Ministry for Primary Industries
Manatū Ahu Matua

Source Attribution Studies for Campylobacteriosis in New Zealand

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A review of work carried out to determine the source of human campylobacteriosis

INTRODUCTION

Attribution is the process of determining how much of a given disease is due to particular sources or pathways. This information is important in working out how to intervene to prevent illness, and to assess the effectiveness of interventions. Data from intensified work on source attribution for campylobacteriosis that commenced in 2005 have enabled the implementation and subsequent modifications of an MPI *Campylobacter* Risk Management Strategy, leading to a decline in human campylobacteriosis cases from 2007 onwards.

Given the pace of change in the science of source attribution and in the patterns of *Campylobacter* infection in New Zealand, a project was commissioned to take stock of this field of work. The objectives were to review and summarise progress with *Campylobacter* source attribution, to examine possible additional approaches to source attribution, to determine what further information on *Campylobacter* is required, to assess whether the current sentinel surveillance site framework remains valid, and to comment on the scope for integrating findings from source attribution with national surveillance and outbreak investigation tools.

CAMPYLOBACTERIOSIS SOURCE ATTRIBUTION IN NEW ZEALAND

Five general approaches to campylobacteriosis source attribution have been used: epidemiological, review of reported outbreaks, comparative risk and exposure assessment, expert elicitation and microbial subtyping.

Epidemiological

Standard epidemiological approaches elicit sources of infection from information on exposures reported by persons who have developed illness. A national epidemiological study found that more than 50% of human campylobacteriosis was attributable to a composite of ‘poultry-related’ variables. Such analyses are weakened by reliance on the recollections of study participants to provide exposure information.

Disease outbreaks

Disease outbreaks are situations where illness in multiple persons may be linked to the same source. Reports of campylobacteriosis outbreaks have been studied collectively. Illness was considered to be predominantly due to contaminated food, although very few reports supported this assumption with strong evidence.

Comparative risk and exposure assessment

Comparative risk and exposure assessment approaches draw on data on concentration of pathogens at different stages in pathways to human exposure. A study using this approach concluded that poultry, and to a lesser extent red meat, were likely to be the most important exposures for campylobacteriosis, followed by occupational contact with livestock.

Expert elicitation

A further method used is gathering expert opinion in a structured manner to develop consensus estimates of the contribution of different sources to specific foodborne illnesses. This is termed 'expert elicitation'. In 2005, this approach estimated that 57.5% (range 37.1% - 69.6%) of campylobacteriosis cases were foodborne, and of these poultry was estimated to contribute 52.9%.

Microbial subtyping

A recent innovation in source attribution makes use of knowledge that different subtypes of specific pathogens can be associated with particular animal reservoirs (termed 'amplifying hosts'). By combining molecular microbiology and mathematical modelling, bacteria collected from different reservoirs, intermediary vehicles and from sick persons are classified into subpopulations based on their genetic signatures, and can then be associated with source reservoirs using mathematic techniques accounting for variation in the genetic signatures over time.

Use of this microbial subtyping approach has focused on the Manawatu region, established as a site for ongoing surveillance from 2005 onward. Output from the programme in 2007 indicated an attribution of campylobacteriosis from poultry ranging from 55.1% - 71.4% depending on model used. Data collected consistently over time has provided a basis for evaluating the impact of poultry-focused interventions: before implementation of interventions, over 70% of human cases were attributable to poultry, and in follow-up years 2008-2010 this estimate had declined to less than 50%.

Further work using the sentinel site data has modelled changes in reservoir attribution over a finer time scale, using a 'dynamic attribution' approach. Findings have emphasised the overall reduction in poultry-associated cases after interventions introduced in 2007, with the further observation that the summer peak in cases, largely attributable to poultry, has been maintained, albeit in a diminished form; cases attributable to ruminant reservoirs have continued unabated.

SOURCE ATTRIBUTION DATA GAPS

Principal areas identified where the current knowledge base on campylobacteriosis source attribution could be improved were listed as follows.

