interferon test used in series after tuberculin test to detect bovine tuberculosis

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FRANCE has been officially bovine tuberculosis (bTB) free since 2000, but an increase in the number of outbreaks has been observed in some areas. bTB is detected through inspections at the slaughterhouse and cervical skin test (ST) performed in farms. When non-negative (i.e. positive or doubtful) results to single intradermal tuberculin (SIT) test or single comparative cervical tuberculin (SICCT) test occur, animals are either culled for bacteriological diagnosis or retested with SICCT test at least 42 days later in order to avoid desensitisation phenomenon. During this period, suspect herds are locked up: movements and sales of cattle or products are forbidden. The low predictive value of non-negative results to ST and the cross-reactions with non-pathogenic mycobacteria engender multiple false-positive results. The consequences are economic losses and demotivation of veterinarians, farmers and veterinary officers.

$\gamma$ -Interferon (IFN) test is known to be an alternative test to detect bTB (De la Rua-Domenech and others 2006). It has been used in European countries, in parallel to ST, to speed up the eradication of bTB in outbreaks. On the contrary, its serial use, in the days following non-negative results to screening ST, has not been much studied in literature (Ryan and others 2000, Praud and others 2015) and is not allowed by the European Directive CE/64/432. An experimental protocol validated by the European Commission was developed in France, in order to assess the accuracy of IFN used right after a non-negative result to screening ST as an alternative to SICCT test performed 42 days later. In this context, it was important to ensure that the sensitivity of IFN performed in the days following ST was not lower than SICCT test performed 42 days after, and that a serial use did not endanger the safety of the trade.

Data were gathered on a voluntary basis in farms where non-negative results to screening ST were observed. Animals with non-negative results to ST on day 0 (ST<sub>D0</sub>) were subjected to IFN between days 3 and 8 after the injection of tuberculin (IFN<sub>D3</sub>) and retested with SICCT test and IFN on day 42 (SICCT<sub>D42</sub>, IFN<sub>D42</sub>). Animals with non-negative results to ST<sub>D0</sub>, IFN<sub>D3</sub>, SICCT<sub>D42</sub> or IFN<sub>D42</sub> were slaughtered. Samples of lesions (if observed) and thoracic lymphatic nodes were analysed

(culture and PCR). Animals with positive results to PCR and/or culture for *Mycobacterium bovis* were considered infected. In departments where SICCT test was used as a screening test, cattle with a positive result to SICCT<sub>D0</sub> were immediately slaughtered: such animals were thus not included in the sample studied.

The STs were performed as part of the usual official screening scheme on French farms between 2013 and 2015. Doses of 0.1 ml of bovine and avian purified protein derivative (PPD Avituber and Bovituber, Synbiotics, Lyon, France) were injected. The tests were performed and interpreted as recommended by the World Organisation for Animal Health (OIE) (OIE 2009) and European Directive CE/64/432: the results were positive when the skinfold thickening was at least 4 mm, negative when it was up to 2 mm and doubtful between 2 and 4 mm.

To perform the IFN, whole blood was put in culture in the presence of different mycobacterial antigens: Bovine and Avian Lelystad PPD (Bovigam; Prionics AG, Switzerland) and specific antigens MIX (Peptid Cocktail ESAT-6/CFP-10; Prionics AG, Switzerland). The levels of  $\gamma$ -IFN released were compared using an ELISA method. IFN was performed according to the manufacturer's recommendations and as described by Faye and others (2011). Optical densities (ODs) were transformed in percentage values by comparing test-sample ODs with control ODs. Tests were performed by local laboratories approved by the National Reference Laboratory for tuberculosis (Anses, LSA, Maisons-Alfort, France) after interlaboratory tests and interpreted according to Table 1.

Data were managed using Excel and Access (Microsoft). McNemar's tests for paired data were performed using R and considered as significant when  $P < 0.05$ .

In the database, 2851 animals had obtained non-negative results to screening ST. Finally, the detailed results of the four studied tests were available in the database for 40 infected animals from 29 farms (Table 2).

The sensitivities of the tests conditionally to a non-negative result to ST<sub>D0</sub> were as follows: Se SICCT<sub>D42</sub> = 0.45 (0.30 to 0.60)<sub>95 per cent CI</sub> (95 per cent CI), Se IFN<sub>D3</sub> = 0.95 (0.88 to 1)<sub>95 per cent CI</sub> and Se IFN<sub>D42</sub> = 0.98 (0.93 to 1)<sub>95 per cent CI</sub>. When IFN<sub>D3</sub> was interpreted only on the PPD basis, its conditional sensitivity was Se PPD<sub>D3</sub> = 0.89 (0.78 to 1)<sub>95 per cent CI</sub>. Se IFN<sub>D3</sub> was significantly higher than Se SICCT<sub>D42</sub> (McNemar's test,  $P = 1.1 \times 10^{-3}$ ). Forty per cent of the results to these two tests were concordant. Most non-negative results to SICCT<sub>D42</sub> were confirmed by IFN<sub>D3</sub> (89 per cent;  $n = 16/18$ ) and among animals detected by IFN<sub>D3</sub>; 22 animals were negative to SICCT<sub>D42</sub>

