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COLLECTION AND INTERPRETATION OF TRACHEAL WASH AND BRONCHOALVEOLAR LAVAGE FOR DIAGNOSIS OF INFECTIOUS AND NON-INFECTIOUS LOWER AIRWAY DISORDERS

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Introduction

Significant advances in our understanding of equine pulmonary diseases have occurred over the past 20 years with much progress due to improvement in diagnostic testing. Recent development has focused on diagnostic tests which equine practitioners can use 'in field' and include tests to evaluate the lower respiratory tract (LRT) of horses such as tracheal wash (TW) and bronchoalveolar lavage (BAL). Naturally the quality and diagnostic value of any sample collected is directly related to the care and skill used by the practitioner in its gathering. Thus, technical prowess and a solid understanding of the potential errors in the collection, processing, evaluation and interpretation of these tests, will improve the outcomes achieved. This is particularly the case when evaluating these samples for potential infectious diseases.

In addition it should be remembered that these techniques should not replace the procurement of an accurate history and performing a thorough clinical examination. This information will either establish a diagnosis or alternatively dictate appropriate diagnostic tests to be performed. In addition, a lack of specific clinical signs associated with respiratory tract disease, yet evidence of poor performance, may be indicative of subclinical or low grade pulmonary pathology. In this situation ancillary diagnostic testing such as TW and BAL may detect subtle changes in the respiratory tract that are not discernible with routine clinical evaluation.

Indications

Indications for TW and BAL include signs of lower respiratory tract (LRT) disease, fever of unknown origin, poor performance, the presence of mucopus in the trachea and possibly dyspnoea. When choosing one or both of these procedures it is important to remember the differences between these techniques. TWs obtain secretions from the larger airways, including the trachea and bronchi, and also from more distal airways, moved to the trachea by mucociliary clearance. BAL harvests secretions from the smaller, more distal airways and specifically from the region lavaged. In certain instances, where a particular diagnosis has a high degree of certainty based on history and clinical signs, one or other of these techniques may be indicated; e.g. BAL in cases of suspected “heaves” or exercise induced pulmonary haemorrhage (EIPH); TW in suspected bacterial pneumonia, pleuropneumonia or fever of unknown origin. In other situations, where the diagnosis is unresolved, such as cases of poor performance or coughing during exercise, collection of samples using both techniques is recommended to broaden assessment of the health of the lower airways. It is important to note that no significant correlation between TW and BAL cytology has been found, therefore the cell population sampled by one technique is not representative of that obtained by the other. Additionally, recent studies have shown that inflammation of the airways may be regionally localized, therefore a combination of TA and BAL is more likely to detect airway inflammation. When there is an indication to perform both procedures, the TW should be performed 1st, particularly if bacterial cultures are to be collected. This is required as BAL will result in transient bacterial contamination of the distal trachea with oropharyngeal flora. Contraindications for TW and BAL include horses or foals with severe respiratory distress, cyanosis, marked hypovolaemia or presence of significant dysrhythmias. Similarly horses with known defects in haemostasis should be assessed carefully before being subjected to these procedures.

Collection techniques

Samples from the LRT are primarily collected for cytological and microbiological evaluation. The choice of technique and method of sampling can significantly affect numbers of bacteria and cells and types of cells obtained. For this reason, standardisation of technique with regard to type of technique used, time of sampling, volume and type of fluid instilled, sample handling and processing is recommended.
The timing of sample collection may influence results. Large increases in numbers of bacteria and inflammatory cells in the LRT can occur within 6 hours of restricted head movement (cross tying, transportation). This accumulation is usually cleared within 12 hours of horses being released from confinement, although clearance may be prolonged if horses are dehydrated. This change in numbers of cells and bacteria must be considered when interpreting samples collected from horses transported long distances. Similarly, collection of samples after exercise can influence results. Samples collected 30-60 minutes after moderately intense exercise may yield specimens of greater diagnostic value as they are more likely to contain extra secretions, more adequately represent different areas of the respiratory tract and are therefore more likely to reveal the presence of airway disease.

**Tracheal washes**

Several methods for obtaining cytological and microbiological samples from the tracheobronchial tree have been developed, each having advantages and disadvantages. The most important consideration when choosing a technique is whether microbiological culture of the tracheobronchial secretions is indicated, such as in cases of suspected bacterial pneumonia. Aspirates obtained endoscopically invariably become contaminated by upper airway flora and therefore are unsuitable for microbial culture unless specific precautions are taken. Use of a double or triple guarded catheter passed through the biopsy port of the endoscope may circumvent this problem. Alternatively, the transtracheal (percutaneous) aspiration (TTA) technique may be used.

