General Interactions of the Immune System in Relation to Bovine Respiratory Disease

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Introduction

When the jigsaw puzzle fan starts to work on a puzzle, the jagged pieces strewn around the table top really do not provide much insight into the final appearance of the completed puzzle. At this point, just sneaking a look at the picture on the cover of the box helps with the initial organization of related pieces, and speeds assembly of the puzzle.

The bovine respiratory (BRD) complex resembles a gigantic and frustratingly difficult puzzle. Those of us who wrestle with potential solutions to this complex problem often become preoccupied with its individual pieces. Possibly this is because we have yet to visualize how all these pieces fit together. Possibly this is because there is no picture on the cover of the box.

Veterinary scientists from all disciplines have contributed significantly to our joint understanding of many of the individual pieces of BRD, however, in recent years, the most exciting new findings are being reported from the field of immunology. While many other scientists have studied bits and pieces, immunologists are now beginning to suggest ways that these parts may interact and fit together.

The body's immune system reacts directly with all other organ systems internally, and with the body's environment externally. Even in the early stages of embryonic development, the chemical messages that direct how cell types develop and differentiate into tissues and organs seem to be immunological mediators. Immunology is the study of interactions.

Therefore, any discussion of those factors which affect the incidence or severity of BRD must center largely on concepts in immunology. Even more so, as we include discussion of potential interactions, we must rely heavily on immunological parameters.

Eventually, with improved understanding of these interrelationships among the puzzle pieces, we may be able to draft a crude or preliminary sketch of that picture on the cover of the box.

Some General Relationships

1. *Genetic*
   There is direct genetic control over the nature of the immune response. Prior to birth, each individual is programmed for relative resistance or susceptibility to particular types of disease. Just as there can be inheritable defects in the body's physical structure, there can be defects in the immune function. Plant geneticists have long sought disease resistant varieties of domestic plants, or sought to produce them by hybridization. Advances in bovine immunogenetics are expected as a result of research programs underway at the Meat Animal Research Center in Nebraska, and elsewhere. This new knowledge will prove exciting.

2. *Nutritional:*
   Again, even prior to birth of a calf, we need prenatal care programs for the pregnant cow. A most significant aspect is nutrition. We need adequate energy level and protein precursors. In addition, balance of trace minerals supplied or available to the pregnant cow and to the neonatal calf is essential. Interestingly, these minerals, such as calcium, phosphorus and magnesium are cofactors, or otherwise involved with the enzymatic reactions of the immune system. Others, such as copper and selenium are necessary in that they aid in minimizing some otherwise damaging side-effects of an over-active immune response. Nutritional balance is essential for balanced immune response.

3. *Environmental:*
   Largely through the secretory immune system, the calf erects a protective shield against potentially injurious substances or possible pathogens that it may encounter in its environment. We can greatly influence relative health of a group of calves by minimizing environmental stress factors. Doesn't it seem logical that we should carefully consider how crowding, rapid fluctuations in ambient temperature or relative humidity, exposure to dust or chemical irritants impinge on the secretory immune system in general, or upon secretory immunoglobulin (IgA) level and function, specifically?

4. *Management:*
   Consultants in food animal medicine tell us that the single biggest problem in any livestock enterprise is not the animals, but the people involved. To counteract these management deficiencies, we are turning to innovative concepts such as integrated resource management (IRM)

systems. To implement these concepts, we will, of course, be guided most frequently by the principles of immunology. Immunology is the study of interactions. IRM will be applied immunology to a large degree.

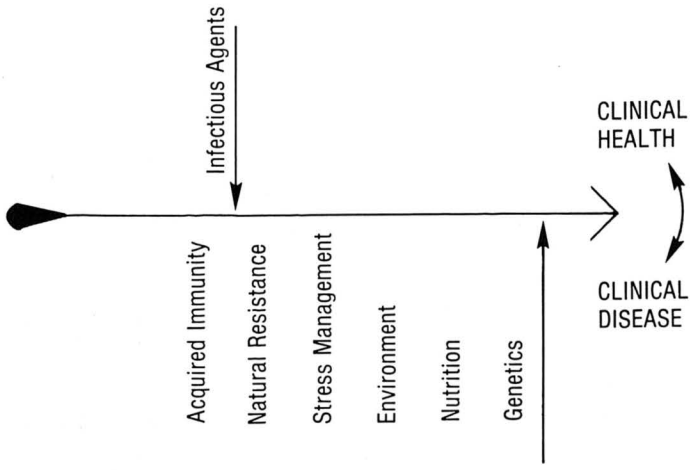
5. *Natural Disease Resistance:*
The calf is genetically programmed to protect itself against harmful substances it will encounter in its external environment. The calf is genetically programmed to regulate the interrelationships and functions of its own internal environment. These are natural, or in-born immune functions. These are augmented by naturally acquired immunity, resulting from everyday exposure to its surrounding environment. These reactive tendencies are often in somewhat precarious balance. To maintain health, the calf maintains that balance. Disease is imbalance.

