Molecular-based surveillance of campylobacteriosis in New Zealand – from source attribution to genomic epidemiology

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Molecular-based surveillance of campylobacteriosis in New Zealand contributed to the implementation of interventions that led to a 50% reduction in notified and hospitalised cases of the country's most important zoonosis. From a pre-intervention high of 384 per 100,000 population in 2006, incidence dropped by 50% in 2008; a reduction that has been sustained since. This article illustrates many aspects of the successful use of molecular-based surveillance, including the distinction between control-focused and strategy-focused surveillance and advances in source attribution. We discuss how microbial genetic data can enhance the understanding of epidemiological explanatory and response variables and thereby enrich the epidemiological analysis. Sequence data can be fitted to evolutionary and epidemiological models to gain new insights into pathogen evolution, the nature of associations between strains of pathogens and host species, and aspects of between-host transmission. With the advent of newer sequencing technologies and the availability of rapid, high-coverage genome sequence data, such techniques may be extended and refined within the emerging discipline of genomic epidemiology. The aim of this article is to summarise the experience gained in New Zealand with molecular-based surveillance of campylobacteriosis and to discuss how this experience could be used to further advance the use of molecular tools in surveillance.

Controlling campylobacteriosis – recent successes

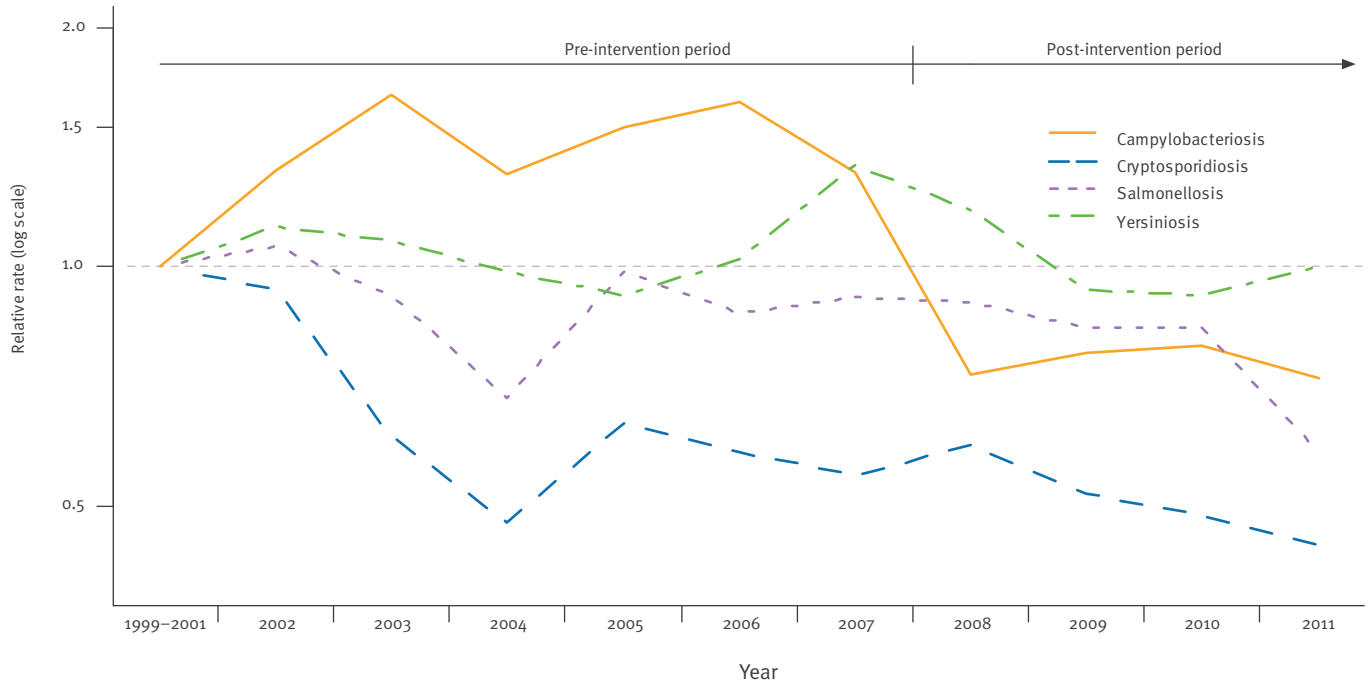
Molecular tools are being used increasingly to inform the control of enteric zoonosis worldwide [1] and to meet a wide range of public health aims and objectives [2-4]. In New Zealand, a country with a historically high rate of campylobacteriosis notifications [5,6], results from molecular-based surveillance in a sentinel site founded in 2005 – where human cases and potential sources were sampled and typed by multilocus sequence typing (MLST) simultaneously over consecutive years [7,8] – provided strong evidence that a large proportion of human cases were linked poultry meat consumption. These findings contributed to a mounting body of evidence [5,9] and stimulated the implementation of regulatory and voluntary control strategies along the poultry supply chain. They were announced in 2007 and fully implemented in 2008 (when they became mandatory) [10], resulting in a 50% reduction in disease incidence of cases in 2008 compared with the previous high level during 2002 to 2006 [10,11].

