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The role of the veterinary diagnostic lab in the management of BRD

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Review

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Abstract

Veterinary diagnostic labs (VDLs) are important service agencies providing essential diagnostic testing for a wide variety of domestic animal species as well as wildlife. They serve key roles in disease monitoring and diagnosis as well as surveillance for diseases of consequence. Of the many roles VDLs serve, one is being a member of the larger team of professionals dealing with the management of the bovine respiratory disease (BRD) complex. VDLs provide a number of services related to the management of BRD. These include disease outbreak investigation, abnormal morbidity characterization, routine monitoring, and biosecurity screening for a variety of infectious agents via methods such as necropsy and histopathology, bacterial culture, antimicrobial sensitivity testing, virus isolation, and serological assays. VDLs continue to look for better methods and assays as instrumentation technology also grows and improves. This is reflected in the growing proliferation of molecular-based assays that provide a high degree of sensitivity and specificity. Professional staff in VDLs work in collaboration with those in academia and private industry to conduct basic research focusing on a different aspect of the BRD complex. VDLs remain a primary source of the varied field-origin infectious agents associated with BRD that are used for research purposes.

Introduction

The veterinary diagnostic lab (VDL) has for decades now been engaged in providing laboratory testing services for a diverse clientele and, for the most part covering all of the major domesticated animal species as well as exotics. Historically, the VDL has been the primary avenue for veterinarians and producers when it comes to diagnostic testing in livestock and this continues to be true today. Full service VDLs offer a broad range of options for disease investigations and more routine health and pathogen screening as well as regulatory testing. This includes a multiplicity of assays in the fields of bacteriology, serology, virology, and pathology, both anatomic and clinical Fulton RW and Confer AW (2012). In areas of intense cattle production, beef and dairy, respiratory disease is a major disease problem and VDLs have and continue to play a significant role in the overall management of the disease complex.

Early on VDLs were involved in basic testing that included necropsy and histopathological examination of animals with pneumonia, bacterial culture/isolation of pathogen(s), antimicrobial sensitivity testing of significant isolates, and virus isolation. Necropsy and histopathology allowed for the identification of gross and microscopic patterns of pneumonia that would help veterinarians servicing the industry to be better equipped to distinguish viral from bacterial from non-infectious types of pneumonia, interstitial versus bronchial patterns, and infectious versus non-infectious and mixed causes.

These basic diagnostic disciplines enabled the veterinary medical community to better understand the complex nature of the bovine respiratory disease (BRD) complex with regard to infectious agents and early understandings of the pathogenesis, most of which still form the 'backbone' in our understanding of BRD.

As the scientific methodology and instrumentation has advanced, it has and continues to offer increased sensitivity and specificity. Advancements in scientific methodologies have allowed for the continued growth in our understanding of the respiratory disease complex through the identification of new pathogens, interactions of previously known or suspected pathogens, and how they influence (if at all), for example, the gross and microscopic lesions of pneumonia.

Professional staff at VDLs have to one degree or another joined with academicians involved in more basic and directed research initiatives tied to various aspects of the complex. These include pathogen characteristics, pathogen metabolic byproduct(s) identification, toxins, virulence gene presence and significance, pathogen interaction with bovine immune system, pathogen interactions, environmental factors affecting pneumonia, host factors that are significant to the BRD complex management and prevention, and development of new and better testing methods and results interpretation.

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One great benefit of VDLs in the management of BRD is that the diagnostic labs are a repository for organisms recovered from field cases of BRD and, thus, serve as an extremely valuable and essential source for in-house and collaborative research. For most isolates, they can be held almost indefinitely under the correct environmental conditions and be available for future study.

Looking more specifically at the various diagnostic disciplines within VDLs, it is interesting to follow the development of testing and the changes that have and are occurring over time to provide better information and service to our clients in the area of BRD management.

Bacteriology

Since early on in diagnostic laboratory work, traditional bacterial culture/isolation methods have been an important component in BRD complex testing. The methodology continues to be a backbone for VDL testing for respiratory disease in cattle. However, new instrumentational technology and molecular testing methods have become more important in the area of organism isolation and identification.

One instrument that is appearing more frequently in VDLs is the MALDI-TOF (Matrix Assisted Laser Desorption/Ionization-Time of Flight). It requires routine culture methods to isolate individual bacterial colonies which are then subjected to high energy to fragment the bacteria creating a 'fingerprint' of sorts that is organism-specific. The instrument keeps a library which then is utilized for identification.