6. *Vaccination:*
Last, and probably least, come vaccination programs. We must understand the normal immune reactions. We must not ignore their presence or their importance. As we superimpose artificial vaccination programs, we must enhance normal functions rather than disrupt them. If out of balance, we must exert antigenic influences in a timely way which tend to restore balanced activity. We must not create further imbalance. That is when vaccination is stressful.

As veterinarians, we tend to be egotistical. We would like to think that the animal recovered primarily because of our treatments. We would prefer to think that it remained healthy because we vaccinated it. We need to exercise some restraint so that it cannot be said, "The biggest problem in bovine immunology turns out to be the people!" Or in the words of the comic strips, "We have met the enemy, and he is us!"

Figure 1 is an attempt to portray some of these interactions which affect general disease resistance of the calf. It is as if a meter contained a pointer that registered either relative health or clinical disease.

FIGURE 1. The Health Meter.



The factors we have just discussed influence the directional swing of the pointer. Good factors support. Stress factors push downward. Only *one* of the stress factors is exposure to infectious agents in the calf's external environment. Consideration of these concepts explains why simple exposure to infectious challenge, in an experimental setting, is seldom successful in reproducing clinical illness that resembles natural field disease outbreaks.

Resistance of the Bovine Lung to Infection by *Pasteurella Haemolytica*

Clinical experience has established that nearly any primary disease process in the bovine respiratory tract very often progresses to, or terminates in, pneumonic pasteurello-sis. The infectious agent most often recovered from "shipping fever" pneumonia is *Pasteurella haemolytica,* Type I[1].

An intriguing experiment was performed several years ago by Walker, Corstvet and Panciera[2] at Oklahoma State University, in which calves were forced to inhale suspensions of virulent *Pasteurella haemolytica* microorganisms from a breather bag. Within minutes after such aerosol exposure, large numbers of these virulent bacteria were found within the terminal alveolar spaces of the lung of experimental calves. Exposure had occurred.

But did the calves develop pneumonia? No! With more elapsed time following exposure, it was found that these bacteria had rapidly disappeared from the lung. They had not persisted there for enough time to establish infection, to replicate, to produce injurious cytotoxins or other products of growth, or to trigger disease processes. It was just as if these pasteurellas had been poured into the funnel, which schematically represents the bovine lung in Figure 2.

But there's a problem with the funnel analogy. Since most calf lungs don't have a hole in the bottom, the rapid disappearance of these invaders must have been a function of immune clearance mechanisms instead.

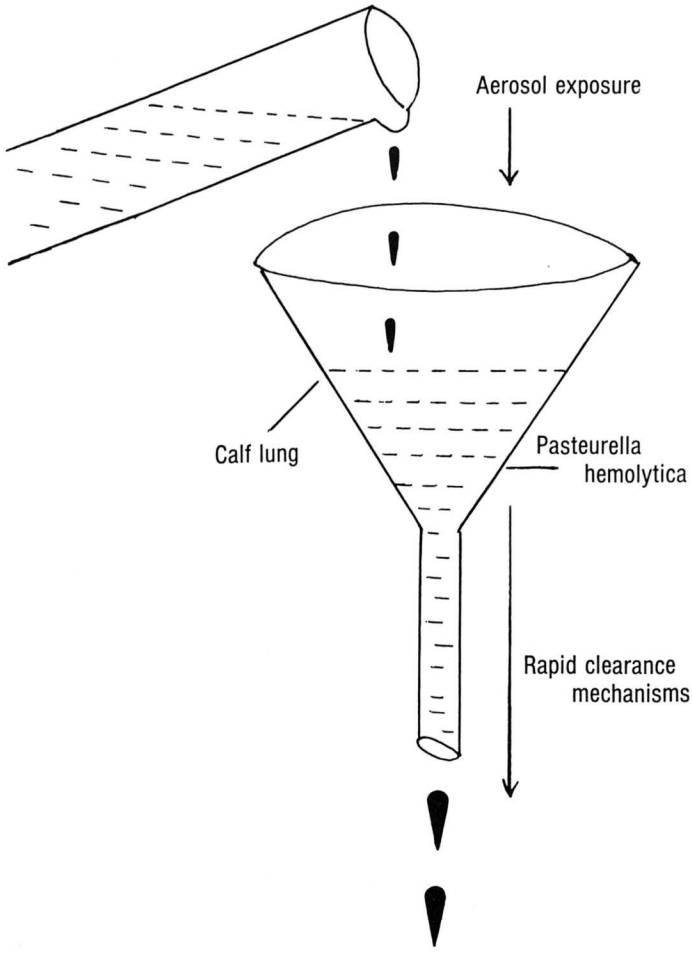
Specific Defense Mechanisms of the Calf Lung

