

# Essential veterinary education in modern molecular tools for the detection of disease: what veterinarians will need to know about genomics and molecular biology and diagnostics (including bioterrorist weapons) in 2025

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## Summary

Future veterinary education programmes in microbiology will undoubtedly include an increasing emphasis on new and existing molecular tools. They should also, however, provide veterinarians with a comprehensive and clear understanding of the types of results that can be obtained using a particular approach (for example, specific diagnostic procedures as against open diagnostic procedures, phenotypic versus genotypic characterisation, etc.). Furthermore, students should gain a sound knowledge of which type of test is the most appropriate in a given clinical or epidemiological situation, and what conclusions can or cannot be drawn from the results. Consequently, each veterinary curriculum should focus on the following items: the principles of molecular biology and genomics; the detection of disease and characteristics of molecular tests; the principles of micro-organism taxonomy, sequence comparison and molecular epidemiology and their applications (such as: taxonomic identification, epidemiological survey, genetic evolution and the traceability of strains); and the role of the veterinarian in the field of zoonoses and human public health.

## Keywords

Bioterrorist weapons – Curriculum – Emerging pathogens – Genomics – Modern molecular diagnostics – Specific versus open diagnostic procedures.

## Introduction

As a medical virologist interested in molecular tools for the detection and characterisation of infectious pathogens, the very first impulse of the author is to declare that nobody can predict what kind of molecular techniques will be used or in development in 2025. Looking back to the late 1980s of the 20th Century, even a clairvoyant could not have

anticipated the technological revolutions that would occur in the field of molecular biology and their consequences for diagnostics. Moreover, a large number of human and veterinary pathogens have been discovered or recognised as emerging agents over the past 20 years and it is certain that the context in which diagnostic techniques will be used in 2025 will be very different. Consequently, anticipating the techniques and thought processes that will

prevail in 2025 and attempting to define 'the essential requirements for veterinary education in molecular methods' are challenging problems that require both imagination and modesty.

## Molecular diagnostics: specific versus open diagnostic procedures

During the past decade, the application of molecular diagnostics to infectious diseases has become an increasingly important tool and this evolution warrants a few comments. The most important contribution of molecular biology has been the development of techniques that enable the direct detection of a specific pathogen in a clinical sample (blood, faeces, biopsies, injuries, etc.). This is based on the specific detection of the nucleic acid of the pathogenic agent. Two main strategies have been proposed to date:

- identification of the targeted nucleic acid followed by amplification of the signal, e.g. hybridisation of a specific probe and amplification of the hybridisation signal using branched deoxyribonucleic acid (DNA) (5)
- genetic amplification of a specific sequence, e.g. using polymerase chain reaction (PCR) (4), or the nucleic acid sequence-based amplification system (or NASBA) (2).

The latter strategy has been the most successful. Specifically, PCR methodology has significantly altered the approach to the application of molecular diagnostics. The most recent significant advance is the development of real-time PCR methods that decrease the risk of false positive results and allow the rapid, sensitive and quantitative detection of specific nucleic acid targets. Importantly, such methods are open to automation (and thus to the development of commercial kits) but can also be designed, developed and optimised in both research and diagnostic laboratories (probably the most important key for their remarkable success). It is noteworthy that the qualitative detection of a given pathogen can be associated in some cases with a strong positive predictive value that this agent is responsible for the observed disease (e.g. the detection of a pathogenic agent in the cerebral spinal fluid in cases of meningo-encephalitis), but, in other situations, does not provide a clear aetiological answer (e.g. in the case of possible asymptomatic infection by a respiratory micro-organism). The quantification of these detection methods will substantially modify and thereby improve the interpretation of such results in the future.

These diagnostic techniques have one important feature in common: they rely on the detection of specific sequences. In other words, the prescriber can only find the pathogen

for which s/he is looking. This is comparable with serological techniques, but contrasts with 'open diagnostic procedures' (such as culture, electron microscopy, etc.) that do not prejudge the result of the analysis. Both approaches obviously complement each other. Future veterinarians must have a global view of the problem and clearly understand:

- what kind of result can be obtained using each approach
- what type of test is the most appropriate in a given clinical or epidemiological situation
- what conclusions can, or cannot, be drawn from the results of molecular tests.

In recent times, challenging strategies have been proposed for a 'more open' approach to molecular diagnostics. First, simultaneous detection of different pathogens is now possible. For example, multiplex PCR systems, or systematic testing for a series of possible pathogens in a particular clinical situation, have been developed. Secondly, generic detection of genetically related viruses has become possible, using degenerate primers or a combination of different primers. In the future, such tests may constitute an intermediate step between 'open' and 'specific' procedures; they still rely on the detection of a predetermined list of pathogens, or groups of pathogens, but also enable the detection of more 'unexpected' agents. Thirdly, the most recent technological developments try to combine non-specific amplification of nucleic acids from clinical specimens (this remains an essential issue in pathogen detection) with large-scale sequencing, for example, pyrosequencing (3) or hybridisation to a very high number of probes, using DNA micro-arrays (1). Such approaches may provide the user with an enormous amount of data and shift the expertise of result analysis from the field of molecular biology to that of bioinformatics.