Specific techniques used to collect samples from the tracheobronchial tree have been well described in texts. An increasingly popular alternative for collection of TWs is via a fiberoptic endoscope. This technique has largely replaced the percutaneous technique since it can be performed less invasively and has fewer side effects. The method is well tolerated by horses and endoscopy allows visualisation of the LRT at the time of sampling. However, it should be re-emphasized that samples collected using unguarded catheters will inevitably be contaminated with bacteria from the nasopharynx and biopsy channel making them unsuitable for microbial culture. More recently, a number of double or triple guarded catheter systems have been developed for collection of uncontaminated samples from the LRT via endoscopy. Again, these guarded catheters are passed through the biopsy channel and samples aspirated whilst directly visualising the collection site. Thorough disinfection of the endoscope, including the biopsy channel, prior to each collection is essential or contamination with organisms such as *Pseudomonas* spp will occur. For this purpose, 2% chlorhexidine in 70% alcohol for 10-15 minutes is effective. The endoscope and biopsy channel should then be flushed thoroughly with sterile saline before sampling.

Although the advantages of guarded catheters are many, there remains some controversy regarding the adequacy of these samples for microbiological culture. Technical prowess definitely influences the quality of sample obtained and factors that help prevent contamination include; rapid sample collection, small volume of instilled sterile isotonic saline (a maximum of 10-15ml), advancement of only the inner (sterile) catheter into the 'tracheal puddle'. In addition, if the horse coughs frequently during collection, the TW has an increased risk of contamination with oro-pharyngeal organisms and is rarely appropriate for bacteriological culture. Finally, isolation of upper airway contaminants or transient bacteria, which are not causing inflammation, is possible with any method of aspiration and correlation of bacteriological, cytological and clinical findings should always be performed. Identification of the presence of squamous epithelial cells and inflammatory cells, quantification of the numbers of bacteria and differentiation of bacterial species is essential when attempting to determine the significance of the cultured isolates.

**Bronchoalveolar lavage**

BAL collects samples from the distal airways and alveoli and is most frequently used for diagnosis of diffuse and/or chronic disease processes. In these cases, samples collected from either side of the lung are considered representative of the entire lung. This constitutes the basis for the use of a 'blind' field technique using a flexible, cuffed naso-bronchial tube. Alternatively, BAL may be collected using an endoscope, which allows more specific selection of the site for lavage especially when there is suspicion of localized lung pathology. Samples obtained by BAL are most commonly evaluated cytologically, although culture of samples, on rare occasions, may also be indicated.
The volume of infused fluid impacts on the total and differential cell counts of the BAL. Smaller volumes typically yield a bronchial wash without retrieving cells from the alveolar space and therefore have a higher percentage of neutrophils. Larger volumes yield samples more representative of the respiratory epithelial lining fluid within the alveoli. A standard volume of 250-500 ml is currently recommended. It is important to observe the presence of white foam (surfactant) on the surface of the sample as this indicates that alveoli have been sampled.

Sample handling, transportation and processing

As samples from the respiratory tract are often collected in the field, there is a necessary delay whilst samples are transported to the laboratory. Every effort should be made to transport these samples in a timely manner to prevent deterioration of cellular morphology and bacterial proliferation. Minimal cellular deterioration will occur if processing occurs within 8 hours of collection when samples are stored at room temperature. This can be extended to 24 hours if samples are stored at 4°C. In addition, bacterial overgrowth of samples rarely occurs over 24 hours in samples stored at 4°C. In contrast, samples that are not refrigerated are at risk of rapid bacterial overgrowth with pathogenic or contaminant organisms, potentially confusing interpretation. Finally, strictly anaerobic bacteria, often isolated from pneumonic processes, rarely survive any storage regimen, particularly if at cooler temperatures. Specimens submitted for viral isolation should be placed in virus transport media during transport.

In all situations it is optimal if smears are made as soon as possible after sample collection. This is recommended as the smears will serve as a reference point for cellular morphology and microbial populations at the time of sampling. Smears should be submitted to the laboratory with all other samples.