An important component of bacteriological testing in VDLs is antibiotic or antimicrobial susceptibility testing (AST) Lubbers (2015). The process has more-or-less been fairly well standardized. Two common systems of reporting are Kirby-Bauer and MIC (Minimal Inhibitory Concentration). In each platform, results are reported as sensitive, intermediate, or resistant. The goal of AST is to look at individual isolates from a diseased lung, for example, and test them against known antibiotics to hopefully find an effective compound to utilize in a treatment regimen. The other side of the coin for AST is the information it provides for detecting abnormal resistance patterns to commonly used classes of antibiotics. This is currently a hot topic in both human and veterinary medicine. VDLs will continue to have an important role in monitoring antimicrobial resistance.

Serology

Serological assays for bacterial and viral agents in BRD investigations continue to be popularly requested tests. It is also a discipline that has seen much growth over the decades. Tests are available for virtually all bacterial and viral agents involved with the complex. Moreover, assays are quick to appear when suspected pathogens are identified. Serological testing is relatively rapid and sensitive. Virus neutralization (VN) and ELISA have become standard testing platforms in the VDL for many serological assays with some applications utilizing the microagglutination protocol. ELISAs may target either host antibody or the antigen. Improved technology has seen the application of more high throughput testing with instruments that can run 96 samples at a time with results available the same day of testing. VNs take a bit longer, requiring 3 days for testing results and are labor-intensive. Toxin neutralization assays are helpful for bacteria like *Mannheimia hemolytica* that secretes a leukotoxin important in the pathogenesis of BRD.

The challenge with serological assays is interpretation. Does the positive result reflect exposure, active infection, or vaccination? Serology is often noted to provide helpful information in terms of pathogen exposure, but can fall short in clarifying actual disease status. VDL professionals can be very helpful in helping to identify the underlying positive reaction.

Virology

Before the advent of other more rapid and sensitive assays for virus detection and identification, virus isolation was the gold standard for virus recovery and identification. The process involves pulling virus from fresh tissue specimens into cells grown in artificial media and then using a combination of visual cytopathic effects via microscopy, electron microscopy, or using fluorescein-tagged antibodies specific for the virus that allow for visual detection in the cultured sample. The culture process can be lengthy and not all viruses grow sufficiently well in artificial media for identification.

In spite of the development and expanding application of molecular diagnostic techniques to organism identification, virus isolation is still an extremely important tool in the VDL as it isolates a viable virus which can be stored and banked almost indefinitely for future study. Diagnostic labs are great resources for field isolates and provide specimens for evaluation of viral genetic changes which may influence virulence and pathogenicity, for example. Such stocks also allow for micro-evolutionary studies to look at viral changes over time and the relationship on viral strains to each other.

Molecular testing

I recall probably 20 years ago a classical bacteriologist telling me that PCR testing would never become accepted in veterinary medicine as a routine diagnostic test. It was too expensive. Basically, it was a waste of effort to develop it.

Well, that proved to be wrong. The big revolution in diagnostic laboratory testing involves a variety of molecular techniques targeting nucleic acids (DNA and RNA). Growth in this area over the past few decades has resulted in the appearance of methods that allow for organism identification in a matter of a few hours rather than days; and the cost has come down and is affordable. Individual PCR assays can be around $25. Moreover, multiplex testing allows for the simultaneous testing for multiple organisms in one batch run. Such batch run assays can be found for $45–$90 depending on the number of targets that are included in the multiplex panel.

For example, a multiplex bacterial assay using rtPCR may include all of the major bacterial organisms of interest in BRD complex testing – *M. hemolytica*, *P. multocida*, *H. somni*, *M. bovis*, and *T. pyogenes*; or in the case of viruses, a multiplex panel could include BRSV, PI3, BVD, IBR, BCoV, and/or Influenza D.

Another area of test development in molecular diagnostics is DNA sequencing Rai et al. (2015). This is the process of determining the sequence of nucleotides in a section of DNA. This can be applied to the nucleic acids in any organism with the appropriate sample. The nucleic acid sequences are highly specific for organisms even allowing differentiation/identification of organisms which make look identical with other testing methods.

