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Microbial forensics for natural and intentional incidents of infectious disease involving animals

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Summary

Microbial forensics is a relatively new scientific discipline dedicated to analysing microbiological evidence from a crime for attribution purposes. It builds on traditional microbiology and epidemiology but within a legal framework. Important motives for forensic investigations include interdiction of criminals, prosecution of justice, and ideally, deterrence of others from committing similar acts.

Forensic capabilities in animal health should focus on building capacity for detection and reporting of increases in infectious disease morbidity and mortality among animals that might reflect a covert release of a pathogen. Suspicion should be raised when epidemiological patterns are different from those expected for the animal population and the pathogen in question. Existing capacities for the detection and reporting of epidemic and even endemic diseases should be an international priority for the prevention of catastrophic losses in animal and potentially in human life. The veterinary community needs to be more aware of the legal requirements related to forensic investigations so that veterinarians will be prepared to handle evidence properly within their own fields.

Keywords

Animal – Biocrime – Bioterror – Forensics – Infectious disease – Law enforcement – Microbial forensics.

Introduction

The vast majority of infections in animals and zoonotic infections in humans happen naturally, without malicious intent or criminal negligence. Nevertheless, rare incidents involving the unlawful and deliberate exposure of susceptible populations to animal and zoonotic infections have occurred. In 1997 for example, persons unknown illegally introduced rabbit haemorrhagic disease virus into New Zealand, apparently to control the large wild rabbit population that was posing a problem for farmers (30). Humans have also been the targets of deliberate zoonotic disease exposure. In 1984, the Rajneeshee cult deliberately contaminated the food supply in The Dalles, Oregon (31). The ensuing outbreak affected at least 751 people and 45 of them were hospitalised. Criminal convictions were obtained for some of the perpetrators. In 2001, *Bacillus anthracis* spores were deliberately distributed through the postal system of the United States of America (USA) in the 'anthrax letters' incidents, resulting in at least 22 human cases of anthrax, five of them fatal (1). Thus far, no convictions have been obtained in the anthrax case.

The above are examples of deliberate exposure with infectious agents; however, illegal activity may also lead to unintentional exposure. For example, Thai eagles infected with highly pathogenic H5N1 influenza were smuggled into Belgium in 2004 (33).

Veterinary microbiologists and epidemiologists are familiar with investigation of naturally occurring outbreaks of infectious diseases, but most have little experience with forensic examination of evidence for the investigation of criminal cases, although some are called upon to provide expert testimony in court. One of the unique features of bioterror (the use of pathogens in terrorism) and biocrime (the use of pathogens in crimes) incidents such as those mentioned earlier, and even hoaxes of a similar nature, is the requirement for attribution of responsibility. Attribution is defined as 'the information obtained regarding the identification or source of a material to the degree that it can be ascertained' (26). Important motives for forensic investigations include interdiction of criminals, prosecution of justice, and ideally, deterrence of others from committing similar acts (8). When a biocrime involving animals is detected, characteristics of the infectious agent that is isolated, along with additional evidence, will be important information for the criminal prosecution of the case and implementation of medical countermeasures (1).

Concerns about bioterrorism, heightened by the 2001 'anthrax letters' incidents in the USA, are reflected in several recent reports concerning the nature of the problem and opportunities for increased detection, control and prevention of future bioterror and biocrime incidents (1, 7, 8, 9, 19, 25, 26). A number of reports (1, 8, 9, 25) give a more complete discussion of microbial forensic issues from the human health perspective than there is space for in this paper. While these reports focus mainly on the human health aspects, some include agricultural pathogens in their lists of potential bioterror pathogens. The reports note the devastation to national economies that could ensue from the introduction of some of these pathogens into susceptible animal populations.

