

Review

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Five postulates for resolving outbreaks of infectious disease

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Outbreaks of infection challenge the surveillance of infectious disease, but they also offer opportunities to improve and refine it. An outbreak may be the first sign of an emerging pathogen or it may draw attention to a new risk group or route of infection. Postulates analogous to those used a century ago by Robert Koch to prove the microbial aetiology of infectious diseases can be employed to verify the existence of an outbreak, demonstrate its cause and pinpoint its origins. In doing this, high-resolution molecular finger printing of micro-organisms has now assumed a crucial role. Without formal analysis based on postulates, the existence, extent and source of outbreaks may be overlooked and public health interventions misapplied or lost.

Introduction

What does, and does not, constitute an outbreak? Even in some developed countries the background data on the incidence and prevalence of infectious disease in man and animals may be so poor as to make the early and accurate recognition of all but the grossest outbreaks impossible. Without this surveillance, novel and unforeseen infection hazards may only slowly come to professional and public attention, and so the opportunity for rapid and appropriate action to contain an outbreak may be lost.

This delay may rob a public or animal health intervention of much of its force. The recent foot and mouth disease (FMD) outbreak in sheep in the United Kingdom, first recognized in February 2001, was within weeks rife in several parts of the country, and became difficult and costly to eliminate. Other pathogens may spread more slowly than FMD, but they do so inexorably, as was the case with HIV in North America and Western Europe in the early 1980s and as, in the UK at much the same time, an outbreak of spongiform encephalopathy spread in the national cattle herd. These examples demonstrate that the eventual human and animal welfare and economic costs can be high if surveillance is weak or if there is delay in identifying the causal organism.

When Society is faced with such a variety of infection hazards and modes of spread it is as well to go back to first principles and reflect on how knowledge of pathogens, host biology and modern technology can best be brought together to recognize outbreaks quickly and intervene most effectively.

Recognizing an outbreak

An outbreak can be thought of as a sudden increase in the rate of transmission of an infectious agent. Its recognition depends on the observation that the incidence of a particular

microbial infection and/or its associated disease is rising, and accurate ascertainment of this will greatly enhance communicable disease control. The proposal advanced below is that this is best achieved by applying 'postulates' analogous to those that for over 100 years have been used to prove that particular microbial species cause specific infectious diseases.

Postulates were first used by Robert Koch and his followers, who described important bacterial pathogens such as *Mycobacterium tuberculosis* and *Vibrio cholerae* (Brock, 1998) and established their associations with disease. Koch's postulates have since been elaborated by the inclusion of immunological proofs of causation. They have also been adapted to fit diseases caused by viruses (Rivers, 1937), and they are still being used to link 'orphan' microbes with diseases of previously unknown cause.

This essay proposes that postulates can also be used as tools in the investigation of outbreaks of disease. In this way, attribution of outbreaks to the wrong microbial species can be prevented and, in the case of very prevalent pathogens, for instance *Salmonella* Enteritidis, the false assumption avoided that a cluster of cases is due to a single strain when in fact different strains and therefore different sources may be involved. These postulates may also avoid outbreaks being described as infectious in origin when they are not. Such mistakes easily arise when there is weak background surveillance, technical error and/or poor analytical methods.

Another reason for applying postulates is that the nature of outbreaks may change, making traditional assumptions and responses inappropriate. Instead of following a classical food-, milk- or water-borne pattern, outbreaks may now take on new guises and spread in ways previously unforeseen. Unusual or even unknown ('emergent') pathogens may be involved. Early recognition then depends upon there being a surveillance infrastructure strong enough to detect simila-

rities between individual cases of infection in man and/or the rest of the animal kingdom, together with laboratory resources that will permit a new pathogen to be isolated and characterized. Because human and other movements, for example of foods, are now so rapid and diverse, modern outbreaks may quickly become very diffuse, and unconfined by administrative or geographical boundaries. This means that the responsibility for, and the costs of, epidemiological and laboratory surveillance must be underwritten nationally, and if possible supported by arrangements in which groups of countries agree to share information. An example of the latter is 'Enter-net' (Fisher, 1999), the forerunner of several planned European Union initiatives in cross-continental surveillance.

