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Molecular epidemiology: the questions and conditions for the interpretation of answers related to strains

B. Toma, J-J. Bénet, Barbara Dufour & Nadia Haddad

Molecular biology tools are now routinely used to identify various microorganisms (bacteria, virus, fungus, parasite...). Results to be used for epidemiological purposes should carefully follow some good practices especially when micro-organisms are not distinguishable. This paper, introducing the thematic meeting on molecular epidemiology and the following conferences, draws our attention on necessary general conditions to monitor results during epidemiological studies targeting several micro-organisms.

Genomic constitution: stability and variability

J-Cl. Manuguerra

In their quest for adaptation to their environment and their conquest of new « territories », conventional pathogens, whose heredity lies within one or many molecules of nucleic acid, undergo three main mechanisms of variations. One of these, called mutation, is continuous and progressive. It corresponds to the change of a nucleotide by another and is associated with the infidelity of the element involved in the duplication of the genetic material. Mutations may cumulate in the course of time and are therefore the basis of the evolution of one or many viral or bacterial component(s). Besides mutations, two events may also occur during the phase of duplication of the genes: one is the deletion of nucleotides; the other is an insertion of nucleotide. The second mechanism of variation is the genetic re-assortment, in the case of viruses, which can be compared with the acquisition or the exchange of plasmids in the case of bacteria. This phenomenon is discrete and may lead to the brutal acquisition of new phenotypic properties (radical change in virulence or in an antigenic profile or acquisition of resistance to antibiotics). Lastly, genetic recombination, the third mechanism of genetic variations, is a powerful drive for the evolution of bacteria and some viruses. The access to the genomes of conventional pathogens and to their variations allows:

- to detect and type these agents generally by targeting stable or poorly variable structures;
- to analyse the genetic information itself and the deduced protein data by targeting variable, or even highly variable, genes.

Whatever genetic analysis is undertaken, it must be interpreted considering, among others factors, the nature of the pathogen itself and the technical strategy used to get results.

Molecular techniques for characterising strains: principles and reliability

H-J. Boulouis, Nadia Haddad & R. Maillard

Molecular biology techniques can analyse the genome without needing a cell culture of the targeted micro-organisms. These techniques are based on four principles: enzymes (polymerases, restriction enzymes...), molecular hybridization, electrophoresis and sequencing. These tools can either target the whole genome (pulsed field electrophoresis, RAPD, ...) or specific DNA sequences (PCR, Southern blotting, RFLP, micro-arrays, ...) from the clones. Depending on the biological material these techniques can compare different strains or evaluate time- and space variations for the same strain. These techniques give information for the diagnostic and strain typing complementing information given

by phenotypical studies. These techniques bring a new dimension for epidemiological studies but technical limitations should be assessed to adapt these tools to individual scientific goals.

Interest and limits of different technics for the study of *Salmonella*

Anne Brisabois

The importance of the salmonellosis infections, associated to the economic and public health consequences implicated the development of numerous typing methods. These were phenotypic in first, then more recently molecular for the characterization of *Salmonella* isolates. This characterization is mainly based on the determination of the serotype, very usually used, allowing to follow trends of evolution of serotypes in various sectors with the aim of *Salmonella* monitoring. The molecular methods are additional tools of investigation allowing to differentiate strains belonging to the same serotype. These techniques, generally longer and laborious, are rather reserved for the epidemiological investigation inquiries in a given ecosystem or during food borne disease or *Salmonella* outbreak. Next to the serotype universally recognized, the choice of the molecular methods should take into account the power of discrimination in a given serotype.

Interest and limits of different technics for the study of strains: example of tuberculosis

Nadia Haddad & B. Durand

In the case of tuberculosis, molecular biology tools have appeared rather recently, with a dramatic development during the last decade. The main techniques used are RFLP techniques and an original technique, spoligo-typing. Others give promising results like VNTR typing, and DNA microarrays techniques should emerge soon. These techniques, which are used for diagnosis or typing purposes, are very precious in the field of epidemiology. For instance, they help to « trace » infections (up and down investigations) or to identify the epidemic dynamic of propagation of certain isolates. But, in order to control these tools and use them optimally, it is important to know as far as possible some of the properties of the genetic regions which are involved, particularly their stability and their polymorphism. As a consequence, the interpretation of the results obtained with these tools should always be cautious and reasoned, in straight consistency with the epidemiological information provided from the investigations on the field. The aim of this paper is then to propose, after a short description of the techniques, some keys for the use and interpretation of molecular biology techniques applied to the epidemiology of tuberculosis.

Molecular epidemiology of foot-and-mouth disease

E. Thiry, E. Baranowski & E. Domingo

Molecular epidemiology provides a methodology allowing to compare foot-and-mouth disease virus isolates based on the analysis of a nucleotide sequence located in the gene encoding VP1 capsid protein. When the nucleotide difference between viral isolates is less than 15%, viruses are considered belonging to the same genotype. This method of analysis was used in several studies. The example of foot-and-mouth epizootics in North Africa (1987-1994) is detailed. The combined use of molecular and descriptive epidemiology is required to determine the temporal and geographical evolution of foot-and-mouth disease virus. Molecular data available for the 2001 European epizootic are also presented.