The veterinary community needs to be more aware of the legal requirements related to forensic investigations so that veterinarians will be prepared to properly handle evidence within their own fields. Are veterinary laboratories prepared to deal with biocrime or are they in a position similar to the Public Health Laboratories (PHL) of the USA in 2001? Diane Barden, the weapons of mass destruction coordinator for the Connecticut State PHL, stated:

'there was one event prior to my start [March 2001]. … A threat letter was sent to an abortion clinic and the Federal Bureau of Investigation (FBI) hand-delivered the letter to the laboratory. That was the first contact we had with the FBI and handling criminal evidence. As you might imagine, it did not go very well' (4).

Veterinary laboratories must be prepared to deal with issues such as chain-of-custody, secure storage of evidence, tracking of individual items of evidence and their derivatives and all the other legal requirements for handling evidence (26). No matter how good a laboratory may be at microbial forensics, a case may well be lost if evidence is not admissible in court due to a problem with documentation of the chain-of-custody (28). The principal objective of this paper is to increase awareness of microbial forensics among those interested in infectious diseases of animals and zoonoses.

Microbial forensics

Forensics is defined as 'the use of science and technology to investigate and establish facts in criminal or civil courts of law' (2). It is a multidisciplinary endeavour involving pathology, toxicology, computer investigation, fingerprint analysis, anthropology, DNA technology and other elements. It focuses on the person or persons who perpetrated the crime, and also on the victims and their related microbiology. Microbial forensics is a new branch of the forensics field and not yet widely recognised, although recent bioterror events, especially the anthrax incident of 2001 in the USA, have highlighted its importance. At the same time, swift action is needed to limit the scale of any outbreak and reassure the public when events are under control. Microbial forensics has been defined as 'a scientific discipline dedicated to analysing evidence from a bioterrorism act, biocrime or inadvertent microorganism/toxin release for attribution purposes' (7, 19).

Microbial forensics builds on traditional microbiology and epidemiology within a legal framework. Investigations of suspected biocrimes have many similarities to epidemiological investigations of naturally occurring infectious disease outbreaks, but also some differences (Table I). In conventional forensics, testing and characterisation of human DNA from crime scene samples is important for the identification of individual persons. Microbial forensics also utilises genetic material, but again there are important differences. Investigation of human-derived specimens involves only one species, and forensics experts are able to use a set of only 10 to 17 microsatellite loci on the genome for most identifications (8). In contrast, microbial forensics is much more complicated due to the large number of potential bacterial or viral species involved, and to the complexity of microbial dynamics, evolution and spread. The large number of microbes that are candidates for biocrimes and bioterror places practical limits on the development of forensic signature assays, specimen archives and databases for use in forensic investigations.

Table I
Comparison of forensic and epidemiological outbreak investigations of infectious disease

Feature	Type of infectious disease investigation	
	Forensic	Epidemiological outbreak investigation
Direction	Police or other law authority	Veterinary or public health officer
Principal goals	Identify source to control outbreak, plus attribution – identify and successfully prosecute the perpetrator	Identify source to control outbreak, plus identify causal factors in order to prevent recurrence
Secondary goals	Deter other perpetrators	Contribute new scientific or risk management knowledge
Case definition	Yes	Yes
Describe and enumerate symptoms in cases	Yes	Yes
Infection distribution in population	Maybe	Yes
Establish mode of transmission	Yes	Yes
Compare odds of disease in exposed and unexposed individuals	Probably	Yes
Collect samples for analysis	Yes	Yes
Laboratory support	Veterinary and public health and forensic	Veterinary and public health
Standard protocols for chain-of-custody	Yes	Sometimes, but not as stringent
Identification of infectious agent	Greatest possible level of detail	To species level at least, may need greater definition
Identify source	Yes	Yes
Involves legal proceedings	Yes	Not usually

