Cattle immunology: vaccination and immunological testing

Charlotte Inman PhD BVSc MRCVS Research Associate
Chris Hudson BVSc CertCHP MRCVS Senior Farm Animal Clinician
UNIVERSITY OF BRISTOL, SCHOOL OF VETERINARY SCIENCE, LANGFORD HOUSE, LANGFORD,
NORTH SOMERSET. BS40 5DU

PRINCIPLES OF VACCINATION
Vaccination has long been established as a preventative measure against infectious disease in both human and animal medicine and it is widely practiced in all farm animal species, including cattle. The principle behind vaccination is to expose the animal’s immune system to a pathogen without causing disease. An effective vaccine must cause the immune system to generate antibodies and T-cells directed at appropriate pathogen antigens. Whilst one pathogen will have multiple antigens against which antibodies will be generated, not all of these antibodies will protect against future infection.

Live and killed vaccines constitute the vast majority of vaccines currently in use. Live vaccines are prepared from attenuated pathogens that are almost or completely devoid of pathogenicity but are capable of inducing a protective immune response. Dead vaccines are produced by killing and administering the whole pathogen, or by using a purified component of the pathogen. There are both advantages and disadvantages of using live over dead vaccines. For example, live vaccines may be able to induce a greater degree of protection for a longer period of time. However, there is also a risk of reversion to virulence associated with the use of a live vaccine; obviously this is not a risk for killed vaccines. Vaccines available for use in cattle in the UK are summarised in Table 1 (NB: This only includes products included in the NOAH Data Sheets Compendium).

Herd immunity
In many respects, farm animals are an ideal target population for vaccination, as large groups of animals are under the control of a single owner and tend to have relatively little contact with animals on other holdings. Compare this to the situation in human or small animal medicine, where the decision to vaccinate is taken at an individual level, so there is little effective ability to control the proportion of the population which is vaccinated against a disease. It is well recognised that this is an important factor in the success of vaccination programmes to protect against infectious disease.

Unfortunately, even in farm situations (where a complete population is under the control of the owner/manager), the phenomenon of ‘herd immunity’ is often not used to our advantage. Many vaccines rely on vaccination of the entire group of animals to decrease level of disease in the population, thereby decreasing the exposure of the animals to the pathogen and making protection provided by the vaccine more effective. Examples of situations in which this opportunity is commonly missed include:

- vaccination against calf pneumonia of dairy-bred heifer calves intended as replacement stock, whilst not vaccinating the beef cross or dairy bull calves that are housed in the same accommodation
- vaccination of a single cohort of animals within the adult herd (first lactation heifers are sometimes vaccinated in herds which do not vaccinate the cows against the same pathogen).