Initial evaluation

Gross Examination

Normal TWs and BAL are clear or mildly turbid, where increased turbidity and presence of flocculent material reflects increased mucus, cells and cellular debris and may be observed in horses with LRT disease. Occasionally, pieces of plant material or debris may be observed in TWs and BALs if the horse had undertaken strenuous exercise prior to sampling. All fluids should be odourless normally. A putrid smell may be associated with anaerobic infections or necrosis of lung tissue and is consistent with a downgraded prognosis. However, absence of a foul odour does not rule out either disease processes. TWs and BALs are normally clear or colourless, with change of colour varying with numbers of red blood cells (RBC) and nucleated cells. TWs or BALs from horses with recent EIPH may appear pink or red, whereas more long-standing haemorrhage may result in brown-tinged fluid due to the presence of haemosiderin.

Mucus

Evaluation of the quantity of mucus within a TW and BAL is best performed in conjunction with endoscopy of the lower airways. This will facilitate accurate estimation of the amount of mucus present in the airways, as opposed to just the quantity of mucus collected. The mucociliary clearance mechanism in normal horses is efficient, such that mucus elimination keeps pace with production. Consequently, the healthy LRT contains little or no muco-cellular material and low numbers of free cells. TW and BAL from normal horses are translucent and light grey, with a few fine strands of clear mucus which may appear as flocculent material. Cytologically, little mucus should be seen.

The amount of mucus in lower airways increases with pulmonary irritation. Specific causes of increased mucus or mucopus include bacterial, fungal or parasitic pneumonia, chronic bronchitis, heaves, and IAD. However, the significance of mild increases in the amounts of mucus in the airways remains unresolved and the point at which increased mucus is significant has not been defined. This is particular the case in horses with increased amounts of mucus, but no, or mild increases in the number of neutrophils and many activated macrophages.
Cell counts

Quantification of the total number of cells/ml of sample retrieved may help indicate overall cellularity as well as assisting interpretation of relative numbers of individual types of inflammatory cells. However, a number of factors will influence the accuracy of these counts, such as variable dilution by infused saline and large amounts of mucus which can trap cells. Despite these shortcomings, total nucleated cell counts (TNCC) and RBC counts should be performed using either a Neubauer haemocytometer counting chamber or an automated cell counter. In general, a TW from a clinically normal horse usually contains < 109 cells/L, with few to no RBCs present. The reference range for BAL fluid is usually < 109 cells/L, though an upper limit of 4 × 108 cells/L is also used. Maximal increases in TNCC usually occur in cases of bacterial pneumonia or pleuropneumonia, heaves or lungworm infections.

Evaluation and interpretation of samples for cytology

Evaluation of samples from the LRT should not be performed in isolation, but be interpreted in association with history, clinical signs and results of other diagnostic tests. In addition, care must be taken when assessing results as controversy remains as to their significance. This is highlighted by differing definitions of normal/abnormal cytological findings between laboratories, unknown significance of mild increases in inflammatory cells and mucus (particularly in performance horses), and variable interpretation in results of bacterial culture.

A sound knowledge of the cells routinely present in various sites and their normal morphological appearance is required for cytological evaluation of samples obtained from the LRT. These cells are well described in a number of texts. In addition, determining the relative proportions (%) of cells aids the interpretation of pathological changes, and is performed via a differential cell count of 200 - 300 consecutive cells. When performing a differential cell count on TWs it may be more difficult to differentiate cells as they are frequently more degenerate and may be trapped in dark stands of mucus. This is particularly the case when pulmonary disease is present as decreased mucociliary rates prevent their clearance. In contrast, cells observed in cytological preparations of BAL are better preserved and usually easier to identify.

Epithelial cells

Samples collected from the LRT reflect the normal respiratory epithelium of this region, together with inflammatory cells present as the first line of respiratory defence mechanisms. TW collect cells from the entire LRT and should thus contain cells from all levels of the pulmonary tree, including columnar and cuboidal epithelial cells, goblet cells and pulmonary alveolar macrophages (PAMs). Limited interpretation of samples is possible if all these cells are not represented.

TWs from normal horses contain low numbers of epithelial cells, although increased numbers may be observed in samples obtained endoscopically. These are predominantly ciliated epithelial cells, with their size reflecting the site of origin. Goblet cells may be observed interspersed among the epithelial cells. Epithelial cells may also be identified in BALs but are less common than in TWs. Squamous epithelial cells should not be present in TWs or BALs from normal horses and when present they represent oropharyngeal contamination.