Need for collaboration and partnerships

Most veterinarians and public health officers have little or no experience with investigation of biocrime or bioterror incidents, and therefore are unlikely to be familiar with forensics. Is there a need to change this situation? To the extent that the societal risk from such deliberate incidents involving animals is low (that is, they continue to be rare and limited in scale), it may be sufficient in some countries to rely on the forensics capabilities of traditional law enforcement agencies. Whether the likelihood of deliberate incidents is increasing or not is not known, but it is known that the potential impact of deliberate introduction of animal disease (e.g. foot and mouth disease) or zoonotic disease (e.g. yersiniosis) is considerable. (For further information see papers in this publication by Hugh-Jones, Wilson *et al.*, Ackerman, Lyra, Woolridge, Ozawa *et al.*, Davies, and Domoradskiy and Orent.) Therefore, in countries that are especially vulnerable, existing animal health surveillance and disease control capabilities should be reviewed in order to enhance detection and management of bioterror and biocrime incidents, and to establish linkages with microbial forensics experts in the human field. It is logical that specialists in traditional and bioterror/biocrime forensics should help each other and collaborate closely. A synergy between the two will increase and widen their collective experience and expertise, building a resource for the future. There is also a pressing need for better integration and cooperation between public health and animal health surveillance programmes (18).

Capabilities in animal health should focus on building capacity to detect and report increases in morbidity and mortality among animals that might reflect a covert release of a pathogen. Suspicion should be raised when epidemiological patterns are different from those expected for the animal population and the pathogen in question. Existing capacities for detecting and reporting even endemic diseases should be an international priority for the prevention of catastrophic losses in animal and potentially human life. In the case of a covert attack (an unannounced intentional release), animal or public health authorities will initially be directing the investigation. Investigations should routinely be conducted in a manner that preserves the integrity of potential crime scenes and evidence for future forensic and criminal investigations. It is important that investigators should consider the possibility of deliberate disease introduction at an early stage, otherwise the opportunity to investigate the incident properly may be delayed or missed.

Veterinary laboratories must be prepared to deal with issues such as chain-of-custody, secure storage of evidence, the tracking of individual items of evidence and their derivatives, and all the other legal requirements for handling evidence (26). Chain-of-custody protocols provide a documented unbroken chain of records showing who had control of the evidence as well as each transfer of its possession by secure and traceable means. This process begins with the original sample, which must be labelled with the date and time of collection as well as the initials of the person collecting it and identifiers (e.g. a code or numbering system that will distinguish this sample from others collected at the scene). Any one of the individuals in the chain-of-custody may be called into court to testify to the identity of the sample (28).

Once at the laboratory the evidence must be kept secure to prevent unauthorised access and the risk of contamination, misidentification or tampering (26, 28). There are several levels of security to consider, ranging from levels of security within the building itself (e.g. restricted access zones and high security zones), methods of controlling access for authorised staff into these zones (e.g. electronic keypads or keys), and security features of the storage unit where the sample itself is held (e.g. exhibit locker, fridge). For example, the storage unit itself should be locked with a high security lock and there should be only two keys per lock. One key is signed out to the individual responsible for the sample and the other stored in a sealed envelope in a safe.

These examples illustrate only a few of the legal requirements needed to maintain the admissibility of evidence at a trial. The chain-of-custody is a complicated series of events with potential for errors at any point. The veterinary community looks at this issue from the viewpoint of providing solid evidence for a trial or legal proceedings; however, viewing the situation from the perspective of a criminal defence lawyer can provide useful insights into possible weaknesses in the laboratory's procedures. A variety of tactics may be used to discredit forensic experts and laboratory results. These may include:

– reviewing past laboratory assessments for deficiencies

– demonstrating conflicts of interest for scientists and staff who own or hold shares in the lab, or in companies that produce diagnostic tests used there

– acquiring laboratory documentation on quality assurance (QA) and quality control

– acquiring laboratory bench notes and using them to develop questions that would be difficult for any expert to answer (17).

When crimes are overt – that is to say, identifiable as such from the beginning or announced in advance by the perpetrator – law enforcement officials will immediately assume direction of the investigation, but may request assistance from animal or public health officials. Whether intentional releases of pathogens and ensuing outbreaks among animals are overt or covert, unobstructed and direct communication between animal health and law enforcement officials is necessary. Also, public health services may be involved to mitigate the effect by initiating medical countermeasures. A report by the American Academy of Microbiology proposed that training in forensics should be given to personnel responsible for the first responses to attacks on human populations (1). Whether such training could in practice be worthwhile for veterinarians is debateable, given the rarity of biocrimes involving animals. The potential impacts of such crimes are highest for foreign animal disease introduction, and it would therefore be prudent to provide some degree of forensics training to veterinarians responsible for the first response to foreign animal disease incursions. As 'war games' practising responses to routine outbreaks can lose their novelty, the occasional and unannounced use of a bioterrorist scenario will provide the opportunity for training in forensic response.