Defense mechanisms of the bovine lung have been reviewed by Liggitt[3] and classified according to the following general outline:

1. *Physical barriers* which impede inhalation of particulate materials; the mucociliary ladder of the trachea and bronchi, as well as the cough reflex which tends to clear the airway of extraneous secretions.
2. *Cellular defenses,* with the most active roles played by the alveolar macrophage, the pulmonary lymphocyte and the neutrophil.
3. *Secretory defenses* include secretory antibodies (IgA) of the upper respiratory tract, IgA and IgG of the lung, interferon induced in response to infection, complement, and the alveolar lining material itself.

So it is obvious that the bovine lung has a significant array of defenses that must be overcome, in order for an oppor-

FIGURE 2. The Lung is a Funnel?



tunistic bacterial pathogen to initiate infection and clinical disease processes. These many defenses serve to explain the reasons for an interesting fact. Despite our impressions to the contrary, infection of the upper respiratory tract of the calf is exceedingly common, usually inapparent, or without production of clinical disease. These defenses are functional, and they are highly protective under most circumstances, and ordinarily prevent pneumonic disease, despite infection.

When we seek to explain the occurrence of respiratory disease, we probably should not bother with explanations of why exposure to potential pathogens happened to occur. We should, instead, attempt to explain *why* that normal infection evaded the normal defenses in this particular calf, at this point in time. Respiratory *disease* is *abnormal*, even if likely exposure to *infection* is *normal!*

The aerosol challenge experiments at Oklahoma State are intriguing for another reason. The physical barriers to infection were readily overcome by this challenge procedure, yet pneumonia did not occur. Of the various pulmonary defense mechanisms, immunological defenses would certainly seem to be the most significant in prevention of pneumonic pasteurellosis, as opposed to the simply mechanical barriers.

Full detail of the whole gamut of interactions among the cellular and secretory components of the immune system as it protects against BRD is not known. Yet, so much detail is known, that it is well beyond the scope of this presentation. An excellent review exists[3], and only selected highlights will be discussed here.

1. *Secretory Immunoglobulin (IgA):*

Just as it does for other external mucous membranes or body surfaces, secretory immunity plays a vital role within the respiratory passages as well. Normally present in the respiratory secretions, in both upper and lower respiratory tract, IgA performs at least two significant tasks:

a) By specifically interacting with potential pathogens, IgA blocks viral access to host cell receptor sites, or discourages attachment to host structures which is a prerequisite for bacterial colonization and growth. IgA is interposed in the most strategic of locations for this role.

b) Of possibly equal importance are some of the things that IgA does *not* do. It neither promotes phagocytosis, nor does it promote involvement of complement in its interactions with foreign antigens. Thus, in performing its first role, IgA possibly also minimizes contact of these foreign substances directly with the general immune system. This can be beneficial.

Thus, IgA appears to be unique among immune substances, in that it is very hard to find any adverse side-effects of over-production or over-reactivity. Yet, since its appearance within the respiratory tract involves active transport across a healthy epithelium, where secretory component is added in the process, presence of adequate antiviral or antibacterial IgA could be uniquely susceptible to environmental stress factors. Its level might be *diminished* at the very moment when it was in *most demand* for host defenses. Consider the probable role of shipment stress on completed synthesis or secretion of IgA. Furthermore, selective IgA deficiency is the most common inheritable immune deficiency disease of children. What about calves?

2. *Interferons:*

In response to viral, mycoplasmal or bacterial infection, synthesis of interferons is induced. The protective role of these proteins, elaborated in response to infection, as they inhibit viral replication in neighboring cells, is being actively investigated.