- Differentiation in the campylobacteriosis attribution among ruminant sources has been hampered by imperfect ability to distinguish between cattle and sheep subtypes.
- Attribution using microbial subtyping has focused on source reservoirs, but data on attribution from different pathways (e.g., exposure to ruminant strains in food *versus* direct contact) may be of additional benefit for policy-makers.

- Some potential reservoirs for *Campylobacter*, such as deer, goats and pet animals, have not been included in sampling and have therefore not been included in attribution models.
- Data on shifts in the molecular signatures of *Campylobacter* from reservoirs other than poultry have not been collected, and may be necessary.
- Data on human exposures in the Manawatu sentinel site have not been confirmed to be representative of other regions in New Zealand.

POTENTIAL IMPROVEMENTS TO THE CURRENT SOURCE ATTRIBUTION APPROACH

Recent developments and future options were identified for possible improvements to the current approach to campylobacteriosis source attribution

- Comparison of characteristics and findings from the Manawatu sentinel site with other regions in New Zealand to determine the validity of continuing to focus on Manawatu.
- Integration of epidemiological techniques with microbial subtyping data would provide a scientific basis for delineating source attribution from different combinations of source and pathway to human illness.
- Software developed to aid detection of disease clusters ('epiclustR') could be integrated with reservoir attribution data to support the operational utility of campylobacteriosis surveillance data.
- Study of other subtyping techniques utilising sections of the *Campylobacter* genome ignored by current tools may provide greater power to discriminate between ruminant strains, between different transmission pathways, and to attribute isolates that cannot be currently associated with reservoirs.

Conclusion

This review shows that New Zealand studies on the attribution of human campylobacteriosis to sources have provided a strong foundation for disease control actions. Based on these studies, interventions to reduce the proportion of human illness associated with contaminated poultry have been developed, and their efficacy evaluated. Interventions have changed the pattern of attribution of campylobacteriosis, so that a smaller proportion of illness can be attributed to poultry, and a relatively larger proportion can be attributed to other sources, such as strains originating from ruminant reservoirs.

The authors of the review indicate that further work on source attribution methods will be necessary to improve understanding of the causes of human campylobacteriosis in New Zealand. Ministry for Primary Industries is pursuing directions identified in the review:

- A study is underway to validate the appropriateness of the Manawatu sentinel surveillance site, by comparing characteristics of the Manawatu population and *Campylobacter* strains with those of a South Island region.

- The dynamic attribution work for campylobacteriosis in the Manawatu region will continue through 2013 and 2014.
- Work to scope the feasibility of a study that combines epidemiological techniques with microbial subtyping and model-based strain attribution has commenced. The intention of such a study would be to characterise pathways from *Campylobacter* sources to human infection.
- EpiclustR has been piloted by Environmental Services and Research Ltd (ESR). As responsibility for surveillance of human campylobacteriosis rests with the health sector, MPI has offered the software to the Ministry of Health for its integration into the national human disease surveillance system.
- Further steps to controlling campylobacteriosis will require a multisectorial approach, as agencies with responsibility for the environment, primary industries and health all have interests, at both commercial and regulatory levels.

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Report: MAF Agreement 11777, Schedule 1A Source Attribution Studies for Campylobacteriosis in New Zealand

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July 2012

prepared for
the Ministry for Primary Industries

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Report: MAF Agreement 11777, Schedule 1A

Source Attribution Studies for Campylobacteriosis in New Zealand.

April 2012

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1 Summary

The number of notified and hospitalised cases of campylobacteriosis in New Zealand increased steadily to a peak in 2006, when over 16,000 cases were notified. After a major intervention in the poultry industry, the rates were reduced by 50% in 2007/8 and this has been sustained to the present day. Although the incidence has been substantially reduced, campylobacteriosis remains the most notified enteric infectious disease in New Zealand, albeit presenting a very different epidemiological picture; the number of cases attributable to poultry has declined rapidly, and per capita rates in rural areas now exceed those in urban areas.