**TABLE 1: Interpretation of IFN results according to the percentage of optical density (per cent OD) obtained with PPD and MIX antigens**

|                   |                  | PPD (per cent OD)             |                  |           |
|-------------------|------------------|-------------------------------|------------------|-----------|
|                   |                  | PPD < 0.05                    | 0.05 ≤ PPD < 0.3 | PPD ≥ 0.3 |
| MIX (per cent OD) | MIX < 0.03       | Negative                      | Inconclusive     |           |
|                   | 0.03 ≤ MIX < 0.1 | Inconclusive                  | Positive         |           |
|                   |                  | IF bovine PPD > 0.7: positive |                  |           |
|                   | MIX ≥ 0.1        |                               |                  |           |

Calculation of ratios: ratio PBS =  $OD_{PBS} / [3 \times (OD_{PC} - OD_{NC})]$ , ratio PWM =  $(OD_{PWM} - OD_{PBS}) / [3 \times (OD_{PC} - OD_{NC})]$ , ratio PPD =  $(OD_{PPDB} - OD_{PPDA}) / [3 \times (OD_{PC} - OD_{NC})]$ , ratio PPDB =  $(OD_{PPDB} - OD_{PBS}) / [3 \times (OD_{PC} - OD_{NC})]$  and ratio MIX =  $(OD_{MIX} - OD_{PBS}) / [3 \times (OD_{PC} - OD_{NC})]$ . The results were interpreted when ratio PBS < 0.125 and ratio PWM > 0.2. IFN, interferon; MIX, specific antigens ESAT-6 and CFP-10; NC, negative control; PC, positive control; PPD, purified protein derivative; PPDA, avian PPD; PPDB, bovine PPD; PWM, Pokeweed mitogen

Veterinary Record (2016)

doi: 10.1136/vr.103803

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Provenance: Not commissioned;  
externally peer reviewed

Accepted June 21, 2016

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TABLE 2: Crossed results of the four tests performed on the 40 infected animals

| ST <sub>D0</sub> | SICCT <sub>D42</sub> | IFN <sub>D3</sub> | IFN <sub>D42</sub> | Number |
|------------------|----------------------|-------------------|--------------------|--------|
| +                | -                    | -                 | -                  | 0      |
| +                | -                    | -                 | +                  | 0      |
| +                | -                    | +                 | -                  | 1      |
| +                | -                    | +                 | +                  | 21     |
| +                | +                    | -                 | -                  | 0      |
| +                | +                    | -                 | +                  | 2      |
| +                | +                    | +                 | -                  | 0      |
| +                | +                    | +                 | +                  | 16     |
| Total            |                      |                   |                    | 40     |

-, negative; +, non-negative; IFN<sub>D3</sub>,  $\gamma$ -interferon test performed on day 3; IFN<sub>D42</sub>,  $\gamma$ -interferon test performed on day 42; SICCT<sub>D42</sub>, single intradermal cervical comparative tuberculin test on day 42; ST<sub>D0</sub>, skin test performed on day 0

(58 per cent; n=22/38). The average sensitivity of IFN<sub>D42</sub> was slightly higher than IFN<sub>D3</sub>.

Between 2013 and 2015, 192 of the 329 French bTB outbreaks were detected because of screening tests. The studied sample represented 15 per cent of these outbreaks. It can be considered as a representative of French cattle with non-negative results to screening STs, even if a selection bias linked to the voluntary inclusion of farms could not be avoided. Furthermore, the fact that animals with positive results to SICCT<sub>D0</sub> (which reacted most strongly to ST) were culled and thus not represented in this sample probably leads to an underestimation of the sensitivities.

In conclusion, IFN performed between three and eight days after a non-negative result to screening STs was significantly more sensitive than the usual test (SICCT test, 42 days later). Most cases detected by SICCT<sub>D42</sub> were also detected by IFN<sub>D3</sub> and a high number of cases undetected by SICCT<sub>D42</sub> reacted to IFN<sub>D3</sub>. The serial association of screening ST with IFN<sub>D3</sub> could thus be used to shorten the lock-up of suspect herds without subdetecting infected cattle. This scheme would, nevertheless,

be less specific than the usual one. Even if the results of published studies about the effect of a tuberculin injection on the result of IFN performed in the next few days are not consensual (Schiller and others 2010), further analyses are currently carried on authors' sample to address this question.

### Acknowledgements

Local tuberculosis coordinators, veterinary laboratories and veterinarians are duly acknowledged for having performed the tests and collected the data. The authors also acknowledge Marina Beral and Axelle Scoizec for the constitution of the database and the French Ministry of Agriculture for providing the data.

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