The postulates

It is proposed that five criteria or 'postulates' should be satisfied before the existence of an outbreak of communicable disease is confirmed. They are that:

(i) there is a discernable pattern of time, place and action

An outbreak, like a story, should have a coherent plot. In classical drama, Aristotle required there to be unity of time, place and action. Similarly, for the existence of an outbreak to be plausible, the cases must have a consistent timing, distribution and evolution. Typically a particular food or behaviour, a shared holiday destination, or some other common exposure or susceptibility is involved. Often this is obvious enough to immediately raise suspicion of an outbreak and allow intervention, but even then early recognition may depend on active surveillance. Only this continuous surveillance, at present so varied in strength from one country to another, can detect the small changes in incidence that may signal an outbreak (Farrington & Beale, 1993). It also makes it easier to hypothesize as to the cause of any outbreak, and avoid false assumptions.

(ii) there is shared mode of spread, usually from a common source of infection

The nature of the illness reported is frequently the key to understanding an outbreak. It suggests the mode of spread, the possible origins and the initial microbiological investigations that are needed (though the possibility of a chemical or biotoxic cause should not be overlooked). An unusual micro-organism may be involved, but the outbreak may also be due to a change in circumstances that favours an already endemic pathogen. For example, quiescent infections may break forth when asymptomatic human carriers begin a new activity, or when they are immunosuppressed so that microbial shedding increases and infections occur in contacts. Climatic or environmental change may favour the dissemination of a water-borne infection such as cryptosporidiosis.

Knowledge of how each known pathogen commonly spreads, how frequently there is clinical expression of infection and what intensity of exposure is required for infection and for spread between individuals is often essential

for outbreaks to be effectively managed. This knowledge should be within the collective experience of the interdisciplinary investigatory team formed to manage an outbreak. Three factors in particular – route of spread, level of clinical expression and efficiency of transmission – may need to be understood for control to be rapidly established, though rarely are all three precisely known.

New technology can be expected to play an increasingly important role in outbreak investigation. For example, modern techniques for quantifying micro-organisms can indicate the risk of transmission. This may avoid the application of unjustified, often costly, control measures and prevent overcautious advice being offered to the public. A pathogen may be able to spread by several routes, but in a particular outbreak one mode of spread will usually predominate. If that can quickly be identified, control measures can be better targeted. Most challenging are those outbreaks involving pathogens that can spread in unpredictable ways, especially if asymptomatic infection is frequent and/or incubation periods are long. The emergence of HIV offers a prime example of this; were it not for the pre-existing model of hepatitis B infection, it might have taken a long time to recognize its modes of spread, and exclude all others.

(iii) those affected in an outbreak are among the exposed, and not among the unexposed

This truth might seem too obvious for further comment, but it remains important for establishing the cause of an outbreak. Such investigations must be well done if erroneous conclusions are to be avoided. For example, when investigating a food-borne outbreak detailed dietary histories should be taken from all those possibly exposed, whether affected or not. Merely to inquire whether the recognized cases ate a particular food under suspicion is not enough. It must also be borne in mind that some who were exposed may be immune, or received a dose insufficient to provoke symptoms. Cases may be mistaken in their recall of what they ate or drank, and records of exposure to particular vehicles of infection, for example biological products, banked blood or breast milk, may be inadequate. The recreational use of injected drugs may be falsely denied. It is telling evidence if an individual who was unaffected received a different food or other preparation to those affected, or was absent at a critical time. Such nuggets of 'negative' information are often more valuable than additional positive associations.

(iv) there is evidence of a unique microbial strain

Recent advances in microbial fingerprinting have made this a most important tool for resolving outbreaks, and it represents the main contribution that the laboratory can make. Some pathogenic species are capable of rapid mutation, especially under environmental pressures such as the consumption of an antibiotic by their host. These pressures favour the emergence of an epidemic clone (i.e. a widespread microbial strain, all the isolates of which have an identical

genome), and clonal strains have become a frequent cause of, for example, hospital outbreaks of methicillin-resistant *Staphylococcus aureus*.

Strict proof of clonality may, however, be difficult to establish. The simplest tests can show that microbial isolates differ, but the fullest molecular analysis may be needed to prove that they are identical, i.e. clonal. Isolates can share an easily identified phenotype such as the same phage type or 'antibiogram', but this may be a type or resistance pattern that is prevalent in sporadic infections unrelated to the outbreak being investigated. A more highly resolving technique is then needed to reveal the source of the outbreak.

Ideally, laboratory evidence for every outbreak will include the isolation and characterization of a demonstrably identical pathogenic strain from every case, but in practice this is rarely achieved. Even if the meaning of the term 'isolate' is widened from the successful culture of a micro-organism to include detection of segments of its genome or of a specific antigen, it is unusual for every case in an outbreak to manifest this direct evidence of microbial infection. The bacteraemic or viraemic interval, or period of microbial excretion, may be too short, and the cases may not be in phase so that only the current cases, and any carriers, yield an isolate.