Next generation sequencing (NGS) is one application of this technology and is being used to very specifically identify

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organisms; even those with very minute differences. NGS makes it possible to look for mutations, drift, and even differentiation of wild-type versus vaccine strains of organisms. It is commonly used in research applications and is making its way into VDLs.

Another aspect of molecular testing is genomics which for the purpose of this presentation broadly examines the bovine genome looking for genetic variations responsible for susceptibility/resistance to BRD. Currently, this is a research application and although it carries intriguing prospects, it remains to be seen what if any significance it will have for BRD diagnostics in the future.

Finally, the concept of microbiome has made its appearance related to the respiratory tract. What is that normal resident populations of microbes in the respiratory tract? What happens to it in disease? Does a disturbance or alteration of the microbiome allow for pathogen proliferation and, thus, aid in the pathogenesis of BRD? What are the effects of antimicrobial administration on commensal populations in the respiratory system? These are some of the questions being asked. It remains to be seen if VDLs will eventually have a 'screening test' of sorts to evaluate the respiratory tract microbiome.

Epidemiology

One thing that has been relatively new for us at TVMDL is having a professional on site with an interest in and skills in dealing with data management. VDLs are storehouses of information. Years and years of data are kept in everything from paper files, microfiche, and electronically. Commercial producers of LIMS seem to come and go like restaurants. Designs vary quite a bit and it can be difficult to adjust to changes made to the programming. It just seems like there is no single, complete LIMS that does everything VDLs need. The beauty of the electronic data storage systems is that they have made it a lot easier to access information, a lot of information, for analysis. Of course, there is quite a bit of work to do on the front end to ensure the data is thorough and complete. It seems statisticians do not always think highly of the external validity of VDL data. Maybe that is why they say it has to be 'cleaned up' or that it is 'messy'? Since VDL's data does not represent anything like a controlled study in the classical sense, it seems too often have a lot of 'holes' in it because many of the retrospective analyses do not match the original purpose of testing. The data is inherently incomplete. The VDLs have no control (or sometimes knowledge) of why a sample is submitted, what the sample represents in the population or disease process, or what testing is performed on that sample. Also, the historical and geographical information is often not included with the submission data when it was initially captured by the medical records technicians. Of course, it goes back farther to the veterinarian or clinic technician or administrator that filled out the forms for sample submission and testing.

It does seem though that the new LIMS programs are becoming more robust and have much better abilities to record, categorize, and store data that can be subjected to any variety of algorithms for drawing out conclusions. It seems that more and more VDLs are hiring an epidemiologist for staff positions. Of course, VDLs affiliated with colleges of veterinary medicine where there are one or more epidemiologists on faculty will cooperate to draw out useful information from the bank of data in the VDLs records.

The data may represent the occurrence of a specific disease, a specific organism, the performance of a specific diagnostic test, or antibiotic sensitivity/resistance patterns in the case of bacteria or parasites, for example. In the case of BRD which has a complex and multifactorial causality, such data analyses will be helpful in looking at not only at the organisms and the testing associated with their presence (or absence) but if we can do a better job of capturing information on the very front end of the process where cattle are entering the pipeline for beef or dairy production.

VDL staff are reaching out and joining efforts with producers and scientists to look at the diverse nature of beef/dairy production in order to identify factors that influence the health of cattle and predisposing them to develop respiratory disease.

Telemedicine

In our region in the Panhandle of Texas, our veterinarians deal with clients widely spread over the region and even into surrounding states. It is not always possible for them to be on site when a disease outbreak occurs or maybe it is just something 'uncommon' or 'not routine'. In those cases, it may be helpful for the veterinarian to be able to have the feedyard necropsy technician or ranch hand/owner contact the VDL diagnostician to get 'real-time' consultation. Beside simple dialogue over the phone, I believe there is an interest in face-to-face contact where the producer or rancher would be able to project actual live video feed to the VDL staff member.

One example of this would be direct live video feed during the actual performance of a necropsy. We do often get photographs of gross postmortem lesions submitted by veterinarians and rarely producers.

One very important addition to our professional staff at TVMDL has been a group of veterinarians that have considerable clinical practice experience and skills. We have two in the livestock field, one in equine and wildlife, and one in small animal. In the livestock arena, these individuals because of their practice backgrounds are key to interacting and fielding questions related to diseases in cattle, like BRD, for example. Because of their clinical experience, they have the ability to dissect and identify key aspects of the clinical history from our client and provide the best diagnostic plan going forward.

