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Diagnostic Medicine: The Challenge of Differentiating Infection from Disease and Making Sense for the Veterinary Clinician

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I. Introduction

Diagnostic medicine has taken on a new, broader meaning in the 1990s and reflects an expansion of clinical investigation from the diagnosis of disease to include detection of infection (Evermann, 1998). This leads to an entirely new perspective on how veterinary clinicians and diagnosticians view laboratory tests and how test results are interpreted. One must consider not only the specificity and sensitivity of the test, but the predictive value of the test, which relates directly to the clinical utility of the result (Jacobson, 1997).

The definitive diagnosis of infectious diseases relies on a combination of clinical symptoms, history, and laboratory analyses of ante-

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mortem and/or postmortem specimens (Evermann, 1998). Disease diagnosis has customarily used diagnostic assays for early recognition of disease and rapid implementation of therapy in an individual animal basis, and when appropriate use of corrective management (segregation, culling, vaccination, etc.) on a population basis.

The detection of infection during preclinical stages has become more important as one considers the consequence of long-term infections that have prolonged incubation periods and inapparent transmission to susceptible animals in the population. This includes life-threatening diseases, such as feline infectious peritonitis (FIP), rickettsial and ehrlichial diseases and canine herpesvirus (CHV) infections. Of equal, if not more so, importance for the early detection of infection has been the increased recognition of zoonotic infections, such as rabies virus, *Salmonella typhimurium* DT104, and *Escherichia coli* O157:H7 (Evans and Davies, 1996; Slutsker *et al.,* 1997; Smith, 1996).

Together with the necessity to detect infections earlier during the preclinical stages, there has been a remarkable expansion in the availability of assays that can detect infectious microorganisms in low quantity. This increased sensitivity has been primarily through the detection of nucleic acid sequences after amplification by polymerase chain reaction (PCR, Relman and Persing, 1997). PCR can allow not only early detection of infection, but rapid speciation of organisms as well as strain typing for epidemiologic analyses (Fredricks and Relman, 1996; McDade and Anderson, 1996).

The assessment of preclinical infections allows the veterinarian the opportunity to determine the relative risk of disease occurring, and to take preventive steps to reduce or eliminate the risks depending on the consequences of the disease in the animal and/or human (if zoonotic) population.

This chapter focuses on one main issue, and that is differentiating the detection of infection from diagnosis of disease. In the course of differentiating infection from disease three questions will be addressed: (1) How early do we want to detect infection? (2) What are the consequences of the results? (3) Where are we heading with veterinary diagnostics?

II. Differentiating Infection Detection from Disease Diagnosis

Historically, the primary aim of the diagnostic laboratory was to assist the veterinarian in the diagnosis of disease. This is presented in

Fig. 1. This type of approach initially ignored the origin of the causative microorganisms and focused on the accurate diagnosis of the disease agent. An example of this type of approach was the testing of cats that were clinically ill for feline leukemia virus (FeLV). If tested positive, they were segregated or euthanized. Further examples include FIP, CHV, Johne's disease (*Mycobacterium paratuberculosis*), and the mucosal disease form of bovine viral diarrhea (BVD) virus. Expanded use of diagnostic results by the veterinarian and client allowed for some corrective management steps to be taken. These included the use of vaccination when available or segregation and culling to reduce the source of the infection in the population. Based on this latter principle, the reduction of the source of the infection, a different approach has been taken. One may consider this an epidemiologic view of the disease process (Susser and Susser, 1996a).

With a combination of more sensitive diagnostic assays, the veterinarian's concern to know the state of the preclinical infection, econom-

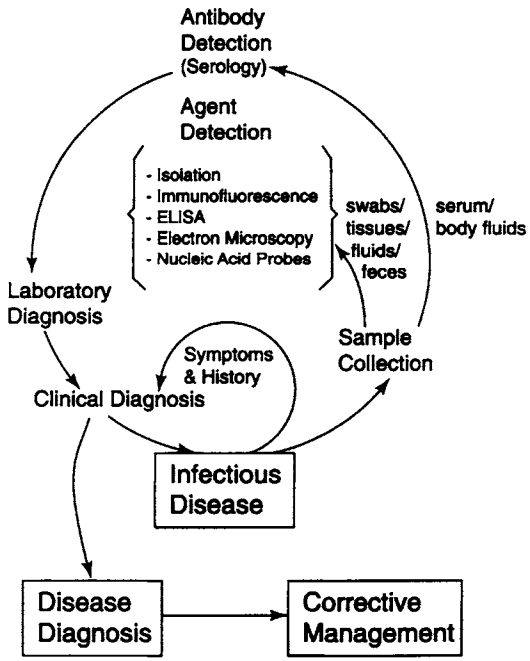

FIG. 1. Schematic depicting the historical interactions between the laboratory diagnosis of infectious disease and the steps leading to corrective management. (Modified from Evermann, 1990, with permission from W. B. Saunders Company.)