For virus infections especially, laboratory evidence of an outbreak may mostly rely on the presence of specific antibody of the IgM class. Tests for these antibodies have to be directed towards pre-selected pathogens and because of cross-reaction they may be hard to interpret at the subspecies level. The successful isolation of the same microbial strain from at least a few of the cases in an outbreak is therefore invaluable. Furthermore, tests of clonality can only be applied if sufficient of the genome of the implicated isolates is available.

When isolates are available, their examination by discriminatory methods will precisely define an outbreak. Speciation is rarely enough, but a shared genomic sequence, identical sets of fragments from an amplified genome, or the same serovar or phage sensitivity, i.e. indistinguishability by the most highly resolving tests available, will signify that there is a strain representative of the outbreak. If there is close similarity between isolates that falls short of identity, then a judgment has to be made whether they belong to a single outbreak. At this point, pre-existing laboratory observations on the intrinsic variability of the species and its prevalent strains are invaluable.

A few examples will illustrate the point that for effective outbreak surveillance of common pathogens high-resolution typing is needed. An outbreak due to an unusual *Salmonella* serovar is easily recognizable, but a cluster of cases due to one of the common *Salmonella* serovars, even if of the same phage type, may not constitute an outbreak at all. In the UK about a third of all recent cases in man have been due to a single phage type of *Salmonella* Enteritidis. In the 1990s, multiresistant nosocomial staphylococcal infection predominated in the UK and elsewhere, and much of it was due to a single phage

type of methicillin-resistant *Staphylococcus aureus*, clones of which are hard to distinguish and call for genotypic analysis as well as phenotypic tests. Other species, such as mycobacteria and some DNA viruses, display so little heterogeneity that epidemiologically unconnected isolates may appear identical even after the fullest possible genomic analysis. For example, many isolates of the *Mycobacterium tuberculosis/bovis* complex have only one or a few copies of the transposon, IS6110, the number and position of which are generally used to characterize them (Stanley & Saunders, 1996). Without further analysis failure to find genotypic differences between such isolates cannot be taken to confirm that they represent a cluster of infection (Geng *et al.*, 2002). Increasingly, therefore, sequencing of large gene fragments is likely to be used to establish the identity of isolates and allow the inference that a group of isolates are part of an outbreak.

Work in many microbiology laboratories is now focused on ways to compare the genomes of pathogens at the infra-species level. Progress has generally been greater for viruses than bacteria and it is often feasible to sequence large parts of viral genomes, usually after amplification by PCR. These data can be compared with those available in international sequence databases derived from the same genomic targets. Bacteria, however, have genomes a hundredfold or more bigger than most viruses, and so the same amount of PCR-based sequencing falls far short of demonstrating identity. Even the currently most favoured analytical method, which is to sequence seven or more 'house-keeping' genes (Maiden *et al.*, 1998), will sample less than 1% of a bacterial genome. This is a flimsy basis on which to conclude that groups of isolates are indistinguishable.

A PCR-based bacterial fingerprinting technique that has recently come to the fore is fluorescent amplified fragment length polymorphism (FAFLP) analysis (Savelkoul *et al.*, 1999), and because it is relatively rapid and cheap and highly resolving it may assume a prominent role in outbreak investigation. Representative sets of genomic fragments are generated from isolates by digestion of the whole bacterial genome with two restriction enzymes, and a single PCR is used to amplify the fragments. In the case of species that have been fully sequenced the observed fragment sizes can be compared with those predicted by reference to published whole sequences (Arnold *et al.*, 1999). The number and size of the fragments amplified by FAFLP (typically 50–100 fragments of 50–500 or more base pairs in length) can be regarded as a 'fingerprint' of each bacterial isolate and used to resolve outbreaks. Examples are *Streptococcus pyogenes* M type 1 (Desai *et al.*, 1998), the virulent clone complexes of *Neisseria meningitidis* (Goulding *et al.*, 2000), *Staphylococcus aureus* (Grady *et al.*, 2000) and *Escherichia coli* O157 (Smith *et al.*, 2000).

Not all outbreaks are attributable to a single microbial strain with a unique fingerprint. In some, more than one pathogen may be involved in the same incident, with the same source and mode of spread. When, for example, sanitary measures break down in a region where enteric infection is endemic,

Salmonella Typhi and *Salmonella* Paratyphi infections can be simultaneously involved in an outbreak, a phenomenon originally termed 'typhoide intriquée'. Similarly, experts have interpreted the present day hyperendemic pattern of human campylobacter infection as reflecting the effect of mixtures of virulent strains of the genus derived from the same chicken, pork or other raw meat sources. This may explain why the recognition of point source outbreaks of campylobacteriosis (not necessarily due to a single strain) has proved so difficult, though such outbreaks doubtless occur.

