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Veterinary Microbiology, 24 (1990) 409–417
Elsevier Science Publishers B.V., Amsterdam

Applications of nucleic acid probes in veterinary infectious diseases

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ABSTRACT

Paul, P.S., 1990. Applications of nucleic acid probes in veterinary infectious diseases. *Vet. Microbiol.*, 24: 409–417.

Nucleic acid probe technology is increasingly being used in basic research in veterinary microbiology and in diagnosis of infectious diseases of veterinary importance. This review presents an overview of nucleic acid probe methodology and its applications in veterinary infectious diseases. The major applications of nucleic acid probes include detection of pathogens in clinical samples, especially those organisms which are fastidious and difficult to cultivate, differentiation of virulent from avirulent organisms and vaccine strains from wild type isolates, typing of microorganisms, mapping genes, screening libraries of cloned DNA for specific genes, detection of latently infected or carrier animals, study of mechanisms of pathogenesis, epidemiological studies and food safety.

INTRODUCTION

Nucleic acid probe technology is being increasingly used by veterinary medical researchers and diagnosticians. This has resulted from the increased awareness, commercial availability of reagents for the preparation of probes, the need for more sensitive methods for the detection of fastidious organisms, the need for more specific methods for the detection of nucleic acids, and recent advances in nucleic acid probe technology.

The key to the development of nucleic acid probes is to identify nucleotide sequences which are unique to the particular organism of interest. These sequences may be genomic DNA, plasmid DNA or ribosomal RNA. The nucleic acid which contains such sequences is isolated and amplified by recombinant DNA technology. The unique nucleotide sequence is then cleaved and is labelled with a reporter molecule such as a radioactive (^{32}P, ^{35}S) or nonradioactive (biotin, digoxigenin) molecule. Alternatively, complementary DNA, RNA or synthetic oligonucleotides may be labelled and used as probes. The labelled DNA or RNA in single-stranded form is then hybridized to single-stranded nucleic acid (DNA or RNA) in tissues (in situ hybridization), on

0378-1135/90/$03.50

paper, or in solution. If the nucleotide sequences in the nucleic acid probe are complementary to those in the sample, hybridization occurs and results in double-stranded nucleic acid being formed. Nonhybridized single strand probe is removed. Hybridization is monitored by autoradiography (exposure to X-ray film) in the case of probes labelled with radioactive molecules, or colorimetrically or visually for probes labelled with nonradioactive molecules.

The basic principles of nucleic acid hybridization and the effect of different hybridization conditions such as probe size, salt concentration, and temperature have been reviewed by Gillespie (1990). Gillespie also presents an overview of different hybridization formats which have either been developed or are being developed. Readers are also referred to reviews on nucleic acid probes by Minson and Darby (1982), Edberg (1985), Kulski (1985), Pereira (1986), Norval and Bingham (1987), Paul (1988), and Tenover (1988, 1989).

Major drawbacks of utilizing nucleic acid probes in the past have included sensitivity limits of hybridization technology, safety and short half-life of radioactive probes. The introduction of new technologies for nucleic acid amplification such as the polymerase chain reaction (Oste, 1988) have revolutionized probe technology. Sensitivity is no longer a major problem as target sequences can by amplified prior to hybridization. Amplification of target sequences by the polymerase chain reaction and transcription amplification system (TAS) is reviewed by Gingeras et al. (1990). Alternative methods for amplification are covered by Gillespie (1990). A number of nonradioactive detection systems are currently available and some of them are reviewed by Pereira (1986); however, improved technologies need to be developed for nonradioactive detection of nucleic acids in clinical samples, especially feces. Some of the potential applications of nucleic acid probes in veterinary infectious diseases are presented in this review.

DETECTION OF PATHOGENS IN CLINICAL SAMPLES

Conventional approaches of detecting etiologic agents of infectious disease include the isolation of organisms, direct detection of organisms in clinical samples by electron microscopy, and direct detection of antigens in tissue sections and clinical samples by immunofluorescence, immunocytochemistry or ELISA. The availability of monoclonal antibodies has provided specific detection of many infectious agents in clinical samples. Monoclonal antibodies will continue to play a significant role in diagnostics, however, in some instances monoclonal antibody based tests are unsuitable and alternative methods are needed. Monoclonal antibody assays may be ineffective on samples contaminated with other organisms either due to the condition of the samples or the sensitivity of the assays. Nucleic acid probes provide an alternative diagnostic tool for the detection of infectious agents. Nucleic acid