ic incentives to minimize disease by effectively controlling the infection, and concern over potential zoonotic diseases, laboratory diagnosis has taken on a different strategy. This is presented in Fig. 2. The primary emphasis in this scheme is to view animals preclinical and determine the disease risk and/or zoonotic potential of the infection. This has been the approach for some retroviral infections (Evermann and Jackson, 1997; Knowles, 1997) and bacterial infections with public health concerns, such as *E. coli* O157:H7 and *Salmonella* spp. infections (Evans and Davies, 1996; Firstenberg-Eden and Sullivan, 1997; McDonough and Simpson, 1996). The ultimate goal of the assessment of preclinical testing is to initiate a preventive management type of control. This type of approach places more emphasis on early testing and management of infected animals rather than on diseased animals.

With the shift in emphasis to preclinical testing, the knowledge of

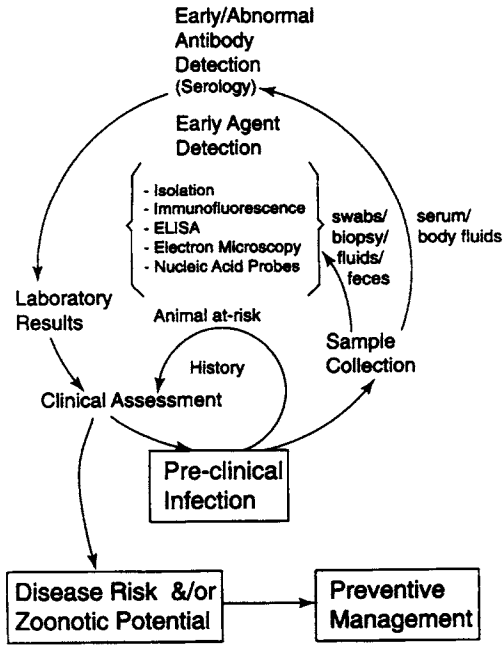

FIG. 2. Schematic depicting the current and future interactions between the laboratory testing of animals-at-risk to determine if preclinical infection has occurred, and the steps leading to preventive management. (Modified from Evermann, 1990, with permission from W. B. Saunders Company.)

the ecology of the infectious microorganism has become very important in our overall understanding of how successful the control program may be (Susser and Susser, 1996b). The control of infections with a low degree of transmissibility and narrow host range, such as caprine arthritis-encephalitis (CAE) virus, is much more realistic than the control of diseases with a wide host range, such as chlamydia and *Salmonella* spp., or those agents spread by arthropod vectors, such as arboviruses and rickettsia (Gregory and Schaffner, 1997; Hewinson *et al.,* 1997; Knowles, 1997; Raoult and Roux, 1997; Saluzzo and Dodet, 1997). The ecology of infection provides the veterinarian with vital information with which to make decisions. The ecology of six different agents is listed in Table I. The ecology of infection is divided into infection rate, attack rate (progress to become clinical), and mortality rate. It can be seen that the infection rate usually exceeds the attack rate and mortality rate in the majority of cases. Exceptions to this generalization are the mucosal disease form of BVD that occur in cattle that are tolerant to BVD and persistently infected (PI), and rabies infections in mammals (Innocent *et al.,* 1997; Smith, 1996).