(v) when the source of the implicated micro-organism is contained or eliminated the outbreak wanes

Though a delayed proof, this last postulate can be the most convincing evidence that the true cause of an outbreak has been found. Precautionary measures that remove the source of a particular micro-organism, for example the withdrawal of an implicated foodstuff, prophylaxis or successful treatment of cases with an antibiotic or immunization with a specific vaccine or immunoglobulin will, if they bring an outbreak to an end, confirm its cause. If a recurrence is to be guarded against it is important to know why an outbreak ended. An example of an intensive study of a declining outbreak is the attention currently focussed on bovine spongiform encephalopathy (BSE) in the UK. Only the total disappearance of cases will confirm that both proven and unproven sources of infection, and alternative routes of spread, for example cow to calf or (even more worrying) from cow or animal feed to sheep, have been identified.

Discussion

Those who investigate outbreaks of infectious disease today are exercising skills developed over at least two centuries, and they do so with enabling microbiological techniques that were not available to the pioneers. On the other hand, they must meet higher public expectations and are working in a more complex environment and often rapidly changing circumstances. Population growth and movements, air travel, intensive farming, new ways of preparing food and supplying drinking water, invasive surgery, transfusion and transplantation, sophisticated medical treatments involving immunosuppression or the use of biological products: all these have added to the variety of outbreaks and extended the potential for spread.

Fortunately, pathogenic micro-organisms can now be characterized with great precision, and this makes it possible to resolve outbreaks that might previously have gone unrecognized. This is true not only for developed countries but elsewhere in the global village, as shown by the achievements of peripatetic investigating teams of specialists from, for example the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA). Even so, it should be acknowledged that many of the illnesses that seem to form an irreducible body of sporadic infection in the community may be part of unrecognized outbreaks which, if delineated, could be controlled. The principle should be that any

infection is preventable if only the means can be found to interrupt its chain of transmission, usually by identifying a pattern of spread and a unique infecting organism.

Recent advances in characterizing pathogens have been accompanied by expectations that outbreaks will be promptly detected. In the UK, substantial progress has been made in this respect through the work of the Communicable Disease Surveillance Centres and the Scottish Centre for Infection and Environmental Health, and other European countries have followed suit (Petersen *et al.*, 2000). Most of the achievements of the two British centres are nevertheless unsung. There are no plaudits for quenching outbreaks before the public has become aware of their existence, and most often it is continuous low-profile surveillance that is the key to outbreak prevention and control. Surveillance is also the basis for successful contingency planning, for example to respond to an antigenically shifted strain of influenza A virus or to a bioterrorist threat. The first signs of some outbreaks may be so insidious that they will not otherwise be recognized, and surveillance must therefore not be interrupted as a result of administrative upheaval or compromised by inadequate funding.

Two recent examples among very many can illustrate the value of surveillance. In 1997 an international outbreak was recognized in which *Salmonella* Anatum had contaminated infant formula dried milk. The feed was withdrawn and infant lives probably saved. In 1998, evidence of an outbreak of hepatitis B affecting 29 patients in an alternative medicine clinic in London first emerged as a single case of hepatitis in Derbyshire, over 100 miles away from the source. In subsequent weeks, widely scattered cases of hepatitis were shown to be related. All were patients of the same London clinic and had identical sequences in the surface and core genes of the infecting virus (Webster *et al.*, 2000). The closure of this clinic and termination of the outbreak was due to pre-existing national surveillance based on laboratory reports of apparently sporadic cases of jaundice.

A further benefit of continuous surveillance is that the professionals involved in it become adept at distinguishing true from factitious outbreaks. The latter may arise when the investigation of sporadic infections becomes more intense without their real incidence rising. This can happen when an energetic clinician, microbiologist or research team appears on the scene, or a more sensitive test is introduced. Such phenomena are easily misinterpreted as outbreaks.