## Molecular diagnostics: phenotypic versus genotypic characterisation

In addition to detecting micro-organisms, molecular techniques have also been widely used to characterise the agents. In technical terms, the current methods identify short, specific molecular patterns (e.g. by genetic amplification and specific hybridisation methods) or longer gene sequences (by sequencing methods). The objective may be the phylogenetic characterisation of the agent (e.g. its genotype, mainly for epidemiological purposes), but also the identification of a genetic signature associated with a specific biological property (e.g. virulence or resistance markers). The problem here is

similar to that described in the previous section. Whilst the presence of a specific genetic signature is generally associated with a particular phenotypic characteristic, its absence does not exclude this phenotype. For example, the absence of a classical genetic marker of resistance to an antibiotic does not mean that the bacterium has a sensitive phenotype, since multiple distinct mechanisms can lead to a similar phenotype.

In other words, in certain clinical situations, the genetic characterisation of the agent may be of interest but the 'open' phenotypic approach may remain both necessary and less expensive. Again, future veterinarians must clearly understand the advantages and limitations of each approach, and what kind of test is the most appropriate in a given clinical or epidemiological situation.

## Identification of micro-organisms: taxonomy, genomics and molecular epidemiology

Understanding the taxonomic classification of micro-organisms is a major challenge for future practitioners. First, the number of known pathogenic agents that can be detected with molecular techniques is increasing rapidly. Secondly, in the future, taxonomic identification of agents will frequently be performed using methods based on the comparison of relevant molecular sequences. Specifically, genomics (the characterisation and analysis of genomes) has become a crucial element of taxonomic analysis. Events have moved so rapidly that taxonomy should no longer be considered a tedious discipline. It is now a convenient tool with which to identify, on the one hand, the specific names of the infectious agents and, on the other, to specify the essential biological properties of the pathogenic agents. In other words, taxonomy provides the right words to describe a micro-organism and to memorise its essential characteristics. From this point of view, modern taxonomy is essential for both teachers and students. Given the number of existing pathogens and the constant emergence of new agents, strong efforts to reinforce the teaching of taxonomy constitute a priority in the veterinary curriculum.

Nowadays, the methods of identifying micro-organisms that are taught to students rely mainly on the identification of phenotypic characteristics. In the future, such identification should increasingly include methods based on sequence analysis. However, the rules that allow a robust taxonomic identification based on genetic sequences are not simple, and not currently taught to students, for example:

- which gene should be analysed?

- should nucleotide or amino acid sequences be used?
- which method and which database should be used for sequence comparison?

These methods are very similar to those used for the epidemiological characterisation of circulating strains. It must be recognised that the bioinformatics analysis of sequence data will become an essential component of molecular diagnosis in microbiology. Consequently, sequence analysis should become an important item in the teaching programme of veterinary students.

## Emerging pathogens and bioterrorist weapons

An increasing number of emerging pathogens have been identified in the fields of both veterinary and human medicine. The factors that have led to these new occurrences are various and include:

- changing anthropological behaviour patterns
- commercial transportation of manufactured goods, such as scrap car tyres
- commercial transportation of vertebrates and invertebrates
- genetic evolution
- inter-species transmission
- modification of eco-systems
- resistance to anti-infectious molecules, etc.

Diagnosing these emerging agents is intrinsically difficult, since they are unexpected and specific tools may not be available. Such diagnosis is, however, extremely important and so this topic is developed elsewhere in this volume. Current molecular techniques that aid in the detection and characterisation of emerging pathogens have not yet been adapted for routine diagnosis. However, as described above, pan-specific identification techniques should become more widely available in the future. The detection of emerging pathogens will then rely on efficient interpretation of the genetic data obtained. This requires a basic understanding of:

- a) bioinformatics and taxonomic identification procedures
- b) the principles of genetic evolution.

In the past, the study of possible bioterrorist weapons has focused on severe human pathogens (some of which are of zoonotic origin). However, the malicious dissemination of highly virulent pathogens targeting cattle, poultry or

plants may also lead to disastrous consequences. The problems raised by such pathogens are similar to those of emerging pathogens. In addition, the traceability of strains is an issue of major importance to detect the deliberate dissemination of pathogens by humans. The bioinformatics and genomics tools referred to above may also be used for that purpose.

## Zoonoses

Forty years ago, a large number of medical microbiologists were educated in both animal and human pathogen microbiology. After decades of hyper-specialisation, the discovery that most emerging human pathogens are zoonotic agents has brought new importance to the interface between animal and human microbiology. In particular, veterinarians should be aware that many animal micro-organisms present serious threats to human health (even if most of these micro-organisms are not pathogenic for their animal hosts). Veterinarians are therefore an important component in the organisational infrastructure of human public health and should be fully trained in all that is required to manage the molecular diagnosis of zoonotic pathogens.