Campylobacteriosis notifications in humans were markedly above the reference value until 2008, when the incidence dropped considerably (Figure 1); a likely effect of a reduction in poultry-associated cases due to the implementation of the control strategies in the poultry supply chain [10,11]. No comparable changes in the annual incidence of other enteric notifiable diseases were observed over the same time period (2002–2011) (Figure 1). Sustained decline in campylobacteriosis case numbers has been shown to have additional health and economic benefits by, for example reducing the incidence of Guillain–Barré syndrome, an autoimmune condition associated with prior *Campylobacter* spp. infection [12].

Furthermore, in the New Zealand sentinel surveillance site, a dominant poultry-associated MLST sequence type of *C. jejuni* (ST-474) was identified that, to date, has been reported rarely from other countries. Before the implementation of the poultry interventions, ST-474 accounted for 30% of human cases in the sentinel site [14,15], but in 2010–11, it was isolated from less than 5% of cases [16]. Figure 2 shows the dramatic reduction in two major poultry-associated genotypes, ST-474 and ST-48 (Figure 2, panel A), and provides a comparison with other STs over the same time period (Figure 2, panels B and C).

Figure 1

Relative rates[a] of notification of campylobacteriosis, cryptosporidiosis, salmonellosis and yersiniosis, New Zealand, 2002–2011 compared with 1999–2001



[a] Rates were calculated using a negative binomial model, which was used to estimate the change in incidence between each year from 2002 to 2011 and the reference period of interest, 1999–2001, as previously described by Henao et al. [13]. Values above the reference line indicate increases in notification incidence and points below the line show decreases, relative to the 1999–2001 reference period.

The pre- and post-intervention periods refer to the implementation of a number of control measures in the poultry supply chain by the regulatory authority. The annual incidence of other enteric notifiable diseases (cryptosporidiosis, salmonellosis and yersiniosis) over the same time period is displayed to show that notification rates were stable for other comparable disease and that the drop in campylobacteriosis notifications was not a surveillance artefact.

Focused molecular epidemiological studies have been contributing to our understanding of the epidemiology of this widespread disease both in New Zealand and elsewhere [7,14,15,17]. For example, the association between ruminant-associated genotypes and preschool-age children (0–5 years of age) in rural areas has provided evidence for direct contact with faecal material being the foremost infection route in this high-incidence group [14].