Forensics laboratories may not have microbiological capabilities, and especially the proper facilities to handle samples contaminated with dangerous pathogens or to isolate and characterise these pathogens. This is a logical area for partnership, with a well-equipped veterinary laboratory or PHL providing the facilities for microbiological work, and the forensics experts providing the expertise in handling and forensic examination of evidence (8). In the USA, such a partnership has been established in the human field, and involves the FBI and the Bioforensics Analysis Center at Fort Detrick, Maryland. The partnership will also include the Plum Island Foreign Animal Disease Laboratory. In the veterinary field, the USA has undertaken a pilot project for a National Animal Health Laboratory Network involving federal and state laboratories with the aim of enhancing the speed of responses to animal health emergencies (32).

Laboratory techniques

An important role of microbiological testing is to compare isolate characteristics (e.g. species, serotype, phage type, genetic profiles) among evidence samples and reference cultures and strains, to determine whether the various isolates are from the same source or lineage, or conversely at least to confirm an independent origin (7). Observed differences may or may not be sufficient to demonstrate that microbes are from the same source or lineage or of independent origin, depending on the accuracy and precision of the test procedure, the characteristics of the test organisms (e.g. laboratory or wild strains) and the expected mutation rates. For example, minor differences at rapidly evolving sites in the genome may not alone justify the conclusion that the organisms derive from different lineages from the reference strains or known recoveries, or are meaningfully different from the other field isolates. Adding to the potential confusion, natural outbreaks can sometimes involve multiple strains; an example would be livestock anthrax from feed containing contaminated bonemeals (8).

Microbial forensics examinations share many techniques with conventional diagnostic or research examinations, including culture and speciation of isolates, phenotyping, phage typing, fatty acid composition analysis, and genetic characterisation (9). Newer techniques, such as microarrays and isotope analysis, may also be important. For example, in the investigation of the 2001 anthrax bioterror incident in the USA, a multilocus variable number of tandem repeats analysis was used to identify the *B. anthracis* spores as belonging to the Ames strain (8). Other techniques that may be employed include microsatellite and minisatellite loci typing and real-time polymerase chain reaction (PCR) (8).

Although microbial forensics employs genetic tools for strain identification, it is important to draw a distinction between this and the use of human DNA analysis in conventional forensics. Human DNA analyses are important tools in modern forensics. They are used for attribution to specific individuals when matches are found between DNA in crime-scene samples and samples from suspects of the crime. Conversely, DNA analyses may be important in eliminating suspects when mismatches are found. In contrast, the power of genetic analyses in microbial forensics is more limited, due to the clonal nature of microbial populations and the lack of high-quality microbial population and phylogenetic databases (8, 10); it is not possible to distinguish among members of a clone that may be widely dispersed, and microbial databases are not organised with forensics in mind. However, as demonstrated in the examples described below, useful qualitative information concerning attribution may be obtained, and mismatches are quite useful in eliminating individuals or premises from the list of suspects.

Genomics is increasing the power of microbial forensics by assisting in the design of gene-based diagnostic tests and guiding interpretation. The genomes of some of the important zoonotic pathogens (e.g. *Salmonella* Typhimurium and *Escherichia coli* O157:H7) have been sequenced, as have various strains of important threat-level biological agents (e.g. *B. anthracis*) (1, 8). However, the costs and technical demands of sequencing, and the difficulty of identifying distinguishing genetic markers, place practical limits on these techniques for use in animal-derived infectious disease outbreaks. Bioinformatics tools (e.g. software) are important for finding genetically related organisms represented in databases and ascribing statistical confidence limits on matches (8). Suitably validated methods are obviously preferred for forensics purposes because they are more likely to be recognised in courts of law.