Prior to 2006, contamination of the poultry supply was implicated as the most important contributor to the burden of human cases, but despite the evidence provided by earlier epidemiological studies and exposure assessment modelling, efforts to implement a targeted control strategy were hampered by continuing uncertainty about the primary sources of infection. In 2005 a sentinel site was established in the Manawatu and a new approach to identifying the predominant animal reservoirs and pathways of infection was implemented. This approach to 'source attribution' combined new technology in microbial subtyping with advances in mathematical modelling to provide estimates of the relative contributions of poultry, ruminants and other animal hosts, to the burden of disease. Model outputs provided strong evidence to confirm the importance of the poultry supply as the predominant source of campylobacteriosis, and this was crucial for the development and implementation of the *Campylobacter* in Poultry Risk Management Strategy.

This report summarises the changing epidemiology of campylobacteriosis in New Zealand between 2005 and 2012, and the work done to inform the national control policy. Source attribution is an emerging discipline, and many of the techniques deployed in New Zealand have been at the forefront of new developments in the field. The report provides a general framework for source attribution, encompassing reservoir attribution, pathway attribution, exposure assessment and risk factor determination, and attempts to clarify some of the terminology currently in use. In addition, knowledge gaps are identified and recommendations are made for developing the science of source attribution, and for optimising surveillance programmes in New Zealand.

2 Introduction

The following section provides an overview of campylobacteriosis in New Zealand and describes some of the work undertaken to inform decision making.

2.1 Historical perspective

Campylobacteriosis was first included in the list of notifiable diseases in New Zealand in 1980. Subsequently, the number of cases increased markedly to a peak of around 16,000 cases in 2006, and this growing 'epidemic' was drawing attention from health professionals and food safety experts in New Zealand and overseas, raising the fundamental question: what was the source of human infection? Earlier reports of the contribution of different animal reservoirs, pathways and exposures to campylobacteriosis in New Zealand focused on the use of case-control studies [12, 25], analyses of case reports and outbreaks [54] and exposure assessments [28, 33]. These studies unanimously concluded that poultry were likely to be the most important source of human infection. However in 2005/6, despite cumulating evidence over the previous decade, it appeared that sufficient doubt and uncertainty remained[1], and this hampered efforts to implement a targeted control strategy. For this reason a new approach was adopted, combining recently developed techniques in molecular biology and strain typing (genotyping) of bacteria [10], with a new suite of source attribution models [24, 53]. In 2006, in an article that received considerable media attention, a strong recommendation for urgent action was made[3].

[1]For example: The report by McBride et al in 2005 [33] made a tentative conclusion: '... the purpose of the model was to focus on the relative importance of four potentially important exposures to *Campylobacter* infection: food (poultry and red meat, including cross contamination); drinking water (three grades of good, poor and untreated); freshwater swimming; and occupational contact (livestock). We have tentatively concluded that of these, cross-contamination during preparation or storage of poultry, and to a lesser extent red meat, were the most important exposures'. In the report by Lake in 2006 [28] a strong recommendation was made to target the poultry supply as a means of controlling campylobacteriosis, but in the summary of that report it was concluded that 'The transmission of Campylobacter in New Zealand is likely to be complex, with a number of risk factors operating at once. It is possible that no single factor is sufficiently important to provide an opportunity to significantly affect the rate of illness'.

From 2006 onwards, source attribution based on *Campylobacter* genotyping contributed towards the growing body of information on the animal reservoirs and pathways for human infection in New Zealand, and added new data to further inform decision making. Initial genotyping data proved valuable for the implementation of the *Campylobacter* in Poultry Risk Management Strategy in 2006 [48] and the first model outputs provided in July 2007 helped inform subsequent modifications to the Strategy which lead to the decline in cases, predominantly in urban areas, from 2007 onwards (Figure 1). Subsequently reservoir attribution modelling provided quantitative information on the impact of the Management Strategy by summarising the change in the contribution of reservoirs to human cases pre- to post-intervention and through the development and application of dynamic modelling [17, 14, 16, 34]. Insight gained from genotyping, and the probabilistic allocation of 'strains' to animal reservoirs, has furthered our understanding of the epidemiology of campylobacteriosis in New Zealand [34, 35, 38, 40].