Conclusion

Just as Koch's postulates of the causality of infectious disease were elaborated after his time, so future experience may add to the postulates suggested above for solving outbreaks. Other postulates might, for instance, take account of new knowledge of individual host susceptibility derived from analysis of the human genome. More automated and cheaper sequencing of microbial genomes will offer objective and more highly resolving bacterial typing. This, in turn, will afford rapid and forensic proof of whether clusters of cases

share the same infecting strain. Unexpected patterns of infection may in consequence emerge, with one suspected source being absolved and another implicated. Laboratory tests which lessen uncertainty will lead to better targeted control measures so that instead of action being based on the precautionary principle of removing all possible sources of infection, public health authorities can use focussed measures to eradicate sources of communicable disease. Outbreaks all have one thing in common: the sooner they are recognized and the more accurately they are resolved, the smaller the burden they place upon the community. In the wider economic context, therefore, good surveillance and more accurate pathogen identification can pay for themselves many times over.

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References

- Arnold, C., Metherell, L., Willshaw, G., Maggs, A. & Stanley, J. (1999).** Predictive fluorescent amplified fragment length polymorphism analysis of *Escherichia coli*: a high-resolution typing method with phylogenetic significance. *J Clin Microbiol* **37**, 1274–1279.
- Brock, T. D. (1998).** *Robert Koch: a Life in Medicine and Bacteriology*. Madison, WI: Science Tech Publishers.
- Desai, M., Tanna, A., Wall, R., Efstratiou, A., George, R. & Stanley, J. (1998).** Fluorescent amplified-fragment length polymorphism analysis of an outbreak of group A streptococcal invasive disease. *J Clin Microbiol* **36**, 3133–3137.
- Farrington, C. P. & Beale, A. D. (1993).** Computer-aided detection of temporal clusters of organisms reported to the Communicable Disease Surveillance Centre. *Commun Dis Rep* **3**, R78–R82.
- Fisher, I. S. T. (1999).** The Enter-net international surveillance network – how it works. *Eurosurveillance* **4**, 52–55.
- Geng, E., Kreiswirth, B., Driver, C., Li, J., Burzynski, J., DellaLatta, P., LaPaz, A. & Schluger, N. W. (2002).** Dynamics of tuberculosis transmission: changes in the transmission of tuberculosis in New York City from 1990 to 1999. *N Engl J Med* **346**, 19.
- Goulding, J. N., Hookey, J. V., Stanley, J., Olver, W., Neal, K. R., Ala'Aldeen, D. A. & Arnold, C. (2000).** Fluorescent AFLP genotyping of *Neisseria meningitidis* identifies clones associated with invasive disease. *J Clin Microbiol* **38**, 4580–4585.
- Grady, R., O'Neill, G., Cookson, B. & Stanley, J. (2000).** Fluorescent amplified-fragment length polymorphism analysis of the MRSA epidemic. *FEMS Microbiol Lett* **187**, 27–30.
- Maiden, M. C., Bygraves, J. A., Feil, E. & 10 other authors (1998).** Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci U S A* **95**, 3140–3145.
- Petersen, L. R., Ammon, A., Hamouda, O., Breuer, T., Kiessling, S., Bellach, B., Niemer, U., Bindert, F. J., Ostroff, S. & Kurth, R. (2000).** Developing national epidemiologic capacity to meet the challenges of emerging infections in Germany. *Emerg Infect Dis* **6**, 576–584.
- Rivers, T. M. (1937).** Viruses and Koch's postulates. *J Bacteriol* **33**, 1–12.
- Savelkoul, P. H. M., Aarts, H. J., de Haas, J., Dijkshoorn, L., Duim, B., Otsen, M., Rademaker, J. L. W., Schouls, L. & Lenstra, J. A. (1999).** Amplified-fragment length polymorphism analysis: the state of an art. *J Clin Microbiol* **37**, 3083–3091.
- Smith, D. G., Willshaw, G., Stanley, J. & Arnold, C. (2000).** Genotyping of verocytotoxin-producing *Escherichia coli* O157: comparison of analysis of isolates of a prevalent phage type by fluorescent amplified-fragment length polymorphism and pulsed-field gel electrophoresis. *J Clin Microbiol* **38**, 4616–4620.
- Stanley, J. & Saunders, N. A. (1996).** DNA insertion sequences and their use in studying the epidemiology of pathogenic bacteria. *J Med Microbiol* **45**, 236–251.
- Threlfall, E. J., Ward, L. R., Hampton, M. D. & 7 other authors (1998).** Molecular fingerprinting defines a strain of *Salmonella enterica* serotype Anatum responsible for an international outbreak associated with formula-dried milk. *Epidemiol Infect* **121**, 289–293.
- Webster, G. J. M., Hallett, R., Whalley, S. A. & 10 other authors (2000).** Molecular epidemiology of a large outbreak of hepatitis B linked to autohaemotherapy. *Lancet* **356**, 379–384.