This is of high relevance for the development and evaluation of appropriate, country-specific control strategies to decrease the human disease burden. Since the number of human cases linked to poultry has fallen in New Zealand, there has been a relative increase in importance of ruminant strains of *C. jejuni*, and ongoing work is investigating the complex epidemiology of *Campylobacter* in ruminant [18] and wildlife sources [19]. While this article describes the MLST-supported *Campylobacter* surveillance conducted at the sentinel site, other typing approaches are used to increase resolution of the molecular analysis. For example, research is currently underway to further differentiate between exposure to ruminant-associated *Campylobacter* subtypes of food and non-food origin to refine attribution estimates using antigen gene sequence typing [20], ribosomal MLST [21] and targeted genes identified by whole genome analysis [22]. In this article, we summarise the experience gained in New Zealand and discuss how this experience could be used to further advance the use of molecular tools in surveillance.

What have we learned?

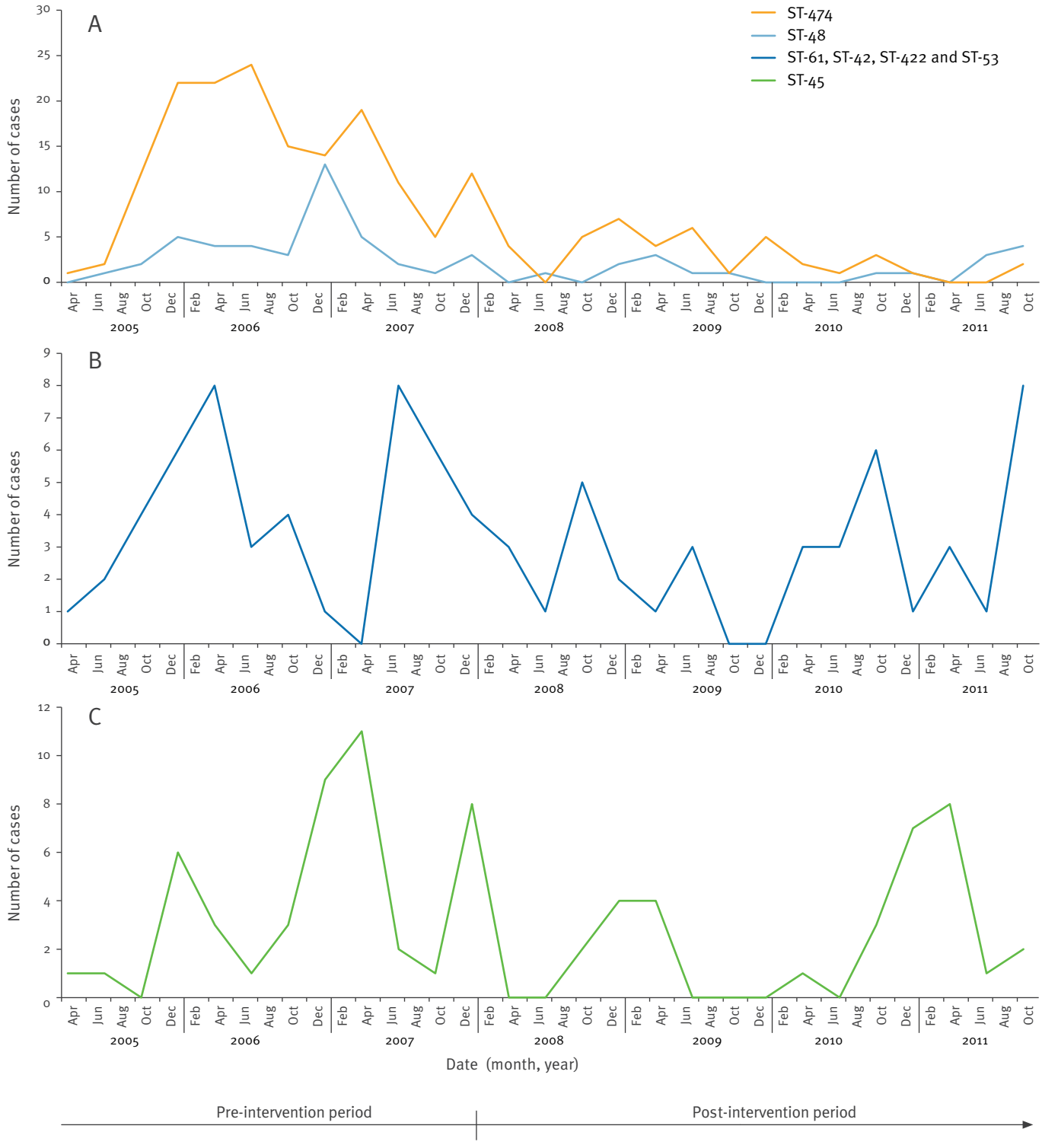
Experience from New Zealand and elsewhere has provided insight into key aspects of molecular-based surveillance. These include the following: (i) its application to both control-focused and strategy-focussed surveillance; (ii) a change in our definition of epidemiological response variables; and (iii) the emergence of genomic epidemiology.