An increase in the number of epithelial cells in samples is relatively rare. Changes to the morphology of epithelial cells are more common and help indicate pulmonary pathology. Mild changes can occur in normal horses and probably represent normal ‘wear and tear’ or turnover of cells. Pathological changes to epithelial cells (epithelial atypia) result from inflammation. In addition, in cases of infectious respiratory tract disease, there may be direct damage to the epithelium by viruses or bacterial toxins.

Inflammatory cells

Large variations in the % of inflammatory cells in TW and BAL from normal horses are reported. This may reflect differences in sampling technique and processing, but also is influenced by the geographical location of the horse at the time of collection. In general, stabled horses will have a higher proportion of inflammatory cells; most likely a response to low level irritation by inhaled agents. These mild
elevations in the proportions of inflammatory cells, often accompanied by a mild increases in mucus, probably represent a normal response to these noxious stimuli and in all probability do not contribute to decreased respiratory function. However, if prolonged, this irritation may act as a risk factor for development of more severe respiratory tract pathology. Clear cut reference values for TW and BAL from normal horses under different environmental conditions need to be established to better define abnormalities.

Defining cut-off values for normal percentages of inflammatory cells is difficult due to considerable variation between studies. In general, however, it is considered that BALs should have < 5% neutrophils, < 2% mast cells and <0.5% eosinophils. Wider ranges in the proportions of lymphocytes (30-60%) and macrophages (40-70%) are reported, therefore ascribing cut-off values for these cell types is more complicated. Tracheal aspirates should have < 20% neutrophils, <1% eosinophils, < 10% lymphocytes and very few mast cells.

**Macrophages, red blood cells and haemosiderophages**

Pulmonary alveolar macrophages (PAMs) are the most abundant inflammatory cell in TWs and BALs from normal horses, therefore, increased proportions of these cells are difficult to detect. Occasionally increased amounts of mucus, increased TNCC and increased numbers of activated macrophages may be observed, but the significance of these changes is currently not clear. Although low numbers of intact RBC are observed frequently in TWs and BALs from clinically normal horses they are usually considered to reflect minor iatrogenic haemorrhage during collection. Following respiratory tract haemorrhage, RBCs within the airways are rapidly phagocytosed by PAMs. The RBCs are subsequently degraded and their haem pigment reduced to haemosiderin, giving rise to haemosiderophages. All causes of respiratory tract hemorrhage caudal to the larynx will result in haemosiderophages, but the most common cause is EIPH. Whilst good correlation has been reported between the presence of haemosiderophages in BALF and the presence of pulmonary haemorrhage in the caudodorsal lung lobes at necropsy, these cells are observed in a large proportion of horses in training, including those that are clinically normal. The 'acceptable' number of hemosiderophages in TAs and BALF is controversial as their number may not reflect the total amount of blood that has entered the airways.

**Lymphocytes**

Lymphocytes are present in low numbers in normal TAs, but occur in higher proportions in BALs. Interpretation of increased lymphocyte ratios is complicated by the wide range of proportions observed in BALs from normal horses. Although increased proportions have been reported in racehorses with exercise intolerance and in other horses with chronic coughing, their significance remains unclear. A narrower range of lymphocytes occurs in TWs, but no consistent correlation has been made between an increase proportion and a specific disease processes.

**Neutrophils**

Although a population of well-preserved neutrophils resides in horses' airways, the relative proportion of these cells is considered to be normally low. However, neutrophils respond to a variety of stimuli, and their numbers may fluctuate rapidly. In addition, neutrophils generally are found in higher proportions in TW than in BALs from normal horses. This possibly reflects the greater exposure to noxious influences occurring in the larger airways.

Neutrophils are usually the most common cell present in samples where there is an increase in TNCC. For example, they are the predominant cell observed in horses with bacterial pneumonia or pleuropneumonia and the percentage of neutrophils is usually > 40%, often exceeding 90% in acute cases. Elevated total and relative numbers of neutrophils also may be observed in cases of IAD, EIPH, acute viral infections, chronic bronchitis and summer pasture associated obstructive pulmonary disease (SPAOPD). However the increase in percentage is variable. Cases of interstitial pneumonia usually have low neutrophil numbers in TAs, but these may be elevated in BALs. Horses with clinical heaves will demonstrate elevated total numbers and proportions of neutrophils in BALs, but horses in remission will have normal BALs. The presence or absence of degenerative and toxic changes to neutrophils may assist in the interpretation of increased % of this cell line. In some disease conditions (e.g., heaves, EIPH and some cases of IAD) neutrophils are mostly mature with no degenerate or toxic changes. In contrast,
degenerate and toxic neutrophils are observed commonly in samples from horses with bacterial or fungal pneumonia or pleuropneumonia. In these cases, careful examination of neutrophils for intracellular organisms is often diagnostically rewarding.