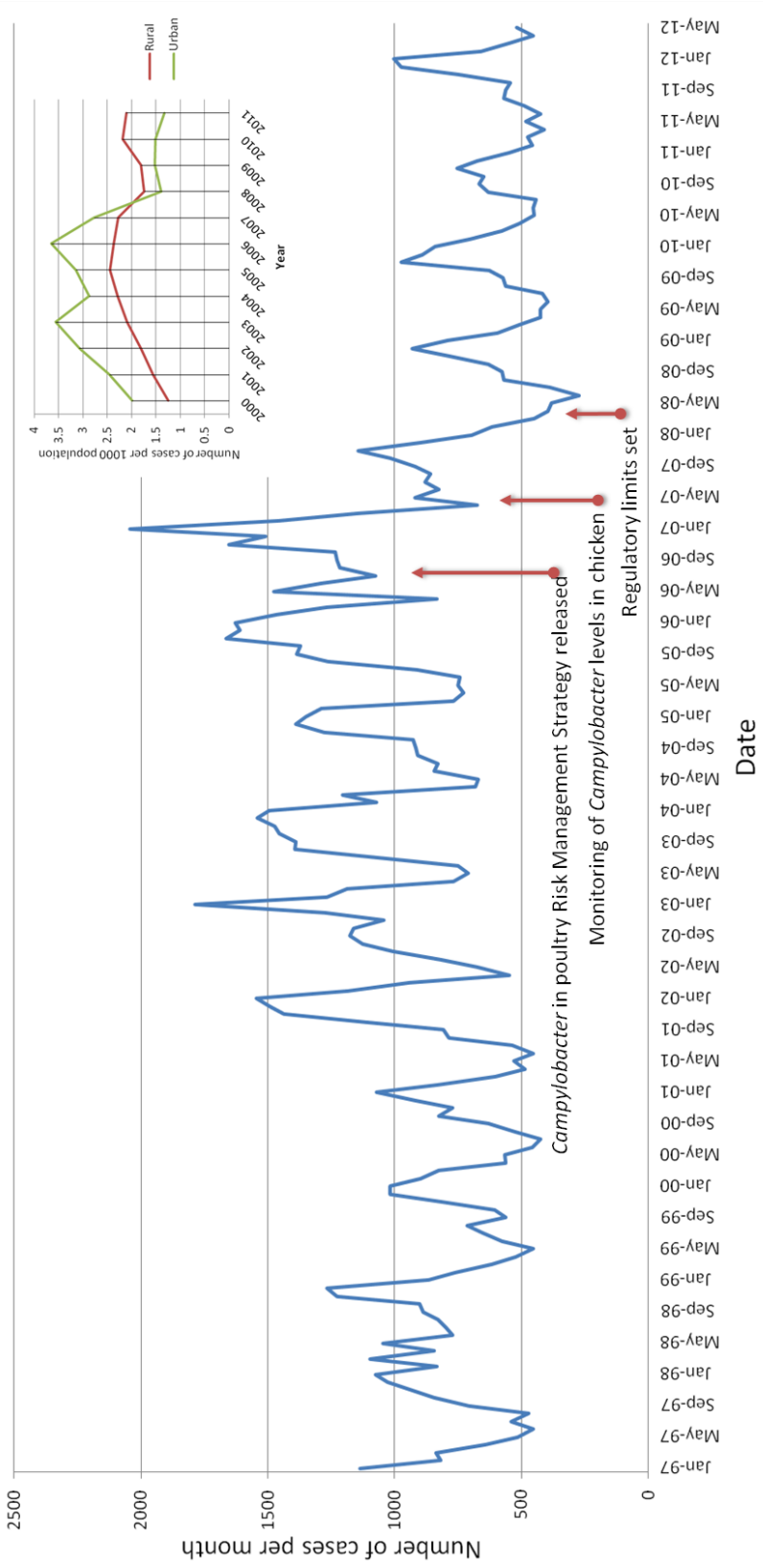


Figure 1: The number of notified campylobacteriosis cases in New Zealand since January 1997 and the dates of regulatory changes. The inset shows the annual rates of notified cases per 1000 people in rural and urban areas from 2000 (denominators were derived from National census data and projections).

3 What approaches have been used in New Zealand and overseas, and what additional approaches are currently available?

Source attribution, as applied to campylobacteriosis in New Zealand, has been carried out using an array of approaches and techniques including: the calculation of population attributable risk from case-control studies [12]; the use of risk and exposure assessment simulation modelling [33]; modelling genotyping and epidemiological information using a suite of models applied to data collected in the Manawatu sentinel surveillance site [17, 16, 14, 15, 20, 19, 18, 39, 40, 41], and an integrated approach to pathway modelling [27]. These New Zealand studies are described in more detail in section 4. In this section we provide a general framework for approaches to inform decision making for the control of campylobacteriosis (and other foodborne pathogens), and outline some advantages and disadvantages of the different techniques available for source attribution.

3.1 A general framework

Figure 2 is an attempt to provide a general framework for understanding how the different data sources and models can be used to inform policy for the control of zoonotic diseases such as campylobacteriosis. It is difficult to classify these approaches into clearly defined categories, due to the overlap in both process and outcomes, so Figure 2 is by necessity an oversimplification of a complex system. Others have used different ways of categorising approaches to attributing human foodborne disease to specific sources, reflecting that this is a rapidly developing area in the field of food safety: Pires at al. [6] categorises attribution methods into 'epidemiologic' and 'microbiologic' and provide a table of approaches to food attribution illustrated with key references and a summary of advantages and disadvantages. Both Pires et al. [6] and Mangen et al. [30] highlight the importance of the 'point of attribution' defined as the location in the food chain addressed by a particular approach. This is also addressed in the framework described below.