probes have been used to detect viruses, bacteria, and parasites in clinical samples. Among viruses of veterinary importance detected by nucleic acid probes are adenovirus (Jouvenne et al., 1987), African swine fever virus (Tabares, 1987), blue tongue virus (Dangler et al., 1988), bovine viral diarrhea virus (Brock and Potgieter, 1990), enterovirus (Bruce et al., 1989), infectious bursal disease virus (Jackwood, 1990), foot-and-mouth disease virus (Rossi et al., 1988), porcine parvovirus (Ridpath et al., 1987; Harding and Molitor, 1988; Oraveerakul et al., 1989), pseudorabies virus (Gutekunst, 1979; Maes et al., 1990), rhinovirus (Gama et al., 1988), rotavirus (Dimitrov et al., 1985; Johnson et al., 1990; Rosen et al., 1990), and transmissible gastroenteritis virus (Shockley et al., 1987). Bacteria for which probes have been developed include *Campylobacter* spp. (Gebhart et al., 1990), *Escherichia coli* (Moseley et al., 1982; Maddox and Wilson, 1986), *Leptospira* sp. (Zuerner and Bolin, 1990), *Listeria* sp. (Wesley et al., 1990), *Mycobacterium paratuberculosis* (McFadden et al., 1987, 1988), *Mycobacterium tuberculosis* (Eisenach et al., 1988; McFadden et al., 1988), *Mycoplasma gallisepticum* (Geary et al., 1988) and other *Mycoplasma* spp. (Razin et al., 1987). Nucleic acid probes have also been developed to detect *Anaplasma marginale* (Eriks et al., 1989; Aboytes-Torres and Buening, 1990; Goff et al., 1990) and *Histoplasma capsulatum* (Keath et al., 1989).

The most important application of nucleic acid probes is the detection of fastidious organisms. Many microorganisms, such as *Mycoplasma* spp. and *Mycobacterium* spp., cannot be cultivated easily or require 3 weeks to 3 months of culture. Nucleic acid probes have been developed and are being successfully used for their detection either directly in clinical specimens or following a short culture period. Probes have also been used to detect specific microorganisms in tissues by in situ hybridization (Brigati et al., 1983; Dunn et al., 1986; Brown et al., 1990; Collisson et al., 1990).

DETECTION OF CONTAMINANTS IN CELL CULTURES

Mycoplasmas, parvovirus and bovine viral diarrhea virus are frequently present as contaminants in cell cultures. Their detection is sometimes difficult by inexperienced workers. Nucleic acid probes offer standardized reagents for their detection and such probes have been developed for these microorganisms (McGarrity and Kotani, 1986; Oraveerakaul et al., 1989).

DIFFERENTIATION OF VIRULENT FROM AVIRULENT ORGANISMS

The differentiation of virulent from avirulent organisms and vaccine strains from wild type isolates is extremely important in diagnostic medicine and epidemiological studies. This can be accomplished immunologically by monoclonal antibodies, and genetically by restriction endonuclease analysis, se-

quence analysis and nucleic acid probes. *Treponema hyodysenteriae*, causative agent of swine dysentery, can not be easily distinguished from a nonpathogenic treponeme, *Treponema innocens*, which is commonly present in normal swine. A nucleic acid probe prepared from a plasmid associated with *Treponema hyodysenteriae* was successfully used to differentiate pathogenic and nonpathogenic treponemes (Joens, 1988). Similarly, probes have been developed to detect virulence genes. Moseley et al. (1982) used probes for the first time to identify organisms carrying genes for enterotoxins. Such probes are now routinely being used to determine potential pathogenicity of *Escherichia coli* by screening for genes of enterotoxins (Moseley et al., 1982; Maddox and Wilson, 1986; Karch and Meyer, 1989).

TYPING OF MICROORGANISMS

Typing of microorganisms has usually been based on biologic and antigenic characteristics. These classical procedures have been found to be unsatisfactory in many cases. Monoclonal antibodies have provided useful methods for typing many organisms where conventional reagents have not worked. Production of type specific monoclonal antibodies is often difficult, time consuming and sometimes an impossible task, especially when bacterial and parasitic organisms are used due to the large number of proteins. Genetic tools have been useful in typing organisms where serologic tests were unsuccessful, tedius or gave ambiguous results. Nucleic acid probes have been used to type pillus and enterotoxin gene types to differentiate *Campylobactor* spp. (Chevrier et al., 1988; Gebhart et al., 1990), *Escherichia coli* (Maddox and Wilson, 1986), *Leptospira* spp. (Zuerner and Bolin, 1990), *Mycobacterium* spp. (McFadden et al., 1988), and rotavirus groups, subgroups and serotypes (Dimitrov et al., 1985; Johnson et al., 1990; Rosen et al., 1990).