**Eosinophils**

Low numbers of eosinophils are present in TWs and BALs of normal horses. Large increases in the % of eosinophils may be observed in lung worm infection or during ascarid migration. Smaller increases in % of eosinophils may be observed in allergic respiratory disease. Frequently these elevations, particularly of eosinophils, are transitory and may not be observed in samples collected 24 hrs later.

**Mast cells**

In normal horses, mast cells in TWs are rare, in contrast to BALs where higher numbers of mast cells may be observed. This difference may be explained by the predominant distribution of equine mast cells within the smaller airways and alveoli.

An increased percentage of mast cells in BALs has been correlated with increased airway hyper-reactivity and decreased exercise tolerance.

**Evaluation and interpretation of samples for infections**

Bacterial cultures of TWs samples are helpful in diagnosing LRT infections and ideally should be performed in all suspected cases of pneumonia or pleuropneumonia. Samples collected by BAL may occasionally be cultured, particularly when collected from a suspected lung abscess and when the endoscope is guided into an airway where mucopus is draining. In foals, this technique may also be used to diagnose Pneumocystis carinii infections, which is interstitial in nature. BALs collected 'blindly' are never of use for microbial culture as they are contaminated with oropharyngeal flora. In addition, this BAL technique also harvests cells from the caudodorsal lung fields, which are rarely involved in infections. Ideally, any sample submitted for microbial culture should be obtained prior to antimicrobial administration or after a sufficient period is allowed for tissue clearance of previously administered antimicrobial agents (at least 24 and preferably up to 72 hours).

Aerobic and anaerobic culture should be performed on TWs which have cytological evidence of airway inflammation. Quantitative cultures, which determine the number of colony forming units (cfu) for each bacterial species, provide additional information where normal horses or from horses with airway inflammation without a bacterial aetiology usually yield < 103 bacteria (cfu)/ml and frequently no bacteria at all. If > 103 cfu/ml are cultured, it is likely that these bacteria are contributing to the disease process, and identification of each species present in high numbers will assist in interpretation of their significance. Furthermore, the greater the cfu/ml isolated, the more likely the significance of the bacteria isolated. Identification of isolated bacteria allows differentiation of possible pathogens from probable contaminants.

The normal bacterial flora of the URT ceases at the larynx and the LRT is considered a sterile site. However, bacteria may occur transitorily in the LRT, especially post-exercise or after transpiration, or may be introduced at the time of sampling. Thus, isolation of bacteria from TWs or BALF may represent infection, a transient lower airway population or contamination at the time of sampling. It is essential for appropriate management of these cases to differentiate between these scenarios. Differentiation can be assisted by; presence of clinical signs consistent with pneumonia or pleuropneumonia, identification of isolates as known pathogens of the LRT in sufficient numbers (ie >103 cfu/ml), and cytological evidence of inflammation. Clinical signs consistent with pneumonia include fever, signs of depression, tachypnoea, abnormal respiratory sounds, coughing and nasal (often purulent) discharge. However, absence of these signs does not preclude bacterial infection, especially in milder cases of IAD caused by bacteria. Neutrophilic exudates are consistently observed in samples from horses with bacterial infections of the LRT and are not present if non-pathogenic species or transient bacteria are present. TWs from horses with bacterial LRT infections will have increased mucus, increased TNCC, and increased relative and absolute neutrophil counts with possibly degenerative neutrophils and intracellular bacteria. No significance can be ascribed to bacteria isolated without this cytological evidence of inflammation. In addition, it is preferable not to culture samples with large numbers of squamous epithelial cells, even when there are many neutrophils present, as this is evidence of oropharyngeal contamination. If such samples are cultivated, and large numbers of bacteria
are isolated, it is not possible to assign any significance to these isolates. Re-collection of the sample is recommended.