It is recommended that the term 'source attribution' be used as a collective term to describe the general approach and techniques outlined in Figure 2, comprising: 'reservoir attribution', 'pathway attribution', 'comparative exposure/risk assessment' and 'risk factor modelling'.

Reservoirs: In Figure 2 we define these as animal reservoirs or 'amplifying hosts'. These can be grouped or subdivided into epidemiologically meaningful categories depending on the question being addressed. For example cattle and sheep may be grouped into 'ruminants' if it is not important to distinguish between the two, or if it's not possible to determine their independent contributions. Alternatively, chicken may be subdivided into differing poultry suppliers if it is possible and important to determine their independent contributions. Reservoir attribution models, provide estimates of the relative contribution of the amplifying hosts to the burden of human disease for the purpose of targeting interventions. In reservoir attribution modelling it may also be convenient to use a non-animal source to capture the contribution from an unmeasured host or group of hosts - such as the use of environmental water to capture the contribution from wildlife hosts.

Pathways: The next level in the chain of events leading to human infection is the primary pathway, which may be considered the route (broadly defined) by which *Campylobacter* shed by reservoir/amplifying hosts can reach and infect humans. Again these can be grouped or subdivided according to the question being addressed, but at this level the most meaningful categories for informing policy - such as for prioritising resources or identifying the authority responsible for control - may be: food, environment (including waater) and direct contact. For example, this may determine whether the primary responsibility for control lies with the agency responsible for food safety or environmental pollution. A range of techniques have been used to estimate the contribution of different pathways to human infection. These may deploy a 'top-down' approach, which can be achieved by subdividing the contribution of amplifying hosts into food and environmental pathways as described in [17], or a 'bottom-up' approach by combining the contributions from different exposures and risk factors using, for example, outbreak [28, 54] and surveillance data [27], risk assessment modelling [33] or population

attributable risks calculated from case-control studies [12].