Forensic testing errors have become increasingly evident, since human DNA testing has supported the wrongful conviction of numerous individuals on the basis of inappropriately used forensic evidence (involving hair, ballistics, fingerprints and similar tests). Such cases have cast doubt on some long-accepted procedures, many of which had not been fully validated (25). There has also been a belated recognition of poor standards in individual laboratories, unfortunately sometimes of long standing. In the USA the current standard for admissibility of scientific evidence in the courtroom is the so-called 'Daubert test', which states that the admissibility of scientific testimony should be based on sufficient facts or data, and that these data should be the product of reliable, internally validated methods which have been stringently applied (25). Thus, great emphasis is placed on the methods used in forensics.

Information networks

Localised outbreaks of disease may be quickly identified by alert veterinary and public health officers. However, special systems may be helpful for rapidly detecting spatially and/or temporally dispersed outbreaks, and for identifying local clusters as part of larger outbreaks, such as food-borne outbreaks that are spread nationally by the wide distribution of a contaminated product. Some systems of this type have been developed for naturally occurring disease; one example is the PulseNet System for subtyping food-borne pathogens (e.g. *E. coli* O157:H7) by pulsed-field gel electrophoresis (PFGE) (29). PulseNet is a US national network of local, state and national PHLs with its headquarters at the Center for Disease Control (CDC) in Atlanta, with collaborative networks in other countries and regions, such as Canada, Latin America and Europe. Using standardised PFGE protocols, participating laboratories can submit DNA patterns of strains of *E. coli* O157:H7, *Salmonella*, *Shigella, Listeria* or *Campylobacter* to the CDC database. The database is open for use by participants, which facilitates rapid comparison of PFGE patterns. There is a need in animal and human healthcare for additional systems that can track infectious disease incidents in real time to improve detection capability and the speed of response.

Quality assurance

Quality assurance is an important aspect of forensic examinations, and the recent emergence of the field of microbial forensics has benefited from QA experience in human DNA testing (19). Recently, a Scientific Working Group on Microbial Genetics and Forensics was created to develop laboratory guidelines (26). The recommendations cover many features familiar to conventional diagnostic laboratories, including documentation of laboratory organisation and management, personnel qualifications and training, sample control, analytical procedures, standard operating procedures, calibration and maintenance, documentation and report writing, and auditing, among other aspects. Special considerations for forensics include the need for personnel security clearances, facilities for the secure storage of evidence, tighter procedures for sample control (e.g. records of the names of individuals involved in sample collection and transport) and chain-of-custody within the laboratory. Method validation is very important for the credibility of forensics data in legal proceedings.

Examples involving animal or zoonotic infections

The 2001 'anthrax letters' incident in the USA has already been mentioned as highlighting the importance of microbial forensics and is a good example of the type of situation for which law enforcement and public health officials need to prepare (1). Fortunately, nothing like this has recently been detected in the animal health sector. There have however been several historical and recent incidents involving animals where criminal activity or criminal negligence may have directly or indirectly threatened the health of livestock populations or the public. A few are briefly summarised here.

Deliberate infection of animals in time of war

During the First World War the Germans carried out a variety of biological attacks on different types of livestock. Unlike chemical warfare programmes, this biological programme was not well documented and is the subject of some debate (27). The best-known example was the case of Dr Anton Dilger, a German-American physician living in a northwest suburb of Washington, D.C. Supplied with seed stocks of *B. anthracis* (anthrax) and *Burkholderia mallei* (glanders) by the Imperial German government, he set up a small laboratory in his home and is believed to have produced about a litre of agent. Assisted by Captain Frederick Hinsch, this effort was claimed to have resulted in the infection of 3,500 horses, mules and cattle waiting to be shipped to Allied forces in Europe (13, 34). The Germans were also reported to have successfully infected 4,500 mules in Mesopotamia with glanders. Other attempts by German agents were aimed at livestock in Romania, France, Norway and Argentina (5, 13, 16).