Interestingly, over-production of interferon may be associated with a feeling of malaise or depression, with anorexia, and that "ache-all-over feeling" associated with viral infection and illness. While generally beneficial in aiding recovery from infection, there can be adverse side-effects of too much interferon activity.

3. *Alveolar Macrophage:*

The alveolar macrophage is the most populous cell type in the alveolar spaces in the normal calf. Macrophages protect the lung from bacteria by engulfing and killing invading organisms. They also release a long list of biochemical mediators that quickly recruit assistants to the scene—both lymphocytes and neutrophils. These intermediates thus trigger the inflammatory response and can cause much tissue damage.

Just as with interferon, only more so—a little bit of activity is great, but a lot is downright damaging, to the very lung tissue that the macrophage is supposed to protect. The alveolar macrophage is a very important example of why the immune response must be balanced to be beneficial. Balance is beautiful!

Auxilliary Defenses—More Harmful Effects

The beneficial action of other immune substances or cells is well known, and most frequently emphasized. Too seldom are the adverse side-effects of the immune response considered. We will do so here.

1. *Humoral Antibodies (IgM and IgG):*

These immunoglobulins are certainly beneficial in that they are known to promote phagocytosis, agglutinate bacteria, neutralize viruses, neutralize toxins, and activate complement. IgG, for example, undergoes rapid increase in concentration within the alveoli of the lung during exudative processes of inflammation. But is it *always* beneficial there?

IgG complexes with antigen and complement. These immune complexes, when present in significant amount, are responsible for Type III hypersensitivity mediated tissue damage. Hypersensitivity pneumonitis may result, accompanied by acute respiratory disease syndromes. Experience suggests that bacterial pneumonia often follows quite readily.

2. *Complement:*

Present as a group of proteins in normal bovine serum, complement enhances phagocytosis of bacterial invaders such as *Pasteurella haemolytica.* By promoting lysis of bacteria, complement can trigger endotoxin release and promote Type II hypersensitivity reactions. The peptides activated during fixation of complement in the antigen-antibody reactions are potent causes of inflammation. Complement, when active in too great amounts in the lung, therefore contributes to severe pulmonary edema and other aspects of inflammation. Inflammation of the lung by another name is pneumonia.

3. *Phagocytic Cells and Reactive Oxygen Intermediates:*

cells Nearly all phagocytic cells (e.g. macrophages and neutrophils) release substances from lysosomes which are potent chemicals, capable of killing bacteria, and which are collectively called reactive oxygen intermediates. Unfortunately, these same substances are also exceedingly harmful to the calf's own tissues. Fortunately, the normal calf also manufactures some defensive enzymes which inactivate these substances, converting them to harmless hydrogen peroxide or to carbon dioxide instead. These beneficial enzymes require copper, or contain selenium. Thus trace mineral deficiency could result in harm to the calf, actually caused by its own cellular defense mechanisms.[4]

Interestingly, the lesions of inflammation in the calf lung which we call pneumonia are *not* actually caused by the invading opportunistic pathogen, but instead by the way in which the calf's own immune system *responds* to that infection. Futhermore, the way in which it responds is governed by other modifiers and stress factors superimposed. (Refer again to Figure 1). Once more, it is no surprise that simple challenge with the infectious agent often fails to result in typical disease syndromes, as seen in field cases.

Immunosuppression

With the recent concern and popular press attention given to acquired immune deficiency syndrome (AIDS) in man, and with recent commentary (true and untrue) about lentivirus infections of sheep, cattle and horses, immunosuppression has become a household word.

Bovine virus diarrhea (BVD) virus infection has been observed and reported[5,6] to increase susceptibility to, or to increase the relative severity of, concurrent *Pasteurella haemolytica* infection of the calf. This may, or may not, be a true example of immunosuppression.

Parainfluenza 3 (PI-3) virus and the bovine respiratory syncytial (BRS) virus have also both been cited as primary viral infections that might increase the probability of secondary bacterial pneumonia. This enhancement exists[7,8], but again it may be immunosuppression, or it may not.

Interestingly, it does not require a viral infection or even a living agent to immunosuppress. There is recent indication, for example, that some killed bacterial antigens, such as *Haemophilus somnus* bacterin may actually possess rather potent immunosuppressive activity, as measured by its specific reaction with lymphocytes or phagocytic cells of the calf.[9] So when it comes to immunosuppression, "killed" is not necessarily "good," and "modified-live" is not necessarily "bad!"