Another way to view the ecology of an infection is demonstrated in Fig. 3. The schematic allows the veterinarian to readily use a graphic approach with clients to explain the differences between infection and disease. Rabies virus is used as an example of a microorganism with a low infection rate, but high mortality. (This figure would be different if one were to diagram the ecology of rabies in bats, the natural reservoir for rabies in the United States.) The CAE virus is used as an example of an infection in goats with a high infection rate, but lower attack rates, and much lower fatality rates (Fig. 4). With retroviruses, such as CAE virus, bovine leukosis virus (BLV), and equine infectious anemia (EIA), the ecology can also be subdivided into progressor (progress onto clinical signs and/or fatality) or nonprogressor (remains clinically normal, but infected and potentially contagious). With persistent bacterial infections, such as *Salmonella* and many members of the Rickettsiaciae, the ecology can be subdivided into clinical disease leading to mortality or clinical disease leading to a chronic carrier state. This chronic carrier state can then be further subdivided into inapparent infections with constant shredding and inapparent infections with intermittent shedding. With potentially zoonotic diseases, such as *Salmonella,* rickettsioses, and psittacosis (*Chlamydia psittici*), the ability to shed or transmit the organism into the environment or vectors becomes particularly relevant (Evans and Davies, 1996; Gregory and Schaffner, 1997; Raoult and Roux, 1997).

TABLE I

ECOLOGY OF INFECTION IN RELATIONSHIP TO DETECTION OF INFECTION AND DIAGNOSIS OF DISEASE

Transmissible agent	Ecology of infection: Infection rate (%)	Ecology of infection: Attack rate (%)	Ecology of infection: Mortality rate (%)	Detection of infection*	Diagnosis of disease	Vaccine available
Prion induced disease (scrapie)	Unknown (variable)**			IHC (biopsy) genetic typing	Clinical signs, histopath	No
Coronavirus induced disease (FIP)	85	2	99	Serology, PCR, genetic typing?	Clinical signs, histopath	Yes (40–80%)
Lentivirus induced disease (CAE)	85	30	<10	Serology, PCR, genetic typing?	Clinical signs, histopath	No
Pestivirus induced disease (BVD)	85	10	<5	Serology, PCR	Clinical signs, histopath, IHC	Yes (80–90%)
BVD-PI (immunotolerant)	1–2	50	90	PCR, VI	Clinical signs, histopath, IHC	Yes (variable)
Herpesvirus induced disease (EHV-1)	85	10	5	Serology, VI, PCR	Clinical signs, histopath, IHC	Yes (variable)

*Antemortem.

**USDA-APHIS survey in progress.

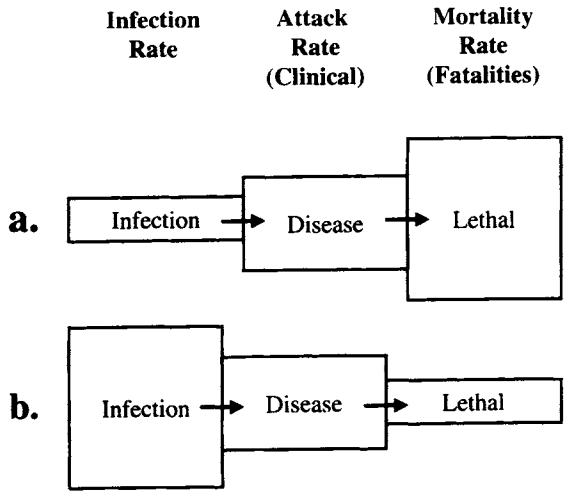

FIG. 3. Schematic depicting the conceptual view of infection rate, attack rate, and mortality rate. (a) An infection with a low infection rate, i.e., rabies virus, but a high mortality rate. (b) An infection with a high infection rate, i.e., caprine arthritis encephalitis virus, and a low mortality rate.

III. How Early Do We Want to Detect Infection?

Early detection of infection is now feasible with a number of microorganisms affecting animals. The detection may take the form of specifically identifying the nucleic acid of the infectious agent, such as bovine herpesvirus-1 in semen samples, BLV provirus is selected blood cells populations, and foodborne bacteria in dairy products (Batt, 1997; Masri *et al.,* 1996; Xie *et al.,* 1997). Although this form of early microbial detection is preclinical at this time, with further research it may be determined that these "subclinical infections" are actually causing alterations in cell structure and function leading to endocrine imbalances and decreased productivity. This form of disease has been referred to a "lesionless pathology," and will be the subject of further research (de la Torre and Oldstone, 1996).