Exposures: The primary pathways can be subdivided into a number of secondary exposures. For example the food pathways can be divided into meat and milk, and environmental contamination of surface water may impact drinking and recreational water. The relative contribution of the exposures may be determined by the use of exposure or risk assessment models, such as that described in [33]. Exposure models describe the level of the hazard to which humans are exposed, such as the number of bacteria consumed per litre of drinking water. Risk models extend this by using the level of exposure determined by hazard modelling, combined with a dose-response model, to estimate the number of human cases. There are many other examples of the application of quantitative microbial risk assessment models that use Monte Carlo simulation of different exposure pathways to determine both the relative contribution of different exposures and the impact of possible interventions on human health, and some are referred to in the review article [6]. As mentioned above, the estimates of the relative exposures can be grouped to provide estimates of the relative contribution of different pathways, such as food, environment and direct contact.

Risk factors: In population based epidemiological studies, such as case-control studies, we measure variables that describe specific determinants of risk, known as risk factors, attempt to estimate the magnitude of risk associated with these factors, and conduct statistical tests of association. In our oversimplified diagram in Figure 2, we represent these as a further subdivision of pathways and exposures. For example cattle (source) may contaminate the food chain (pathway) resulting in hazard in the milk supply (exposure) which manifests itself as an increased risk associated with the consumption of raw, unpasteurised milk (risk factor). Note this cascade is analogous to the 'point of attribution' defined in earlier reviews [30, 43]. The relative contribution of these risk factors may then be calculated as population attributable risks expressed as percentages (PAR%) or fractions (PAF), and these may be combined to provide estimates of the relative contributions of exposures, pathways, and even reservoirs as described in section 4.1.