During the Second World War, in 'Operation Vegetarian' the United Kingdom (UK) developed and produced 5 million anthrax-laden linseed-oil 'cattle cakes' that were intended to be dropped from aircraft over German pastures to infect and kill beef and dairy cattle (16, 24). Each cake was 2.5 cm in diameter, weighed 10 g and contained 5×10^8 anthrax spores in a glass capillary tube (6, 14, 24). Prior tests showed that the cattle would die within 5.25 days after consuming a cake and it was estimated that about 80% of distributed cakes would be consumed within two weeks of being airdropped (14). Although everything was in place to carry out the plan in the summer of 1944, the Normandy invasion had taken place and the operation was cancelled. At the end of 1945 the cattle cakes were incinerated at Porton Down in the UK (24).

Following the Second World War the USA biological warfare programme tested hog cholera (classical swine fever) and Newcastle disease, using experimental bombs that released virus-coated feathers that would float down into farms (16).

An unusual anthrax outbreak occurred in Zimbabwe from 1978 to 1980, which may have been due to deliberate spread. Nass has suggested that:

'[an] explanation for the sudden peak of anthrax in the Tribal Trust Lands beginning in November, 1978, is that one or more units attached to the Rhodesian military may have airdropped anthrax spores in these territories. This action would expose cattle to the disease through ingestion or inhalation (or both) of anthrax spores' (20).

However, it is also possible that the outbreak was caused by natural events such as excessive rains, exacerbated by the civil conflict (e.g. failure of proper vaccination, and absence of veterinary services or inspection). *Bacillus anthracis* isolates from this outbreak may never have been characterised, indicating a possible role for microbial forensics. This case is an example of the difficulty of establishing or ruling out deliberate contamination, particularly in time of war.

Bird smuggling and influenza

In October 2004, customs officials at Brussels International Airport seized two crested hawk-eagles that were smuggled from Thailand (33). Virus cultured from the euthanised birds was identified as highly pathogenic H5N1 influenza virus on the basis of haemagglutination inhibition and RT-PCR testing. Follow-up investigations and actions resulted in the destruction of over 650 birds in quarantine, as well as medical examination of more than 25 persons who had been in direct or indirect contact with the birds, followed by oseltamivir prophylaxis. This case demonstrates the potential importance of animal smuggling in the unintentional international spread of important infectious diseases.

West Nile virus in North America

West Nile virus (WNV) is a zoonosis that first appeared in the New York City area in 1999 (11). In retrospect, it is evident that the infection first caused mortality in several wild bird species, then illness and mortality in humans.

Investigations of the outbreaks in humans and birds were conducted independently, and a lack of early and effective collaboration among the public health and animal health programmes probably delayed the timely recognition of the incursion (15). Previously, the virus had been found only in the eastern hemisphere, specifically in Asia, Africa, the Middle East and Europe. Since 1999, WNV has firmly established itself in a large portion of the USA and Canada (22). The epidemic strain of WNV was closely related to a strain circulating in Israel between 1997 and 2000, so the Middle East was the most likely source (21). How the virus arrived in North America is unknown but was the subject of much speculation, including the possibility of deliberate introduction (23). Probabilities include a human traveller infected in another part of the world, smuggling of infected birds, or inadvertent transport by an insect vector in an airplane. The history of WNV incursion into North America is important because it shows that delay in effective collaboration between public health and animal health surveillance programmes inhibits the rapid detection of outbreaks.

Escherichia coli and *Campylobacter* from cattle and contamination of municipal water supply

In May 2000, an outbreak of waterborne illness in Walkerton, Canada, due to *E. coli* O157:H7 and *Campylobacter jejuni*, caused approximately 2,300 cases of illness and seven deaths (3). Epidemiological and hydrological investigations were conducted to identify the source of the contamination. Evidence of bacterial contamination was found in one of the wells supplying the town. Faecal and environmental samples were collected from 13 farms in the vicinity of the town wells. While *Campylobacter* spp. were identified on nine farms, and *E. coli* O157:H7 were found on two farms, on only two farms were both pathogens found, including one farm adjacent to the contaminated well. Strain characterisation of the *Campylobacter* spp. isolates (heat-stable and heat-labile serotyping, phage typing, biotyping, fla-restriction fragment length polymorphism typing and PFGE testing) and the *E. coli* O157:H7 (phage typing and PFGE) showed that samples from that farm were identical to those found in most of the human cases (3, 12).