## Conclusion

It is clear that, in the future, veterinary education programmes should include disciplines dedicated to molecular biological diagnostics. However, it is currently impossible to define the outlines of these programmes precisely. First, it is difficult to anticipate the technological advances that will certainly be forthcoming in this very active and innovative field of research and development. Secondly, veterinarians work in both developed and developing countries, in tropical and temperate regions, etc. – that is, in markedly contrasting situations with different epidemiological environments and available tools. Thirdly, it is difficult to anticipate the cost of the next generation of molecular tests.

For example, retrospective analysis of the evolution of serological tests demonstrates how easy it is to make incorrect and naïve predictions. It was predicted 20 years ago that the cost of individual serological tests would become so low that systematic testing of sera against a large series of antigens would become standard practice. This did not happen. The production costs for state-of-the-art reagents cannot be ignored and methods such as multiplex simultaneous detection of several pathogens, or pan-specific detection of pathogens using large-scale sequencing or micro-arrays, will remain costly and therefore not affordable for all potential users. On the other hand, the development of innovative molecular techniques may lead to low-cost, specific diagnostic field tests particularly adaptable to the needs of veterinarians.

## Recommendations

As a helpful guideline, the author suggests that all veterinary curricula should provide a focus on each of the following:

- an introduction to the principles of molecular biology and genomics
- an introduction to the principles, performance and methods of disease detection and the characteristics of molecular diagnostic tests
- an introduction to the principles of micro-organism taxonomy
- an introduction to the principles of sequence comparison and their application to taxonomic identification, molecular epidemiology and epidemiological survey, genetic evolution and the traceability of strains
- an introduction to the principles and role of veterinarians in the fields of zoonoses and human public health.



# Les fondamentaux de l'enseignement vétérinaire dans le domaine des techniques moléculaires appliquées à la détection des maladies : ce que les vétérinaires devront savoir de la génomique, de la biologie moléculaire et du diagnostic moléculaire (y compris sur les armes bioterroristes) en 2025

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## Résumé

Nul doute que les programmes d'enseignement vétérinaire de demain mettront davantage l'accent que ceux d'aujourd'hui sur les outils moléculaires existants et à venir. Ils devront également permettre aux futurs vétérinaires de savoir exactement quels types de résultats ils peuvent attendre de chaque méthode particulière (par exemple, des méthodes de diagnostic spécifiques par opposition aux méthodes non spécifiques, des caractérisations phénotypiques versus génotypiques, etc.). En outre, les étudiants devront acquérir de solides connaissances sur les types de tests le plus appropriés dans chaque situation clinique ou épidémiologique, et sur les conclusions à tirer ou à ne pas tirer des résultats obtenus. En conséquence, les programmes d'enseignement vétérinaire devront porter sur les éléments suivants : principes de la biologie moléculaire et de la génomique ; détection des maladies et caractérisation des tests moléculaires ; principes et applications de la taxonomie microbienne, de la comparaison de séquences et de l'épidémiologie moléculaire (en particulier : identification taxonomique, enquêtes épidémiologiques, évolution génétique et traçabilité des souches) ; rôle des vétérinaires dans le domaine des zoonoses et de la santé publique.

## Mots-clés

Agents pathogènes émergents – Armes bioterroristes – Diagnostic moléculaire moderne – Génomique – Méthodes de diagnostic spécifiques versus non spécifiques – Programme d'enseignement.



## Año 2025: ¿qué tendrán que saber los veterinarios sobre genómica y biología y diagnóstico moleculares (armas bioterroristas inclusive)? Enseñanza veterinaria básica sobre las modernas herramientas moleculares para detectar enfermedades.

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### Resumen

No cabe duda alguna de que los futuros programas de enseñanza de microbiología veterinaria pondrán cada vez más el acento en las herramientas moleculares, tanto novedosas como ya contrastadas. Pero además deberán aportar al veterinario una comprensión clara y general del tipo de resultados que pueden obtenerse al utilizar determinado método (por ejemplo, procedimientos de diagnóstico específicos frente a procedimientos abiertos, caracterización fenotípica frente a genotípica, etc.). Por otra parte, el estudiante deberá adquirir un sólido conocimiento de los tipos de pruebas que resultan más apropiados en determinada situación clínica o epidemiológica y de las conclusiones que cabe o no cabe extraer de los resultados. En los planes de estudios veterinarios, por consiguiente, convendrá prestar especial atención a los siguientes elementos: principios de biología molecular y genómica; detección de enfermedades y caracterización de pruebas moleculares; principios de la taxonomía de microorganismos, la comparación de secuencias y la epidemiología molecular y sus aplicaciones (por ejemplo identificación taxonómica, controles epidemiológicos, evolución genética y rastreabilidad de las cepas); y función de los veterinarios en materia de zoonosis y salud pública.

### Palabras clave

Armas de bioterrorismo – Genómica – Moderno diagnóstico molecular – Patógenos emergentes – Plan de estudios – Procedimientos de diagnóstico específicos frente a procedimientos abiertos.

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