Application of molecular tools to disease surveillance

The framework developed by Baker et al. [23], which differentiates between control-focused and strategy-focused surveillance, provides a meaningful way to

Figure 2

Human cases of campylobacteriosis caused by poultry- and ruminant-associated *Campylobacter jejuni* MLST types, as well as a ubiquitous ST in a sentinel surveillance site, New Zealand, 2005–2011



MLST: multilocus sequence typing; ST: sequence type.

Panel A shows the time series of human campylobacteriosis cases with two poultry-associated genotypes, ST-474 and ST-48 and illustrates the drop in the number of cases following interventions in the poultry production chain.

Panrel B shows the trend in human campylobacteriosis cases with ruminant-associated genotypes ST-61, ST-42, ST-422 and ST-53.

Panel C shows the time series of human campylobacteriosis cases with the ubiquitous ST-45.

categorise molecular approaches to disease surveillance. Approaches that are suitable for control-focused surveillance, such as those used in an outbreak setting, are potentially of lesser value for strategy-focused surveillance, where the aim is often to monitor long-term changes in epidemiology [24,25], and vice versa.

The purpose of control-focused surveillance is 'to identify each occurrence of a particular disease, hazard, or other health-related event that requires a specific response, and to support the delivery of an effective intervention' [23]. Such surveillance requires methods that have a high degree of timeliness, sensitivity and security (i.e. that can be maintained on an ongoing basis) [23]. The molecular typing tools and associated modelling approaches required for this objective need to be capable of identifying genotypes that indicate a common source of disease or highlight a particular transmission pathway. Often, but not always, this is achieved through highly discriminatory typing tools.

Using a recently developed model-based tool for identifying clusters of campylobacteriosis cases related in space and time [26], eight cases in a small area of New Zealand's North Island were identified as having a high probability (>0.8) of being part of an anomalous cluster (i.e. they were more spatially and temporally localised than would be expected given the average temporal and spatial patterns). Inspection of the epidemiological information linked to each case revealed that they were reported within a single two-week period and typing data showed that they were all the same MLST sequence type (ST-520). When compared with a larger database of over 3,000 sequence types isolated from humans, animals and food in New Zealand it was shown that this type was associated with ruminants in New Zealand, but was a relatively uncommon cause of human infection. This finding triggered a more detailed investigation into the cases, requiring further contact with some affected individuals, which revealed that all cases had consumed unpasteurised (raw) milk – a relatively rare risk factor – and that 7/8 cases reported purchasing the milk from the same source farm. This combination of epidemiological information and typing data lead to a local response and also informed the ongoing debate on the national policy concerning the sale of raw milk.