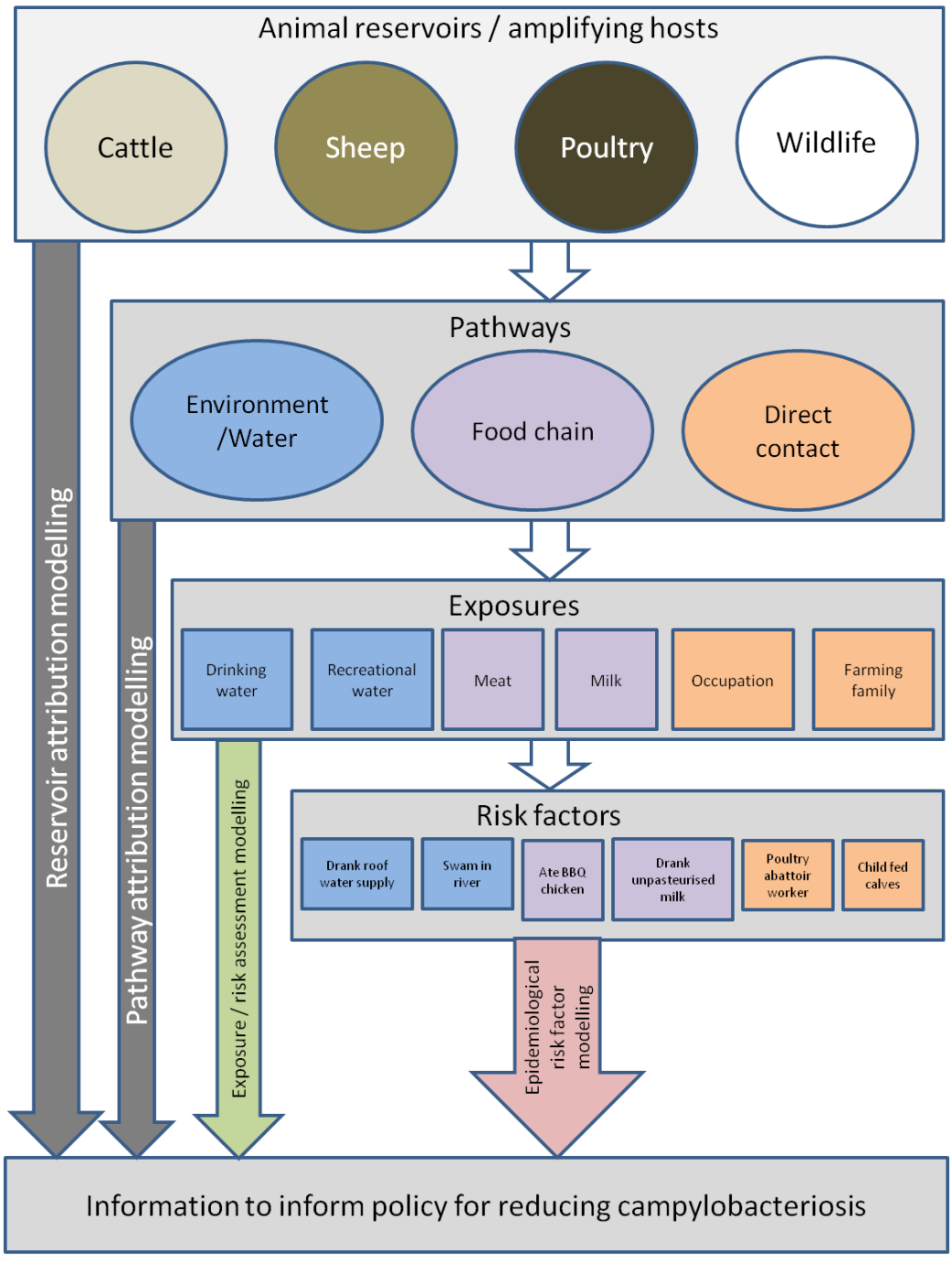


Figure 2: Source attribution: a framework. Diagram showing the sources of information and modelling approaches that can be used to inform decision making for the control of zoonotic diseases such as campylobacteriosis. Note the terms: reservoir, pathway, exposure and risk factor are used here for illustrative purposes to show how various levels of data disaggregation and refinement can be incorporated into different models for informing decision making.

3.2 Different approaches to source attribution and their advantages and disadvantages

Each of the current techniques has its own advantages and disadvantages and some of these are outlined below using the framework described in the 'Overview of methods for source attribution for human illness from food borne microbiological hazards' document produced by the European Food Safety Authority [2]. (N.B. This is not intended to be an exhaustive list either of the methods or of their pros and cons, and can be considered alongside other reviews providing similar summaries [30, 6, 43, 2]).

3.2.1 Epidemiological observations and studies

Using classical epidemiological studies to calculate population attributable risk/fraction. The most common type of epidemiological study used to calculate population attributable risks is the case-control study [11, 12]. This approach compares the characteristics of cases of disease with those of a sample of the population that are not diseased and are representative of the population from which the cases arose (the controls). The variables measured are the risk factors that are of primary consideration, or they may be potential confounding variables that need to be controlled for in the analysis. The analysis leads usually to the calculation of odds ratios or relative risks, adjusted for confounding, and these are combined with information on the prevalence of the risk factor in the population under investigation to calculate the proportion of cases that could be attributed to the risk factor.

These studies can be done rapidly, and provide valuable insight into the role of risk factors, and information at this level can be aggregated to provide estimates of the relative contribution of different exposures, pathways and potentially sources. They have a number of disadvantages: they are prone to reporting bias (particularly the inaccurate recall of risk factors by cases and controls) and, importantly for diseases such as campylobacteriosis, they are inefficient when the population is exposed at a very high rate to the pathogen. In this situation, the onset of a protective immune response in a high proportion of the population means that a large proportion of undiseased controls are likely to be immune to disease, and yet have a similar pattern of

exposure to the cases. This will tend to bias estimates of odds ratios towards the null value (1.0) and reduce the power of the study to detect significant risk factors.