MAPPING GENES

Nucleic acid probes provide a powerful tool for evaluating homology between related DNAs (Howley et al., 1979). This can be accomplished by dot blot, Southern blot or Northern blot hybridization. For example, homology between polyoma and papovavirus was analyzed by Southern blot hybridization (Howley et al., 1979). Similarly, homology between porcine and canine parvovirus was evaluated using Southern blot hybridization (Ridpath et al., 1987). Location of homologous sequences on the physical map of these viruses was also revealed in these studies by using probes from subgenomic fragments. Hybridization has also been successfully used to identify genetic reassortants (Midhun et al., 1987) and gene rearrangement (Paul et al., 1988) among rotaviruses. These strategies can also be employed to map specific genes of interest using well studied genes from related organisms. A complete map

of bovine adenovirus 7 was generated by Southern blot hybridization at low stringency with probes prepared from human adenovirus 3 DNA (Hu et al., 1984). Transcriptional maps for specific genes and identification of microbe-specific RNAs can be identified by Northern blot hybridization. Additionally, coding assignments for genes can be established by a combination of Northern blot hybridization and in vitro translation (Collins et al., 1984). Probes have also been useful for screening libraries for specific genes (Rigas et al., 1986) and cloning genes by oligonucleotide hybridization (Mayaux et al., 1987). The latter method is being extensively used for cloning specific genes or subcloning regions of interest.

STUDY OF MECHANISMS OF PATHOGENESIS

Nucleic acid probes are an excellent tool for the determination of molecular mechanisms of pathogenesis. Mechanisms of latency induction by herpesviruses have been studied by in situ hybridization. Rock et al. (1987) found that with bovine herpesvirus-1 (BHV-1), causative agent of infectious bovine rhinotracheitis, the nucleic acid is present in ganglionic neurons of rabbits latently infected with BHV-1. Furthermore, only selected regions of the viral genome were transcribed in neurons of latently infected rabbits, whereas all regions of the genome were transcribed in lytically infected cells. These studies indicated that transcription of BHV-1 is restricted during the latent phase of infection and suggested that transcription of specific genes may be important in the establishment and maintenance of latency. The mechanism of persistent infection by equine infectious anemia virus (EIAV) was determined using Southern blot hybridization by Rasty et al. (1990). They found that the degree of provirus integration may be important in the establishment of persistent infection since 30% of provirus was randomly integrated in persistently infected cells, whereas 65–90% of provirus was integrated in lytically infected cells. Hybridization technology was also used to better understand the mechanisms of leukemogenesis by bovine leukemia virus (BLV) (Gregoire et al., 1984). These studies revealed that BLV-induced tumors in sheep were monoclonal in the same sheep, integration was observed at different sites in the animals and there was nonintegrated BLV in tumors. Hybridization procedures can provide information on organs and cell types harboring organisms, the number of genome copies per cell, whether genes are integrated, and the transcription status and regulation of the genes. These studies, combined with immunocytochemistry, can provide valuable information on the presence of viral nucleic acid and antigens in cells and can provide information on the status of virus expression.

EPIDEMIOLOGICAL STUDIES

The development of specific probes specific for organisms, groups of organisms, or specific serotypes provides a valuable tool for epidemiological studies. Such tests can be developed in a few reference laboratories and distributed to diagnostic laboratories to determine prevalence and incidence of a specific organism. Probes will be especially useful for determining the epidemiology of microorganisms which are difficult to cultivate or type.

FOOD SAFETY

There is increasingly more emphasis on the rapid detection and identification of microorganisms in foods. In the past, this has generally been accomplished by the isolation and identification of bacteria using conventional methods that are time consuming and require the presence of viable microorganisms. Nucleic acid probes provide a more rapid method for the identification of microorganisms in food. Nucleic acid probes have been developed and are commercially available for the detection of several microorganisms of public health importance e.g., toxigenic *Escherichia coli*, *Listeria* sp., *Salmonella* spp., and *Yersinia enterocolitica* (Hill, 1989). Probes for additional microorganisms and improved methodology for their detection in foods will enhance their use in food safety.

In this review, some of the applications of nucleic acid probes are highlighted. Additional potential applications will become evident as newer technologies are developed. Automation in nucleic acid probe technology will significantly enhance its acceptance in diagnostic medicine. In the meantime, nucleic acid probes will continue to serve as a powerful tool to researchers and to diagnosticians in selected cases.

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