In these situations, vaccines may work less effectively than would be expected, as there will be less decrease in the exposure of the vaccinated animals to the infectious agent, thereby challenging the vaccinal immunity to a greater extent.
| Disease                  | Product name          | Manufacturer                     | Pathogens included                                                                 | Route of administration | Vaccine type       |
|-------------------------|-----------------------|----------------------------------|------------------------------------------------------------------------------------|--------------------------|-------------------|
| Clostridial Diseases    | Blackleg Vaccine      | Intervet Schering Plough Pfizer  | Clostridium chauvoei, Cl. perfringens Type A, Cl. perfringens Type B, Cl. sordellii | SC                       | inactivated       |
|                         | Blackleg Vaccine      | Intervet Schering Plough Pfizer  | Clostridium chauvoei, Cl. perfringens Type A, Cl. perfringens Type B, Cl. sordellii | SC                       | inactivated       |
|                         | Bravoxin 10           | Intervet Schering Plough Pfizer  | Cl. septicum, Cl. chauvoei, Cl. novyi Type B, Cl. tetani, Cl. haemolyticum          | SC                       | mixed             |
| Covexin 8               | Pfizer                |                                   | Cl. perfringens (welchii) Type C, Cl. perfringens (welchii) Type D, Cl. septici    | SC                       | mixed             |
| Covexin 10              | Pfizer                |                                   | Cl. perfringens Type A, Cl. perfringens Type B, Cl. perfringens Type C, Cl. septici| SC                       | mixed             |
| Tetanus Toxoid          | Trivax T              | Intervet Schering Plough Pfizer  | Cl. tetani, Cl. chauvoei, Cl. novyi Type B, Cl. tetani, Cl. haemolyticum            | IM                       | purified toxoid   |
| Calf Pneumonia          | Bovilis Bovipast      | Pfizer                           | Parainfluenza-3 virus, bovine respiratory syncytial virus, Mannheimia haemolytica  | SC                       | mixed             |
|                         | RSP                   | Pfizer                           | Infectious bovine rhinotracheitis virus, parainfluenza-3 virus                     | SC                       | live attenuated   |
|                         | Imuresp RP            | Pfizer                           | Mannheimia haemolytica                                                            | SC/IM                    | inactivated       |
|                         | Pastobov              | Pfizer                           | Parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhoea   | IM                       | mixed             |
|                         | Rispoval 3            | Pfizer                           | Parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhoea   | IM                       | mixed             |
|                         | Rispoval 4            | Pfizer                           | Parainfluenza-3 virus, bovine respiratory syncytial virus, infectious bovine        | IM                       | mixed             |
|                         | Rispoval              | Pfizer                           | Mannheimia haemolytica                                                            | IM                       | inactivated       |
|                         | Pastuaerella          | Pfizer                           | Bovine respiratory syncytial virus                                                | IM                       | live attenuated   |
|                         | Rispoval RS           | Pfizer                           | Parainfluenza-3 virus, bovine respiratory syncytial virus                          | IM                       | live attenuated   |
|                         | Rispoval RS+PI3       | Pfizer                           | Parainfluenza-3 virus, bovine respiratory syncytial virus                          | IM                       | live attenuated   |
| Calf Diarrhoea          | Lactovac              | Pfizer                           | Rotavirus, coronavirus, Escherichia coli                                           | SC                       | purified antigen  |
|                         | Rotavec Corona        | Intervet Schering Plough Pfizer  | Rotavirus, coronavirus, Escherichia coli                                           | IM                       | Inactivated       |
|                         | Trivac 6              | Intervet Schering Plough Pfizer  | Rotavirus, coronavirus, Escherichia coli                                           | SC                       | Inactivated       |
| IBR                     | Bovilis IBR live      | Intervet Schering Plough Pfizer  | Infectious bovine rhinotracheitis virus                                            | SC (preferred), IM       | live attenuated   |
|                         | Bovilis IBR Marker    | Intervet Schering Plough Pfizer  | Infectious bovine rhinotracheitis virus                                            | Intranasal/IM            | live attenuated   |
|                         | live                  | Intervet Schering Plough Pfizer  | Infectious bovine rhinotracheitis marker virus                                     | SC                       | marker            |
|                         | Rispoval IBR-         | Pfizer                           | Infectious bovine rhinotracheitis marker virus                                     | Intranasal/IM            | live marker       |
|                         | Marker Live           | Intervet Schering Plough Pfizer  | Infectious bovine rhinotracheitis marker virus                                     |                          |                   |
|                         | Rispoval IBR-         | Pfizer                           | Infectious bovine rhinotracheitis marker virus                                     |                          |                   |
|                         | Marker Live           | Intervet Schering Plough Pfizer  | Infectious bovine rhinotracheitis marker virus                                     |                          |                   |
| Tracherine              | Pfizer                |                                   | Infectious bovine rhinotracheitis virus                                            | Intranasal/IM            | live marker       |
| BVD                     | Bovidec               | Novartis                          | Bovine viral diarrhoea virus Type 1                                                | SC                       | inactivated       |
|                         | Bovilis BVD           | Intervet Schering Plough Pfizer  | Bovine viral diarrhoea virus Type 1                                                | IM                       | inactivated       |
|                         | PregSure BVD          | Pfizer                           | Bovine viral diarrhoea virus Type 1                                                | SC                       | inactivated       |
| Leptospirosis           | Leptovoid H           | Intervet Schering Plough Pfizer  | Leptospira interrogans serovar hardjo (hardjo-prajitno), Leptospira borgdetersenii | SC                       | inactivated       |
|                         |                      |                                   | serovar hardjo (hardjo-bovis)                                                     |                          |                   |
|                         |                      |                                   | Leptospira borgdetersenii serovar hardjo                                            |                          |                   |
|                         |                      |                                   | Leptospira borgdetersenii serovar hardjo                                            |                          |                   |
|                         |                      |                                   | Leptospira borgdetersenii serovar hardjo                                            |                          |                   |
|                         |                      |                                   | Leptospira borgdetersenii serovar hardjo                                            |                          |                   |
|                         | Spirovac              | Pfizer                           | Bovine viral diarrhoea virus Type 1                                                | SC                       | inactivated       |
|                         | Bovilis BTBV          | Intervet Schering Plough Pfizer  | Bluetongue virus serotype 8                                                        | SC                       | inactivated       |
|                         | BTVPUR AlScap 8       | Merial                           | Bluetongue virus serotype 8                                                        | SC                       | inactivated       |
|                         | Zulvac 8 Bovis        | Fort Dodge                        | Bluetongue virus serotype 8                                                        | IM                       | purified antigen  |
|                         |                      |                                   | Bluetongue virus serotype 8                                                        |                          |                   |
| Other                   | Bovilis Huskvac       | Intervet Schering Plough Pfizer  | Dicycocalus viviparus, Trichophytan verrucosum                                    | oral                     | live attenuated   |
|                         | Bovilis Ringvac       | Intervet Schering Plough Pfizer  | Salmonella dublin, Salmonella typhimurium                                          | IM                       | live           |
|                         | Bovivac S             | Intervet Schering Plough Pfizer  | Escherichia coli and other enterobacteriae (in mastitis)                           | SC                       | inactivated     |
|                         | Enviracor             | Pfizer                           | Escherichia coli and other enterobacteriae (in mastitis)                           | SC                       | inactivated     |
Fluctuations of immunity