A subsequent public judicial inquiry into the incident concluded that the primary source of the outbreak was manure spread on the farm adjacent to the contaminated well. The inquiry found that the farmer was not at fault, because he had been following good farming practices. Other factors that were thought to have contributed to the well contamination were unusually heavy rainfall, poor well construction, and a failure to ensure proper chlorination and water quality monitoring (21).

Deliberate introduction of rabbit calicivirus disease (rabbit haemorrhagic disease) into New Zealand

In August 1997, rabbit calicivirus disease (RCD) was diagnosed by the Animal Health Laboratory in Wallaceville, New Zealand, using an antigen capture enzyme-linked immunosorbent assay from the World Organisation for Animal Health (OIE) Reference Laboratory in Brescia, Italy (see paper by Peter O'Hare in this publication; 30). An exotic-disease response was initiated by the Chief Veterinary Officer, and the vicinity of the outbreak was designated as a controlled area, with restrictions on animal and human movement. A criminal investigation was initiated to determine if the virus had been deliberately introduced. At a subsequent meeting of farmers and agriculture ministry staff, farmers admitted to widespread use of RCD in inoculated carrots and oats as a biological control of rabbits. Within a few days, additional outbreaks were detected in other regions of the South Island. Agriculture ministry staff concluded that eradication and containment of the infection was not feasible, and controls were lifted. The disease was initially detected only a few weeks after a government announcement that RCD virus would not be legally imported into New Zealand as a biological control agent for rabbits (30). No charges were apparently laid.

Deliberate *Salmonella* contamination of the food supply: The Dalles, Oregon

In September and October 1984, at least 751 persons contracted *Salmonella* gastroenteritis in The Dalles, Oregon (31). Ten restaurants were identified as the source of infection in most of the cases. Eating from salad bars was the main risk factor, although implicated foods on the salad bars differed among restaurants. The epidemiological investigation showed that no common mechanisms, such as specific foods or food handlers, were responsible for the contamination. Suspicious events associated with the outbreak were reported to local, State and Federal police. Police, with technical assistance from the Oregon PHL, investigated the facilities of the local Rajneeshee religious commune and seized a sample of *Salmonella* Typhimurium (indistinguishable from the outbreak strain and subsequently determined to have been obtained from a commercial supplier) from the commune clinic. Criminal investigations showed that members of the commune intentionally contaminated the salad bars. The apparent motive was to test a programme to incapacitate voters in an upcoming local county election. In 1986, two members of the commune pleaded guilty to charges of conspiring to tamper with food products by poisoning food, and were sentenced to 4.5 years in prison.

It is noteworthy that the initial epidemiological investigation did not recognise the outbreak source. It was more than a year later that sufficient evidence was obtained to link the commune to the outbreak. The possibility of intentional contamination was considered early in the epidemiological investigation but was initially rejected for several reasons: no motive was apparent; no one claimed responsibility; the epidemic curves suggested that salad bars were contaminated on various occasions over several weeks; police investigation of initial questionable activities did not establish a pattern; no disgruntled employees were identified; and the investigators recognised that sources of outbreaks sometimes remain unidentified (31). This incident shows the challenges involved in early identification of deliberate contamination.