The purpose of strategy-focused surveillance is 'to provide information to support prevention strategies to reduce population health risk, such as describing the epidemiology of the annual influenza season and the characteristics of the seasonal influenza viruses' [23]. Such surveillance requires methods that have a high degree of representativeness, completeness (referring to the data recorded with each event) and validity [23]. Different molecular and modelling approaches are required in this instance, with the optimal tools providing information on the long-term epidemiology of a pathogen rather than short-term changes. An example is the recent emergence of new approaches to source attribution using molecular subtyping, which has been used successfully in several countries to understand the relative contribution of different sources to the burden of human campylobacteriosis and salmonellosis [27-30]. Source attribution models based on microbial subtyping were initially developed in Denmark as a tool for salmonellosis risk management [31]; they provide estimates of the number of human cases originating from different sources or reservoirs based on a comparison of genotypes [31,32].

In New Zealand, attribution models were adapted to data from the MLST surveillance site. Two models were used, a population genetics-based attribution model [32] and the microbial subtyping-based model by Hald et al. [31], to quantify the contribution of selected sources to the human disease burden. These studies revealed that between 2005 and 2008, poultry was the leading source of human campylobacteriosis, causing an estimated 58–76%, of notified cases [8]. Contributions by individual poultry suppliers showed wide variation and supplier specific strains were detected [15]. The use of these models to monitor changes over time and to assess the effectiveness of interventions is ongoing [10,11].

Re-defining response and explanatory variables using molecular tools – a new epidemiological approach to inform surveillance?

A common starting point of epidemiology is seeking non-random associations between response variables and potential explanatory variables. Regression modelling, for example, may be used to identify statistically significant predictors of increased risk of adverse health effects [33]. However, the use of such traditional methods for quantifying the contribution of different sources of campylobacteriosis to the disease burden in New Zealand (notably case–control studies [9,34], which identified poultry as the major source of human infection) had not provided sufficient compelling evidence for decision-makers to invest in controlling the poultry source. The epidemiology of campylobacteriosis is challenging: as a multi-host pathogen, infection with *C. jejuni* is associated with a large number of risk factors [35] and human cases arising from exposure to different sources may have very different risk factors, some of which may even be protective for some sources and increase the risk for others.

Using molecular tools, pathogen genetics and evolution can be incorporated into epidemiological modelling to make inferences about disease or transmission risks rather than simply relying on the association of response and explanatory variables. Such tools can be used to refine outcome variables, for example by using case–case comparison of poultry- and ruminant-associated cases of campylobacteriosis to identify more subtle associations [14,17] or to investigate the cause of a disease outbreak [24]. However, greater epidemiological gains are likely to be made when models combine pathogen evolution and transmission in

an integrated way [36,37]. This may be best achieved by modelling a relatively low number of isolates with high-coverage sequence data, such as increasingly available full genome data [38] or a larger number of isolates with low coverage such as a 7-locus MLST scheme. The additional information provided even by routinely applied molecular tools such as pulsed-field gel electrophoresis (PFGE) adds to our understanding of epidemiological variables. For example, the level of similarity and relatedness of restriction-enzyme profiles in the analysis of a food-borne outbreak can be directly used to refine epidemiological investigations. It is the synergy between the epidemiological and typing information that makes molecular tools so powerful and novel modelling approaches are constantly being developed to advance research at this interface [39,40].

Into the future: genomic epidemiology

New modelling approaches are being adopted to utilise the abundance of molecular data available [24,39,40]. Bell et al. [41] argue that the enormous volumes of data that can be provided by new technology provides many challenges for data management and analysis, and that we have entered a new area of data-intensive science that requires specialised skills and analytical tools. This argument holds true in the area of molecular epidemiology: next generation high throughput sequencing has vastly increased the availability of pathogen genome sequence data [38] and as the costs decrease, these tools will be more frequently incorporated into epidemiological studies and surveillance. By fitting statistical genetics and epidemiological models to sequence data, and combining these within a single framework [42], new insights can be gained into pathogen evolution, the nature of associations between strains of pathogens and host species, the timing of emergence, origin and geographical spread of pathogens, and aspects of between-host transmission [43]. Furthermore, advances in statistical methods for modelling evolutionary ancestry are resulting in better reconstructions of pathogen genealogies and improved estimates of evolutionary parameters. Although complex in nature, these models can be extremely valuable – for example, they can be used to enable the contribution of different sources and transmission pathways to the human disease burden to be determined [32].