Early detection of infection may take a "back door" approach by analyzing the host animal's genetic predisposition to infection and disease. This interesting approach has already been used in order to control the prion disease, scrapie (O'Rourke *et al.,* 1997). Sheep with a unique chromosome are highly susceptible to progress onto scrapie, an irreversible disease. Animals that are bred for genetic resistance to

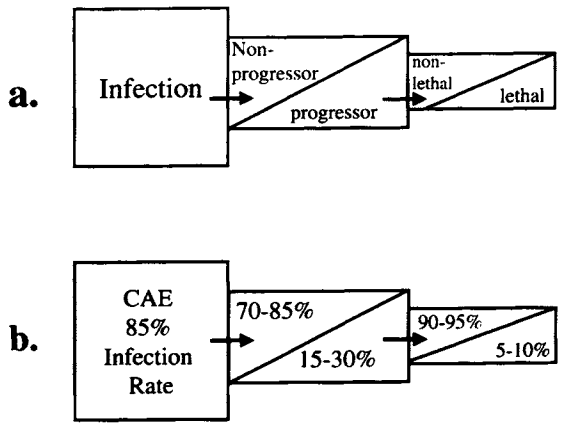

FIG. 4. Schematic depicting the conceptual view of further analyses of the attack rate into (a) progressor and nonprogressor, and the mortality rate into lethal and nonlethal. (b) How CAE virus infection occurs in this scheme.

infection and/or disease will be major factors in disease control in the future (Gavora, 1996; Malo and Skamene, 1994). The utilization of genetic testing is essential for some infections, such as the retroviruses, which serve to activate cellular oncogenes and promote disease. Identifying these cellular oncogenes would be a major step in controlling retroviral-induced diseases (Wiedemann *et al.,* 1991).

It will be essential to clearly define what the diagnostic assay is detecting so that the veterinarian may utilize the information appropriately. Figure 5a graphically presents the use of thresholds to differentiate subclinical infection from clinical manifestations of the disease. Figure 5b shows five potential diagnostic assays, each with varying levels of sensitivity. It would be critical to understand the differences between a test with high sensitivity, which detects *subclinical infection* and a test with lesser sensitivity, but more accurately *diagnoses disease.*

IV. What Are the Consequences of the Results?

This question becomes more difficult the more one employs preclinical testing in preventive medicine programs (Clementi *et al.,* 1995; Jacobson and Romatowski, 1996; Smith, 1995). The predictive value of a positive result may be high when an animal is clinical, such as a cat with FIP.

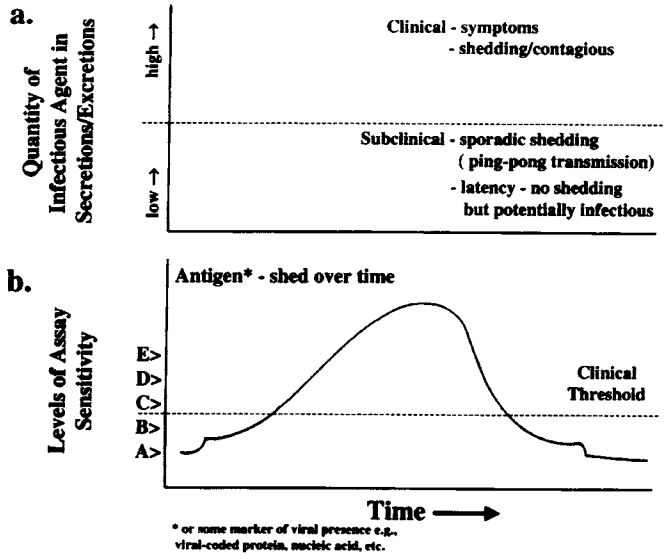

FIG. 5. (a) Schematic depicting the differences between subclinical and clinical infections where infectious agents are detected (panel a). (b) The different levels of sensitivity of detection assays, with assay A being the most sensitive and assay E being the least sensitive.

However, with early testing the problems of detecting cross-reacting viruses (feline enteric coronaviruses) increases, as does the question of whether the preclinical result accurately identifies an animal that is just infected or will progress onto disease (Evermann *et al.*, 1995; Foley *et al.*, 1997). In infections such as EIA, the consequences of infection are just as severe as the horse that has clinical signs of EIA. This is because the infection is regulated by the U.S. Department of Agriculture and all seropositive horses and mules are required to be reported, regardless of their health status. Assays for early detection of EIA infection have been reported to detect viral RNA in plasma samples as early as 48 hours after infection (Langemeier *et al.*, 1996). Similarly in bovine tuberculosis, caused by *Mycobacterium bovis*, the consequences of a positive test result can be economically devastating due to stringent government regulations. This becomes particularly problematic because many tests currently available may cross-react with other mycobacterial species (Essey and Koller, 1994; O'Reilly and Daborn, 1995).