Another important concept is the understanding that immunity (vaccinal or natural) is not a ‘binary’ state (i.e. animals are not simply ‘immune’ or ‘naïve’). Immunity tends to increase after vaccination, peak within a few days to weeks (depending on vaccine type and route of administration) and then gradually decline. Vaccines are often licensed to provide a certain duration of cover, but it is still worth being aware that protection is likely to be more effective early in the period of cover. Good examples of utilising this knowledge can be found in adult dairy cows. Vaccines against bovine viral diarrhoea (BVD) and leptospirosis are amongst the most commonly used cattle vaccines in the UK, and vaccines with label claims for 12-month protection are available against both diseases. However, the principle that animals will be less well protected in the period before revaccination is due can still be useful:

- Infection with leptospirosis is more likely at pasture: therefore peak coverage should logically apply during the grazing season, so annual vaccination should be timed to early spring
- The major aim of BVD vaccination is to prevent cows becoming infected around the time of conception and early pregnancy (as this could lead to failure of conception, embryonic loss or production of a persistently infected calf): therefore vaccination should logically be completed before the breeding period. In seasonally-calving herds, this is a practical possibility (and is often done), but in year-round calving herds would mean vaccinating smaller numbers of cows over the course of the year rather than vaccinating the herd as a batch.

Vaccine handling

The importance of correct handling of vaccine is well recognised amongst the veterinary profession, but this can still be a common reason for apparent vaccine failure in farm animals. The role of farm animal vets in communicating the importance of correct storage and use of vaccine (including the shelf life of the vaccine after the vial is breached or the vaccine reconstituted) is perhaps underestimated.

