Abstract
We have developed a method allowing the identification and quantification of Ag-specific plasma cells and memory B cells in the blood of cattle. Using this model, we were able to build a comprehensive picture of the appearance in the blood stream of both these cell types within individual calves immunised with ovalbumin. During the primary response, we detected a burst of ovalbumin-specific plasma cells at days 6 and 7 post-immunisation, which was followed by the production of specific Ab, whereas a gradual increase of memory B cells was only detected from day 15. As expected, a boost immunisation performed 7 weeks later induced a quicker and stronger secondary response. Indeed, a burst of plasma cells was detected in the blood at days 3 and 4, which was followed by a strong increase in Ab titres. Furthermore, a burst of memory B cells, and not a gradual increase, was detected at days 5 and 6 post-boost immunisation. Importantly, we showed a strong correlation between the anti-ovalbumin-specific IgG titres and plasma cell numbers detected in the blood at the peak response after secondary immunisation.

The B cell ELISPOT assay
This assay allows, in the bovine model, the identification and quantification in the blood of Ag-specific plasma cells and memory B cells, using ovalbumin as a model T-dependent Ag (fig 1). As shown below, stimulation of quiescent, memory B cells within PBMC is necessary to induce their differentiation into Ab-secreting cells (ASC) and subsequent detection by the B cell ELISPOT assay.

Results
• Various reagents have been used for inducing the differentiation of human memory B cells, but these are not suitable when using bovine B cells. Here, we show PWM + anti-bovine CD40 mAb + rhIL-2 + rbIL-10 is a potent differentiation stimulus (fig 2A), which was used in all subsequent experiments aiming at the detection of memory B cells. These activated PBMC were mostly T cell blasts, but numerous plasma cells (positively stained with an anti-bovine light chain mAb) were also detected (fig 2B-D).
• We determined the kinetics of the appearance of ovalbumin-specific IgG, plasma and memory B cells in the blood following an immunisation and boost-immunisation (fig 3): (i) Primary response: burst of ovalbumin-specific plasma cells detected at days 6 and 7 post-immunisation (pi), followed by the production of specific Ab. Generation of memory B cells detected at a later time-point (starting between days 15 and 21pi and gradually increasing); (ii) Quicker/stronger secondary response: burst of plasma cells detected at days 3 and 4 post-boost immunisation (pb), followed by a strong increase in Ab titres. Burst of memory B cells detected at days 5 and 6 pb.
• Subsequently, eight calves were immunised with various doses of ovalbumin (results detailed in fig 4A). No correlation was found between the ovalbumin IgG titres and memory B cell numbers detected at the peak response during the secondary immune response (r values = -0.05, n=8, fig 4B).

Conclusion
Using the B cell ELISPOT assay, we precisely determined the kinetics of bovine plasma and memory B cells appearing in the blood in response to an immunisation and a boost immunisation with ovalbumin. We also demonstrated a strong correlation between the anti-ovalbumin specific plasma cell numbers at the peak of the secondary response and IgG titres detected subsequently in the blood.

The detection and quantification of plasma cells and memory B cells following an immunisation/vaccination strategy could constitute a very effective means to predict, at an early time point, the magnitude and maintenance of the Ab response that will be generated afterwards. Such a method could be valuable for determining the potency of new vaccines and immunisation protocols.