To determine what consequences the test results will have on the

animal and the owner it is important to ask five key questions (Table II). Is the infection and/or disease of economic concern, such as EIA or *M. bovis;* is the infection and/or disease of zoonotic concern, such as *E. coli* O157:H7; where is the microbial agent when not causing disease, such as with rabies reservoirs in bat populations; what are the contributing factors to the infection and/or disease process, such as pregnancy for CHV and other herpesviruses; and what factors can animal owners/veterinarians/public health personnel control to minimize or eliminate the risk of infection and/or the disease process? Table III lists some of the consequences of the infection and/or disease process. These range from no sale, as with a goat that is CAE seropositive, to euthanasia if a horse or mule is tested EIA seropositive.

V. Where Are We Heading with Veterinary Diagnostics?

Veterinary diagnostics, like their human counterparts, are already directing efforts toward more sensitive assays, which are capable of detecting infections very early (within hours of initial infection); subclinical infections that are the result of persistent infections acquired during gestation and masked by immune tolerance; latent infections due to herpesviruses and retroviruses; and infections that pose a public health risk (Barrett *et al.,* 1997; Burr, 1996; Clarke, 1997; de la Torre and Oldstone, 1996; Rodriquez, 1997).

The evolution of diseases and the emergence of newly recognized pathogens have placed considerable pressure on new diagnostic technologies. The newer assays will assist in tracking the emerging infections, as well as linking causal association with disease to a firm cause and effect of the disease (Bryan *et al.,* 1994; Holtzman *et al.,* 1997; Hoet and Haufroid, 1997; Lipstich *et al.,* 1996; McDade and Anderson, 1996; Poland *et al.,* 1996).

TABLE II

FIVE KEY QUESTIONS TO ASK REGARDING INFECTIONS/DISEASES OF ANIMALS

1. Is the infection and/or disease of economic concern?
2. Is the infection and/or disease of zoonotic concern?
3. Where is the microbial agent when not causing disease (microbial ecology)?
4. What are the contributing factors to the infection and/or disease process?
5. What factors can animal owners/veterinarians/public health personnel control to minimize or eliminate the risk of infection/disease process?

TABLE III

THE CONSEQUENCES OF THE INFECTION/DISEASE PROCESS ON THE ANIMAL

- No sale
- Public health risk
- Early cull
- Regulatory quarantine
- Shedding of microbial agent to susceptible animals in the population
- Segregation of animal
- Euthanasia of animal(s)

The future of veterinary diagnostics is now. There are at least five directions to be pursued (Table IV), none of which is new, but continuing to evolve as the needs mandate the detection of infection earlier and the diagnosis of disease at a manageable stage (Wilson, 1994). These five directions are the development of (1) assays to monitor

TABLE IV

WHERE WE SHOULD BE GOING WITH VETERINARY DIAGNOSTICS

1. Assays to monitor immune function (immune competence)
   - Foal check
   - Calf failure of passive transfer
   - Llama/alpaca immunoglobulin status
   - CMI response
2. Assays to monitor genetic resistance/genetic susceptibility
   - Cellular receptors
   - Cellular oncogenes
   - Cellular prion proteins
3. Assays to monitor infections
   - In the environment
   - In asymptomatic vectors (potential transmissibility)
   - In asymptomatic carriers (potential shedders)
4. Assays to diagnose disease
   - Prognosis
   - Monitor response to treatment via cytokines (IL-2, IL-4, etc.)
5. Assays to track emerging infections
   - Culture *invitro*
   - Conserved PCR
   - Disease potential
   - Develop new detection assays
   - Develop new vaccines

immune function (immune competence), (2) assays to monitor genetic resistance/susceptibility, (3) assays to monitor infections, (4) assays to diagnose disease and monitor response to treatment, and (5) assays to track emerging infections. As infectious agents continue to evolve, disease expression will change, resulting in the necessity to develop new diagnostic assays (Susser and Susser, 1996b; Wilson, 1994).

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