Molecular epidemiology: example of the equine influenza virus

S. Zientara

The use of the molecular tools is of a high interest in epidemiology. The interest of the association of these techniques and the classical epidemiological analyses will be illustrated by taking the example of equine influenza viruses. The determination and the comparison of the nucleotide sequences allow to characterize the virus strains more precisely than the classical methods and are useful to analyse the evolution of the equine influenza viruses. These methods are also useful to select the relevant strains which will be used in the vaccines.

Molecular tools applied to animal trichinellosis

P. Boireau, Liu Mingyan, Violeta Niborski, T. Roman & Isabelle Vallée

Despite the macroscopic size of the nematode parasite *Trichinella*, molecular tools are very useful and sensitive in epidemiological studies of trichinellosis. The worldwide distribution of *Trichinella* can be explained by the variety of species inside the genus correlated to a various degree of resistance to temperature. PCR techniques are highly sensitive techniques to identify infected animals with *Trichinella sp* and to characterize *Trichinella* species without xenodiagnoses. The various PCR strategies allow the identification of positive samples which remain undetected by other methods. Such tools are necessary to follow the circulation of *Trichinella* species in wild animals and to understand the origin of human contamination.

EPIDEMIOLOGY PAPERS

Initial training in animal epidemiology at the Veterinary School of Alfort

B. Toma, J-J. Bénet, Suzanne Bastian & Nadia Haddad

This paper describes the way of teaching general epidemiology at the Veterinary School of Alfort, France, during the last four years and analyses some parameters. This topic taught to second year students, was previously based on lectures and practical but it is now based on a new problem-solving approach. The new formula is based on working group activities and individual assessment towards the solving of six basic problems. A book entitled « Applied Epidemiology » is used in this *curriculum* and a continuous assessment is performed (assessment after each practical session focusing on learning parameters). The acceptance by the students of this new formula was monitored by an annual questionnaire. Student's satisfaction has improved in the last three years and teachers used the outcomes to improve their teaching. As it is not possible to compare the two different methods it is quite difficult to identify the best one. However, teachers felt that a larger student group achieved academic aims. The second method associating aims, teaching methods and assessment of learning outcomes from the students is clearly a way to improve teaching quality. Furthermore, by exclusively using working group sessions, this teaching method is matching the epidemiology characteristic known as « a way to think ».

West Nile disease in France

S. Zientara, B. Murgue, H. Zeller, Barbara Dufour, S. Murri, J. Labie, B. Durand & J. Hars

West Nile fever is a mosquito-borne *flaviviridae* infection transmitted in natural cycles between birds and mosquitoes, particularly *Culex* species mosquitoes. West Nile virus was first discovered in 1937 in the blood of a native woman of the West Nile province of Uganda. In human West Nile infection is a non-symptomatic or mild febrile illness, however encephalitis cases are reported with some fatalities in old patients. On September 6, 2000, two cases of

equine encephalitis caused by West Nile virus were reported in the Southern France (*Hérault département*), close to the Camargue National Park where a West Nile outbreak occurred in 1962. Till November 30, 76 cases were laboratory-confirmed among 131 equines presenting neurological disorders. The last confirmed case was on November 3. No human case has been laboratory-confirmed among clinically suspected patients. All but three cases were located in a region so-called "*la petite Camargue*" harbouring several large ponds, numerous colonies of migratory and settled birds as well as large mosquito populations. No abnormal mortality was reported in birds. A serosurvey study has been undertaken in horses in the infected area and other studies are in progress.

Incidence and typology of nervous diseases in adult cattle in France – The NBA network

D. Calavas, G. Desjouis, E. Collin, F. Schelcher, S. Philippe & M. Savey

In the European Community, the estimation of the incidence of nervous diseases in adult cattle and its follow up in time are two objectives of the overall epidemiological control system of the livestock towards Bovine Spongiform Encephalopathy that European countries have to set up. Therefore, the Ministry of Agriculture has decided that a Surveillance network of these diseases would be set up in France for a one-year period from the 1st January 2000. It is a pilot network based on « sentinels », which is working thanks to veterinary surgeons who communicate information about the cases of nervous diseases they diagnose during their work. The two main objectives of this network are: to give a first estimation of the incidence and of the typology of these diseases; to analyse the conditions to perpetuate this network and make it become widespread. The analysis of the first year shows a low incidence rate of neurological diseases (0.35%), the preponderance of metabolic diseases and of troubles with locomotive and medullar origins (respectively 34.6% and 38.8% of the cases), and a wide range of observed diseases. The analysis of the way the network is functioning, shows how important the motivation of the veterinary surgeons is and also the importance of the network's leading, especially the feedback to the veterinary surgeons.

Quantitative evaluation of Veterinary Services: ring-test study on marking

B. Toma, P. Bonjour, Barbara Dufour & J-L. Angot

A quantitative evaluation of the Veterinary Services in six countries, based on questionnaires, was studied by analysing differential marking from 10 staff members of the French Veterinary services. This study was able (1) to appraise the diversity in marking results for questionnaires from the same country but evaluated by different experts, (2) to analyse the discrepancy of these results, and (3) to discuss feelings and suggestions from the participants on the actual methodology. The outcome of this study recommended that each questionnaire should be analysed by two experts to reduce errors and allowed the amelioration of the questionnaire and its evaluation.