We also must exercise caution to distinguish immunosuppression from specific immune tolerance, which is the failure to recognize and to react to a specific foreign antigen. Just as we may have become overly impressed by the general subject of immunosuppression, there is an inherent danger in overreaction to recent findings governing the pathogenesis of BVD-MD. Specific immune tolerance may equally as well explain some of the clinical observations in cattle that we associate with bluetongue infections or with malignant catarrhal fever, just as readily as this phenomenon applies to mucosal disease.

Immunoenhancement, Immunomodulation and Hypersensitivity

The principal purpose of this paper is to call attention to the importance of a balanced immune response. (See Figure 3). In so doing, it is an attempt to restore some balance to our collective thinking about the immune system, to possibly cool our ardor for the subject of immunosuppression, and to call attention to the potential for host injury from excesses in the opposite direction. In essence, the other side of the coin. In essence, a look at defenses of the bovine lung from a different perspective—that is, immunology upside down. And a word of caution—it might even be backwards!

Primary viral infection can increase the susceptibility of the calf to opportunistic bacterial invasion by acting to *suppress* normal defensive immunological mechanisms, thus creating imbalance. (See Figure 4).

Primary viral infection can also interact with host defense mechanisms to promote *over-reaction.* And we have just emphasized that the serious tissue damage to the bovine lung is of the calf's own doing. Increased clinical severity of pneumonia is very likely explained by immune enhancement, rather than solely just by immunosuppression. It is probable that upward imbalance is just as damaging as downward, a possibility that we perhaps have too long ignored. (See Figure 5).

FIGURE 3. Balance Is Beautiful

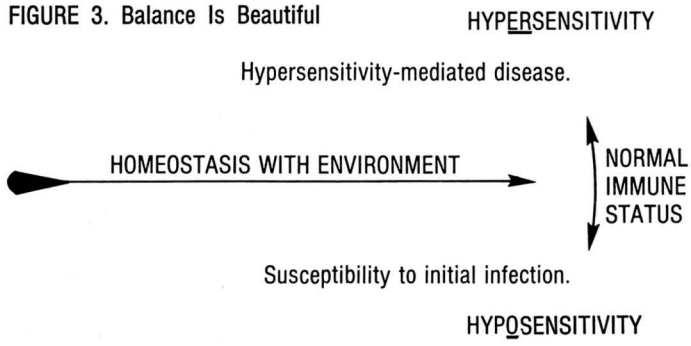


FIGURE 4. Immunosuppression.

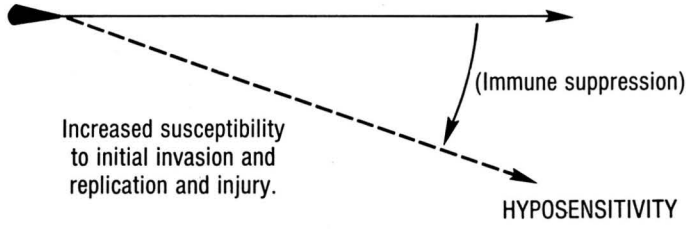
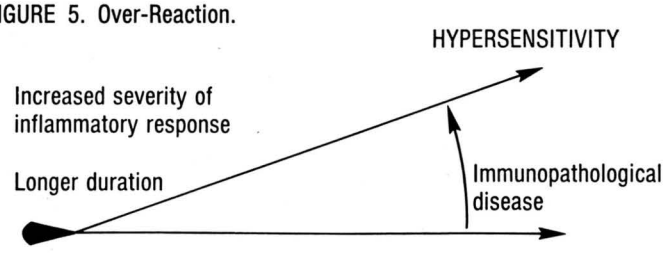


FIGURE 5. Over-Reaction.



How to Fool Mother Nature

Suppose that we wanted to repeat the experiment of Walker *et al.*[2] and that we intentionally set out to experimentally produce typical shipping fever pneumonia or pneumonic pasteurellosis, how could we perhaps improve on their methods? We would first establish several requirements:

1. Viable, virulent *Pasteurella haemolytica* would have to reach the terminal alveolar spaces of the calf lung. (Evade IgA).
2. These pathogens would have to remain long enough to adhere, to colonize and to replicate. (Evade immunological clearance).
3. During growth, we would expect them to elaborate cytotoxins and other byproducts of growth, damaging to cell-mediated immunity, probably directed at the alveolar macrophage.
4. If damaged by bacterial action, we might then expect the alveolar macrophages to respond with release of chemical messages calling for additional helpers.
5. Pneumonia would be triggered, and it would result from the calf's own immunological reactions.