In New Zealand, whole genome sequencing is being used to understand the evolution of epidemiologically important strains of *C. jejuni* and identify potential markers for host association [44]. This may help to improve the discrimination of sources of human infection, such as between cattle and sheep, and result in more precise source attribution estimates. Similarly, full genome sequence data from multiple *Campylobacter* isolates and *Escherichia coli* O157 are being combined with phenotypic microarray data to improve the understanding of the relationship between phenotype and genotype. The identification of genetic markers for stress resistance, such as pH, temperature, oxidative stress, and freeze-thaw [45], could help to determine which sources and transmission pathways strains isolated from humans have been acquired from, further refining attribution studies and strategy-focused surveillance.

By furthering our understanding of host associations with particular strains of pathogens, and the relative rates of transmission between animals and humans, the melding of statistical genetics and epidemiology with partial and full genome sequence data will further inform and refine control strategies for enteric pathogens in New Zealand and elsewhere.

Conclusion

New Zealand provides a distinctive island ecosystem in which to study infectious diseases [46]. The relative isolation and management of farmed livestock has contributed to the epidemiology and population structure of microbial pathogens. For example, the country's poultry industry is structured in a way that is different to most countries, with no importation of untreated poultry products and freedom from several important poultry diseases such as Newcastle disease and *Salmonella enterica* serovar Enteritidis PT4. Furthermore, the production of poultry meat is highly integrated, with three companies supplying about 90% of all chicken meat [15]. In addition a risk management strategy developed by the regulator supports a strong collaboration with researchers and science-based decision-making [47]. While the situation in other countries is likely to be more complex, for example through the presence of federal regulations or the risks associated with poultry importations, lessons learned from New Zealand can be applied elsewhere.

The New Zealand approach, which includes the first large scale implementation of effective regulatory *Campylobacter* control measures in broilers, is of high relevance internationally, including Europe. Findings have been incorporated in scientific opinions of the European Food Safety Authority. In 2008, it was acknowledged that the MLST approach to source attribution developed in New Zealand may be the way forward [48] and the approach is being used in several European countries, including the Netherlands and Scotland [2,49]. The New Zealand experience was also included in an assessment of the extent to which meat derived from broilers contributes to human campylobacteriosis at the European Union level [50].

The molecular tools deployed in epidemiological and evolutionary analyses clearly need to be fit-for-purpose. Ideally, during their development phase, measures of their utility in specific settings, such as discriminatory power and the strength of association between genotype and host, should be considered and attempts made to optimise their performance for the outcome in mind. In the case of 7-locus MLST, for example, retrospective analyses have shown this to be a valuable approach for certain types of surveillance,

including reservoir attribution, but the method was not designed for this purpose and an alternative approach based on a different set of gene targets may perform better and be more cost-effective. Equally important are rigorous sampling size considerations and guidance on the number of samples from different sources to acquire a desired level of precision – for example, in source attribution estimates. Further work will be necessary to develop expert agreement and sound working principles on these matters.

The field of molecular epidemiology is continually evolving and its role in advancing our ability to understand and control infectious diseases will also keep increasing. Its interdisciplinary nature will provide key support to One Health approaches to disease control, by supplementing medical and veterinary expertise with an in-depth understanding of the molecular biology of pathogens. As genotyping approaches and analytical models continue to evolve, an understanding of the complex interface of both disciplines becomes a crucial element of molecular-based disease surveillance. In New Zealand, we have learned that close collaboration between laboratories and epidemiologists is extremely important for the success of molecular-based surveillance: in our example, this started when the sentinel surveillance site was first set up. In a small and geographically isolated country, such early collaboration is likely to be more easily achieved; nevertheless, the general principle still applies and could add value to molecular surveillance in other countries.

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