Conclusions

Incidents of deliberate animal-related infectious disease outbreaks are rare, although the potential health and economic impacts of such incidents on animals and the public are considerable. Enhanced capacity to investigate biocrimes and bioterror incidents will enhance national and international security. The most advanced capabilities in microbial forensics should be concentrated at the national level and focus on bioterror and agroterror. Cooperation is needed between traditional forensics laboratories and laboratories equipped to deal with level-3 and level-4 pathogens of animals and humans that may be used by terrorists or criminals. Only a few countries are likely to have the infrastructure to enable sophisticated microbial forensics capabilities, so there is a need for international cooperation on methods and facilities for the rapid and thorough processing of microbial forensics evidence. There is also need for better integration and cooperation between public health and animal health surveillance programmes. International organisations such as the OIE, World Health Organization and Food and Agriculture Organization have an important role in facilitating this cooperation. While specialised forensics training is of doubtful value for most veterinarians, public health officers and laboratory diagnosticians, these individuals should be cognisant of the possibility of criminal involvement in outbreaks of animal-related infectious disease, and recognise where they need to cooperate with law enforcement and traditional forensics investigators at an early stage. Quality assurance is very important to veterinary diagnostic and forensic laboratories alike. Therefore implementation of credible QA in veterinary laboratories, especially in relation to sample chain-of-custody, should strengthen the role of these laboratories in assisting the investigation of crimes.

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La microbiologie médico-légale lors d'épisodes de maladies infectieuses d'origine naturelle et intentionnelle impliquant des animaux

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Résumé

La microbiologie médico-légale est une discipline scientifique relativement récente qui consiste à analyser les données microbiologiques afin d'identifier les coupables d'un crime. Elle se fonde sur la microbiologie et l'épidémiologie classiques, mais dans un cadre juridique. Les principaux motifs des enquêtes médico-légales sont la recherche des coupables, leur poursuite en justice et, dans l'idéal, l'effet de dissuasion qui peut empêcher d'autres personnes d'accomplir de tels actes.

Dans le domaine de la santé animale, la recherche médico-légale devrait viser surtout à renforcer les capacités pour détecter et déclarer une augmentation de la morbidité et de la mortalité chez les animaux qui pourrait s'expliquer par la dissémination cachée d'un agent pathogène. L'existence de caractéristiques épidémiologiques autres que celles attendues pour la population animale et l'agent pathogène en question doit éveiller les soupçons. Les capacités existantes de détection et de déclaration des maladies épidémiques et même endémiques devraient constituer une priorité au niveau international pour que l'on puisse prévenir des pertes catastrophiques de vies animales et peut-être humaines. La communauté vétérinaire doit prendre davantage conscience des exigences juridiques liées aux enquêtes médico-légales afin d'être mieux armée pour gérer efficacement les preuves qui apparaissent dans son domaine.

Mots-clés
Animal – Biocriminalité – Bioterrorisme – Exécution de la loi – Maladie infectieuse – Médecine légale – Microbiologie médico-légale.

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Microbiología forense para casos de enfermedades infecciosas de origen natural o intencionado que afecten a animales

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Resumen
La microbiología forense es una disciplina científica relativamente nueva que se ocupa de estudiar indicios microbiológicos para identificar al o los autores de crímenes. Se basa en la microbiología y epidemiología tradicionales, pero opera dentro de un marco jurídico específico. Entre las importantes razones que motivan una investigación forense cabe citar la prohibición de entrada o circulación de criminales, las actuaciones judiciales y, en el mejor de los casos, la disuasión de terceros a la hora de perpetrar actos similares.
En el terreno de la sanidad animal, la labor forense debería centrarse en reforzar los medios para detectar y notificar eventuales aumentos de la morbilidad y mortalidad de enfermedades infecciosas en los animales que pudieran ser indicativos de la diseminación encubierta de un patógeno. Cabe contraer sospechas cuando se observen patrones epidemiológicos anómalos en la población animal o el patógeno en cuestión. La capacidad de detectar y notificar enfermedades epidémicas o incluso endémicas debería constituir una prioridad internacional para prevenir pérdidas catastróficas de vidas animales e incluso humanas. Los círculos veterinarios deben conocer mejor los requisitos legales relacionados con las investigaciones forenses y estar así preparados para manejar correctamente las pruebas dentro de su propia jurisdicción.

Palabras clave
Animal – Aplicación de la ley – Bioterrorismo – Crimen biológico – Enfermedad infecciosa – Medicina forense – Microbiología forense.

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