Handouts detailing the key points of this advice can be useful when dispensed along with vaccine.

Client information and expectations

What protection is provided

Another area where it is important for the farm animal vet to communicate effectively with clients is in promoting understanding of exactly what protection is provided by a vaccine. Many vaccines prevent only a single strain of a disease, and may provide limited or no cross-protection against other strains. A good example of this is vaccination against bluetongue virus (BTV). The currently licensed vaccines in the UK protect only against BTV-8 (the strain that has been diagnosed in the UK); other strains of the virus are present in continental Europe and could easily enter the UK. Although many farmers are well informed about BTV, there are still a large number that are unaware that the currently-used vaccines do not protect against other strains which may be a threat. This could potentially lead to disillusionment with the vaccination campaign should another strain enter the UK.

Active vs. passive vaccination

It is also important to be aware of the distinction between active and passive vaccination. Active vaccination is the process described above. Passive vaccination effectively involves providing protection against a disease by giving exogenous antibody to the recipient – in practical terms in cattle, this means vaccinating the dam in order that the calf is protected via colostrum. The available vaccines against calf diarrhoea are good examples of this. It is obviously important when practising passive vaccination to ensure that the calf has a good colostral intake in order to maximise benefit from the vaccine.

Diagnostic testing using immunological methods

The most commonly used immunodiagnostic tests in cattle include enzyme-linked immunosorbent assays (ELISAs), the serum neutralisation test (SNT), the complement fixation test (CFT) and the microscopic agglutination and serum agglutination tests (MAT and SAT).

Enzyme-linked immunosorbent assay (ELISA)

The basic ELISA technique can be used to detect either antigen from or antibody to specific pathogens (Figs. 1A, 1B and 1C). Antibody ELISAs are commonly used to diagnose exposure to or infection with diseases such as infectious bovine rhinotracheitis (IBR) and Johne’s disease, while an antigen ELISA is used to detect circulating BVD antigen.

As an example: in order to detect antibody against Mycobacterium avium subsp. paratuberculosis (MAP), MAP antigen is coated on to the surface of plastic wells. Serum from test animals is then added to the wells. Specific antibody produced in response to infection with MAP will then bind to the antigen on the plate. Excess non-specific antibody is washed off and a second (anti-bovine immunoglobulin) antibody conjugated to an enzyme is added to the plates. This will bind the antigen-specific antibodies. The excess of the second antibody is washed away and a colourless substrate (degraded by the enzyme to a coloured product) is added. It is then possible to quantify the amount of antibody present in each well by measuring the absorbance of light by the coloured product (Fig. 1A).

Detection of antigen in a sample is possible in a similar way. Samples to be tested are coated on to the surface of the wells and antibody specific to the antigen is added to the plate; non-specific binding is
prevented. This detection antibody is conjugated to an enzyme and addition of a substrate makes it possible to quantify the amount of antigen present in the sample (Fig. 1B). A modification of this technique, called a sandwich ELISA, allows detection of soluble or secreted products. Here, antigen-specific antibodies are used to coat the plate where they can bind antigen when the test sample is added. A second detection antibody that binds to a different epitope on the antigen is then added (Fig. 1C).

**Serum neutralisation test (SNT)**

The SNT is sometimes used as an alternative to an ELISA for detection of antibody (for example against BVD). Serum samples from test animals are serially diluted and each dilution is incubated with the same quantity of virus. Any anti-BVD antibody present will bind to the virus, ‘neutralising’ it. BVD susceptible cells are then added to the samples; if the virus is neutralised, no cytopathic effect on these cells will be visible on microscopy. The dilution of serum (or its log equivalent) at which virus neutralisation no longer occurs is the reported result.

**Complement fixation test (CFT)**

The CFT (Fig. 2) is most commonly used to detect antibody against (and therefore exposure to) pathogens such as *Brucella abortus* and *Histophilus somni*. The test relies on the activation of the complement cascade via the classical pathway (Inman & Hudson, 2009).