Obviously, to fool Mother Nature is to fool the calf's own immunological response. We would like to manipulate these mechanisms in the proper direction at the appropriate time. *First,* nudge them downward, in order to immunosuppress them, and to allow *Pasteurella haemolytica* time for colonization. In doing so, we would target IgA and the alveolar macrophage activities.

Then, and only then, nudge them upward to enhance the severity of response and to facilitate lesion development by immune enhancement. Bring in lots of neutrophils, IgG and complement in response to pleas for help from the alveolar macrophage.

Note that we would have to totally fake Mother Nature out of her socks, with the head and shoulder move in one direction, but with the final body and leg movement going the opposite way! It's not easy to fool Mother Nature! We're talking about coordinated effort and timing.

How to Help Mother Nature

Isn't this the real objective? But evaluation of the problem suggests that it is also difficult to improve upon Mother Nature. Our own clinical experiences with the BRD complex tend to confirm this viewpoint.

Attempts to artificially intervene, or to redirect the calf's immune response can be troublesome, as we have found. It is obvious that the good timing required to fake her out, may also be required to provide the needed assistance. The good and the correct immunizing antigen might prove to be detrimental, rather than beneficial, if the natural immune response of the calf is nudged in the wrong direction at the wrong time.

We need a very clear picture of the cover of the puzzle box, and a clear understanding of the interrelationships

among the many various puzzle pieces, if we are going to be successful in our approach to artificial immunization against BRD. Then it will be nicer to fool around with Mother Nature.

Summary

As veterinarians,we like to think that the calf recovered because of our treatments. Or better still, that it didn't ever get sick, because we vaccinated it. But wait.....

Immunity is not *all good!* Uncontrolled overresponsiveness can be as bad or worse than immunosuppression. For example, development of lesions in the calf lung which we call pneumonia is due to the calf's *own immune response* to infection. So infection is very common. Disease is rather rare. Balance is beautiful!

The immune responsiveness of a calf is often in rather *precarious* balance. As we seek to intervene, we must have an understanding of the interactions which maintain that balanced response. Otherwise, we can find ourselves guilty of doing the right thing, for the right reason, but perhaps at the wrong time. *Oops!*

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Abstracts

Protection against respiratory disease in calves induced by vaccines containing respiratory syncytial virus, parainfluenza type 3 virus, *Mycoplasma bovis* and *M dispar*

C. J. Howard, E. J. Stott, L. H. Thomas, R. N. Gourlay, G. Taylor

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A field trial to assess the ability of two vaccines to protect calves against respiratory disease was carried out on a large beef rearing unit in southern England over the two winters of 1983 to 1984 and 1984 to 1985. A quadrivalent vaccine containing the killed antigens of respiratory syncytial virus, parainfluenza virus type 3, *Mycoplasma bovis* and *M dispar* or a vaccine containing only the respiratory syncytial virus component were inoculated into 246 and 245 calves, respectively; 245 calves remained as unvaccinated controls. The calves were reared in seven batches and outbreaks of disease occurred in five; significant protection was achieved in the four batches in which disease was associated with respiratory syncytial virus and *M bovis* infection, together or independently. The death rate from pneumonia was 9 per cent in the control group, 2 per cent in the calves inoculated with the quadrivalent vaccine ($P<0{\cdot}001$), a protection rate of 77 per cent, and 3 per cent in the calves inoculated with the respiratory syncytial virus vaccine ($P<0{\cdot}01$), a protection rate of 68 per cent. The proportion of calves receiving treatment for respiratory disease was 38 per cent in the control group, 25 per cent in the calves inoculated with the quadrivalent vaccine ($P<0{\cdot}001$) and 27 per cent in the calves inoculated with the respiratory syncytial virus vaccine ($P<0{\cdot}01$). The results show that protection against respiratory disease can be achieved by parenteral vaccination of calves with the appropriate inactivated microorganisms.

The effects of selenium, housing and management on the incidence of pneumonia in housed calves

M. Phillippo, J. R. Arthur, J. Price, G. J. Halliday

Veterinary Record (1987) **121**, 509-512

The occurrence and incidence of pneumonia in housed calves were not related to the selenium status of the herd as measured by blood glutathione peroxidase activity nor were they affected by selenium treatment of calves during the neonatal period. Pneumonia was related more closely to herd size and building design.