A variety of bacteria can cause LRT infections and frequently these infections are mixed. Bacteria that are most commonly isolated from uncomplicated lower airway infections in adult horses include *Streptococcus* (both α and β haemolytic), *Pasteurella* spp, *Actinobacillus* spp and occasionally *Bordetella bronchiseptica*. Bacteria in the Enterobacteriaceae family (e.g., *E. coli*, *Klebsiella pneumoniae*) are more commonly secondary invaders and may be isolated following induction of antimicrobial therapy or when pneumonia is severe. Anaerobic bacteria (e.g., *Bacteroides* spp, *Fusobacterium* spp, *Peptostreptococcus* spp) may be isolated from cases where sufficient necrosis of lung tissue has occurred such as within lung abscesses or when pleuropneumonia is present. *Mycoplasma equi* and *M. feli* have been implicated in some outbreaks of equine respiratory infection. While it is difficult to culture these agents, some laboratories now perform serological evaluation in order to assist diagnosis of these infections. Pneumonia in foals may be caused by all the isolates causing disease in adults, as well as *Rhodococcus equi*. Pathogenic bacteria that rarely cause LRT disease but are common contaminants during sampling include coagulase positive *Staphylococcus* spp, *Pseudomonas* spp, and *Proteus* spp. In particular, *P. aeruginosa* is a major contaminant commonly residing in inadequately sterilized endoscopes. This organism is rarely involved in LRT infections. Isolation of non-pathogenic bacteria (e.g., *Bacillus* spp, coagulase negative *Staphylococcus* spp), especially if part of a mixed culture, indicate contamination at the time of sampling.

Occasionally, pathogenic fungi may be cultured from TWs or BALs. The most frequently identified pathogenic fungus from the LRT of horses is *Aspergillus* spp, although the primary pathogens *Blastosomyces dermatitidis*, *C. immitis*, *Histoplasma capsulatum* and *C. neoformans* also are occasionally isolated. Contaminating fungi, especially *Alternaria* spp, must be distinguished from true pathogens. The presence of an appropriate inflammatory reaction, together with free and ingested fungal elements, will help with this distinction. The fungal parasite *Pneumocystis carinii*, can also cause acute interstitial pneumonia in immunosuppressed foals or secondary to *R. equi* infections. Mixed inflammatory reactions (neutrophils and macrophages) together with the distinctive intact cysts can be identified in TWs or BALs. Finally, the majority of respiratory fungal infections in horses are secondary to immunsuppression, other severe diseases of the lung, or severe systemic diseases. Therefore, investigation of potential underlying disorders should be conducted in order to appropriately assess and treat these secondary infections.

Parasitic bronchitis and pneumonitis has decreased in prevalence due to improved anthelmintics. The most likely parasite involved in pulmonary disease of foals and young horses is the round worm, *Parascaris equorum*. In contrast, the lung worm, *Dictyocaulus arnfieldi*, occurs predominantly in adult horses pastured with donkeys. Parasitic pneumonitis may be difficult to definitively diagnose, but a history of a poor anthelmintic regimen or close association with donkeys may provide clues. In addition, large numbers of eosinophils and activated alveolar macrophages may be observed on cytological preparations of TWs and BALs, but are not specific for parasitic infections as they also may be observed in other forms of eosinophilic pneumonia.

A presumptive diagnosis of viral respiratory tract infections may be based on history and clinical signs. However, confirmation requires viral isolation or detection by serological or molecular techniques. Positive results should be discussed with the laboratory in order to apportion appropriate significance to this finding. Viral isolation or detection of viral antigens or nucleic acid will confirm the presence of specific viruses but is rarely performed in routine cases. A number of serological tests for respiratory viruses have been developed and include assay for EHV-1, -2, -4, equine influenza, equine rhinoviruses A and B, equine arteritis virus and adenoviruses. The specific type of test, their availability, and the interpretation of results will vary between laboratories. However, in most cases, a 4-fold or greater increase in titre between acute and convalescent serum samples is considered significant and may be used to indicate retrospectively that a viral infection has occurred. Alternatively, a lack of an increasing titre does not rule out a viral infection as titres rise rapidly during infection and may be increased at the time of onset of clinical signs. Although serological diagnosis of viral infections may not be of immediate value in the clinical management of cases, it may yield valuable information on the aetiology of infections, particularly if virus isolation has proven unsuccessful. Such epidemiological information has apparent advantages in monitoring and designing effective vaccination programs, particularly for large susceptible groups of horses, such as those kept in training yards.