In discussing services with clients, over the years, direct contact with lab professional staff whether in bacteriology, virology, or pathology remains an essential aspect of the service VDLs provide. Veterinarians and producers continue to rate this as a top service they expect from their VDL.

Client education

One of the goals of TVMDL and most VDLs has to do with outreach and client education. With the advancement of electronic media, there is an ongoing revolution of sorts in clinical practice which has necessitated changes in the way VDLs interact with and provide information to our clients. Labs are finding better ways to communicate important information. VDL websites and apps are a key part in this, but also how labs package the various tests they offer for diagnostic purposes. The website also allows VDLs to attach any variety of resource documents or links to other sites to be helpful.

Members of our lab have worked to develop a bovine diagnostic reference manual. It partially reflects recently implemented diagnostic plans for key bovine disease syndromes, but includes a significant amount of other information related to appropriate

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sampling, safe packaging and shipping, essential supplies, and a whole section on the necropsy procedure.

Professional staff at VDLs may also be involved with extension activities which may include demonstrations, online webinars, conferences, and publications to list a few.

In our region, TVMDL is heavily involved with the 'Panhandle Livestock Professionals' (PLP) that meets monthly with a featured speaker in some field of livestock production. This group includes lab staff, veterinarians in practice, nutritionists, agricultural and environmental engineers, biopharma, animal scientists, graduate students, and university faculty. It allows for a free flow of information and discussion on important topics to the industry. Factors involved with BRD have been the source of a number of these meetings.

Client needs

In talking with bovine-oriented veterinary clients of TVMDL about their practice needs from a VDL, several themes were recurring. These include: (1) accessibility to a full-service lab with experienced professional staff in the various disciplines for in-depth consultations about testing and lab results, (2) access to a full range of tests and testing methods, (3) rapid turnaround of results, (4) pathologists to discuss postmortem lesions, (5) serving as teachers/educators for new graduates entering into practice for both disease analysis and test selection, (6) availability to help with 'wrecks' quickly, (7) have veterinarians on staff with good clinical experience, and (8) being able to help identify specific situations which are atypical.

Areas of potential growth for VDLs include: (1) collaborative 'investigations' into the role of performance parameters, feed components, and drugs and their role or interaction with animal health, (2) the use of technology to provide 'distance diagnostic service', and (3) educational programs for feedyard staff tasked with animal health.

Quality assurance

Finally, a component of the VDL that seems to get little outside recognition from its clientele, but actually plays a significant role in the overall operations of the lab to ensure uniform and consistent, validated results is the quality assurance (QA) program. In the early days of VDL work, no such organized system was present. It may or may not have existed in any form. Even into the 1990s, VDLs did not necessarily have a QA department. However, accredited VDLs under the American Association of Veterinary Laboratory Diagnosticians (AAVLD) are required to have a Quality Management System (QMS) meeting high standards which are based upon ISO 17025. In fact, some labs go beyond this standard utilizing an electronic QMS for QA fundamentals such as document control, corrective actions, training and competency of personnel.

Conclusion

VDLs have over the decades evolved to continue to keep pace with services required by veterinarians working in the area of respiratory disease management in cattle. This includes staffing the lab with highly qualified professionals directing the development of services in the various disciplines and providing necessary consultations to field veterinarians. One group that is integral to the VDL mission and working alongside the other staff is the clinical diagnostician whose practice experience provides a highly appreciated dialogue in dissecting health issues in a population of cattle.

VDL testing services continue to expand as new organisms appear and are tied to pulmonary disease in cattle. This is driven by new methodologies, better reagents, and improved instrumentation. The explosion of highly technical procedures and the equipment to perform them is most exhibited in the field of molecular diagnostics. We will continue to see advancement in this area.

Other areas of growth for VDLs are in the area of client services especially related to distance diagnostic medicine and identifying educational platforms that will work for remote sites. We will continue to see these develop and mature in the next decade. Moreover, if there is one important aspect of VDL services that is voiced repeatedly, it is summed up in the word 'relationship'. Clients demand and highly appreciate the working, professional relationships they have with their VDL. It provides a significant and meaningful element in their practice of veterinary medicine.

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