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Molecular Insights into Zoonotic Diseases: Understanding the Interface Between Animals and Humans

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ABSTRACT

Molecular epidemiology is a progressive advancement within the field of epidemiology that utilizes molecular biology tools to provide insights that are unattainable through traditional methods. These tools aid to investigate zoonotic outbreaks, to establish surveillance systems, and to conduct the rapid diagnostic assays. Genotyping methods, include a diverse set of tools such as Pulse Field Gel Electrophoresis (PFGE), Whole-genome sequencing (WGS) and Restriction Fragment Length Polymorphism (RFLP). These techniques plays a pivotal role in addressing zoonotic diseases, multidrug-resistant organisms, viral infections, and parasitic zoonoses in veterinary and public health contexts.

Keywords: Molecular epidemiology, Zoonosis, Animals, Humans

Introduction

The application of molecular epidemiology in veterinary science and study of zoonotic diseases began back in early 1970's. In 1973, Edwin Kilbourne utilized the molecular biology tools of the time to investigate the pandemic of 1918 influenza, also known as the Spanish flu. His findings established a connection between swine and human related A influenza viruses, H_1N_1. This investigation opened the door for a new era of molecular biology to investigate the issues related to animal health, zoonotic transmission of diseases and foodborne illnesses. Various molecular biology and bioinformatics tools i.e., DNA restriction digestion analysis, gene amplification through PCR, hybridization and most recently the whole-genome sequencing (WGS) have become a useful tool to address different infectious diseases infecting the livestock population [1].

Molecular epidemiology represents a progressive advancement within the field of epidemiology. The use of tools in molecular biology furnishes the valuable insights that may not be provided by traditional epidemiological methods. These tools prove beneficial to investigate the outbreaks, to establish surveillance systems, and to conductthe rapid diagnostic assays, thereby enhancing the precision and timeliness of epidemiological responses. It is crucial to differentiate between genotyping of microbes and molecular epidemiology. Genotyping refers to molecular biology techniques employed for microbial characterization, while molecular epidemiology covers a broader scope, addressing etiological agents, their hosts, environmental factors, disease distribution, determinants of transmission, and population-level disease prevention and control measures [2].

This article underscores the issues within veterinary medicine, mainly focusing on zoonotic disease priorities. It also focuses on the application of genotyping methods and their role in interpreting the epidemiology of various diseases of one health concerns, including multidrug-resistant organisms, highly prevalent but neglected parasitic zoonoses and emerging and endemic viral infections.

Genotyping Methods used for Major Zoonotic Diseases

Genotyping techniques are used for categorizing organisms beyond their prominent characteristics, by utilizing molecular biology methods often referred as "DNA fingerprinting" due to their dependence on DNA as the primary material. Apart from epidemiology, these techniques have vast application in forensic medicine and evolutionary biology studies. These techniques generally have higher sensitivity to differentiate different organisms as compared to other phenotyping methods and have greater reproducibility, typeability, and throughput, although exceptions exist. These methods typically involve a combination of molecular tools such as amplification, restriction digestion, hybridization, and sequencing. Although numerous genotyping methods are in use with different objectives, yet certain methods are particularly prevalent to address the zoonotic livestock diseases [3]. Table 1 provides a summary of the numerous methods used in veterinary medicine, illustrating their distinctive attributes regarding applicable genome size, core molecular techniques and other relevant factors.

Table 1: Common genotyping methods used in veterinary science with key features [2]

Genotyping method	Genome size	Fragment size	Electrophoresis system	Comment
Pulse Field Gel Electrophoresis (PFGE)	Bacteria or larger	30 kb->10 Mb	CHEF	Surveillance of common bacterial infections
Restriction Fragment Length Polymorphism (RFLP)	Broad genome size ranging from viruses to bacteria	Variable	Regular horizontal agarose gel system	Broadly used to study various genome sizes
Amplified fragment length polymorphism (AFLP)	Bacteria or larger	50-500 bp	Polyacrylamide gel electrophoresis	High resolution
Repetitive element sequence-based PCR (REP-PCR)	Bacteria or larger	Variable	Regular horizontal agarose gel system	Variable reports of higher discriminatory power
Random Amplified Polymorphic DNA (RAPD)	Any	Variable	Regular horizontal agarose gel system	Generally used for phenotype/genotype correlation
Multilocus sequence typing (MLST)	Bacteria	Sequence based	Capillary system	Generally used to study population genetics
Multiple Locus Variable-Number Tandem Repeat Analysis (MLVA)	Bacteria	-	-	-
Spoligotyping	Tuberculosis specific/ *Mycobacterium bovis*	Hybridization based	-	-

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Pulsed-field gel electrophoresis (PFGE), a macro-restriction genotyping technique introduced in 1984, stands as one of the primary methods employed for typing livestock-associated bacterial pathogens, particularly those involved in foodborne illnesses. PFGE generally operates on the principle of fragmenting an intact genome through restriction digestion using a rare-cutting restriction endonuclease. This process yields several large restriction fragments, typically ranging from 30 kb-10 Mb in size, which offers a significant advantage in analysis. It serves as the primary method within CDC and other regulatory agencies in USA, notably in the PulseNet system, extensively employed for investigating outbreaks involving *Salmonella*, *E. coli*, and various bacterial pathogens [4].

Restriction fragment length polymorphism (RFLP) represents another widely used genotyping method in veterinary science. RFLP relies on profiling restriction DNA fragments. However, classical RFLP methodology incorporates probe hybridization based on repeated genomic elements, such as the insertion sequence IS6110 in *Mycobacterium tuberculosis*. An alternative approach gaining attraction is PCR-RFLP, which involves amplifying specific DNA segments, often at defined gene loci, followed by digestion of the resulting amplicons with one or more restriction enzymes to generate restriction profiles. This technique generally does not necessitate the costly laboratory equipment setup [5].

Random amplified polymorphic DNA (RAPD) is genotyping method used for the first time in 1990. This technique is used for amplification of random targets found within the organism genome. This is considered as a simple technique that does not necessarily require any previous knowledge of nucleic acid sequence of any selected organism. This method is considered as the simplest method to set up in the laboratories [6]. Repetitive palindromic PCR (REP-PCR) is a subtyping method based on the known repeated elements existing in different eukaryotic or prokaryotic genomes. Amplified fragment length polymorphism (AFLP) is a newly developed method used to map various eukaryotic genomes. In this method, fragments of genome are digested by the help of two restriction enzymes i.e., EcoRI and MseI (cutter enzyme) followed by amplification [2].

Multi-locus sequence typing (MLST) is another newly developed method used to investigate various zoonotic diseases. In this method, short sequences of housekeeping genes (HKG's) are compared. This method is widely used to investigate those zoonotic agents whose sequences have already been submitted to global database [7]. Whole-genome sequencing (WGS) is the newest method used widely in the investigation of various diseases in recent times. Recent examples of using this technique are in *E. coli* O_{104}:H_4 outbreak in Germany [8] and *Salmonella* serovar Bareilly outbreak in 2 states of USA [9].

Conclusion

The understanding and management of various zoonotic diseases have been revolutionized due to extensive use of genotyping methods in epidemiological investigations. Moreover, the human ability has been enhanced significantly to identify and track the infectious disease threat at human-animal by the use of molecular techniques.

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