Here, the C1q complement protein binds to antigen-antibody complexes, initiating the cascade. Where pathogen-specific antibody is present in serum, the addition of appropriate antigen will cause the

---

**Fig. 1:**

A. Antigen-specific antibody in the test sample is detected using antigen bound to the plate and an enzyme-linked detection antibody plus substrate;

B. antigen in a test sample is detected by coating it onto the surface of the well and adding a detection antibody to bind it; C. a sandwich ELISA is used to detect soluble antigen - antigen-specific antibody is bound to the plate, the sample containing the antigen is added to bind this first antibody and a second enzyme-linked antibody is used to detect bound antigen.

**Fig. 2:**

1. Antigen-specific antibody is present in the test serum but not in the control; 2. antigen is added and binds to the antibody in the test serum; 3. complement proteins are added and are fixed to the antigen-antibody complexes in the test serum but remain unfixed in the control; 4. antibody-coated sheep red blood cells are added. The unfixed complement in the control sample binds to the antibody on the surface of the cells, causing haemolysis. In the test sample, this cannot occur as complement is already fixed.
formation of antigen-antibody complexes. When complement is added to the solution, it will bind to these complexes, or become ‘fixed’. If no antibody is present, these complexes will not form and there will be no fixation of complement. Antibody-coated sheep red blood cells are then added to the test solution. If free complement is present, it will bind to the antibodies on the surface of the cells, initiating the cascade and causing haemolysis. If no free complement is present due to fixation on the antigen-antibody complexes, no haemolysis will occur. The absence of haemolysis therefore denotes a positive test.

Microscopic agglutination test (MAT)
The MAT is commonly used for the diagnosis of leptospirosis and basically allows the recognition of complexes formed from specific antibody in test serum and antigen added to the sample. This involves incubating serial dilutions of serum with known concentrations of live cultures of the serovars of interest. The test is read by transferring a drop from each well on to a microscope slide and examining it by dark-field microscopy. If antibody is present it will agglutinate the live leptospires. Again, the dilution of serum (titre) at which agglutination no longer occurs at a specific level is the reported result. The basis of the SAT is essentially the same except that the agglutination is visualised macroscopically.

Choice of test
There are various reasons that different diagnostic tests are chosen. Broadly speaking, ELISA tests are rapid, inexpensive and have relatively good specificity and sensitivity. They are often the test of choice where an ELISA has been developed for a specific application. Other tests are most often used where there is no ELISA, or where (often older) tests are specifically mentioned in legislation (for example relating to animal exports).

As with all diagnostic tests, it is important to be aware of the properties of the test when interpreting results and to look at data critically before making decisions. In particular, specificity and sensitivity of commonly used tests are important to be aware of (from a practical point of view the positive and negative predictive values are more useful, but these will vary according to the prevalence of disease in the population being tested). Some immunodiagnostic tests, such as the ELISA to detect serum antibodies to MAP in Johne’s disease, have a relatively low sensitivity in certain circumstances (e.g. in animals early in the course of disease), which will decrease the negative predictive value of the test (i.e. decrease the probability that a negative result is correct). Properties of tests may also vary between laboratories depending on reagents and protocols used. Laboratories should be able to give an idea of the sensitivity and specificity of the tests they offer, and this knowledge should be used in conjunction with test results.

CONCLUSION
This series of articles has attempted to put some current immunological knowledge into a clinical context for the cattle vet. A sound understanding of the immunological principles underlying different disease states as well as the practice of vaccination and immunodiagnostic testing is extremely helpful in providing a good quality of service to farm clients.

ACKNOWLEDGEMENT
The authors would like to acknowledge the kind help of staff at the Veterinary Laboratories Agency, Langford, for their help with this article.

REFERENCES
INMAN, C. F. and HUDSON, C. (2009) Cattle immunology: the immune response to bacteria. UK Vet - Vol 14 No 3 May 2009 (29).