Other epidemiological approaches. Other population-based studies, in addition to those based on a case-control design, have used surveillance data and other sources of information to inform reservoir and pathway attribution these are outlined in review articles [6, 30, 43]. They include studies of surveillance data of both sporadic cases and outbreaks (discussed below), cohort studies and the impact of serendipitous interventions (such as the 1999 dioxin crisis in Belgium). All of these approaches can be a valuable way of gaining insight into the disease using existing data, and are potentially a cost-effective and efficient. However, the lack or poor quality of available data and the time taken to produce what are often highly retrospective estimates, can limit the utility of these methods.

3.2.2 Compilation of outbreak data

Although considered a separate category in other reviews (e.g. [2]), the compilation and analysis of outbreak data is in essence another use of epidemiological data. Outbreaks are commonly defined as two or more epidemiologically linked cases, often identified as part of a national surveillance programme or as a result of local public health related activities. The evidence linking outbreak cases will include epidemiological information on the spatial and temporal pattern of cases (i.e. they are often clustered in space and time) and a shared exposure or risk factor - the latter is particularly important for reservoir and pathway attribution. Information provided by the investigation of outbreaks will often identify very specific events arising from contamination of food or the environment, such as undercooked poultry served to nursing home residents, or a batch of contaminated unpasteurised milk. It may be difficult to aggregate information at varying levels of resolution and to identify the precise source of contamination, and this could lead to misclassification due to, for example, complex food exposure comprising multiple ingredients. Large and diffuse outbreaks can also be detected, and these may be due to the widespread dissemination of contaminated food products,

including imported food. Although the vast majority of campylobacteriosis cases are considered to be sporadic in nature (i.e. not epidemiologically linked), outbreaks do occur frequently and can provide valuable information to be considered alongside other approaches, however this relies on the assumption that outbreak and sporadic cases share a similar epidemiology. Estimates derived from outbreak data may be combined with estimates of underreporting to determine the total burden outbreak-associated disease attributable to each pathway and exposure [1].

3.2.3 Comparative risk and exposure assessment modelling

This approach is termed 'comparative exposure assessment' in the review by Pires et al [43], and uses Monte Carlo simulation modelling to determine the relative importance of different exposures, by simulating the prevalence and numbers of pathogens along transmission routes to the point of human exposure [13, 33]. In the context of attribution, these are simplified versions of food chain quantitative microbial risk assessments (QMRAs) that are usually detailed models of the propagation of pathogens along specific food pathways (e.g. Modular Process Risk Models [42]). This approach typically uses information from a number of published studies, expert opinion and small scale experiments to determine the relative exposure for each pathway. When combined with a dose-response model, the exposure assessment becomes a risk assesment, estimating the relative or absolute number of cases arising from each source. This method has the advantages that specific exposures and pathways are considered, and the impact of potential mitigation measures can be assessed. Disadvantages include the lack of good data to inform the model, and the uncertainty associated with dose-response, both of which result in a lack of precision in risk estimates.

3.2.4 Expert elicitation

The formal gathering of structured expert opinion has been used to inform estimates of source attribution, both as a stand alone qualitative exercise and as a method for estimating parameters for other model-based approaches. There have been a number of recent developments in the field of expert

elicitation, including the introduction of quantitative methods. Techniques for reducing bias in expert estimates have been developed for other risk assessments, and are likely to be deployed for source attribution in the future. For a more thorough review of these approaches see [2].

3.2.5 Source attribution modelling based on microbial subtyping

This approach is described in detail in section 4. The advantages of this approach include the ability of these methods to determine the primary animal reservoirs/amplifying hosts, and the contribution of subsets of these to the burden of human disease. Recent advances in reservoir attribution models specifically designed for microbial subtyping data has greatly improved the quality of inference that can be drawn using this approach [39, 53]. The data generated by molecular subtyping is also of considerable value for understanding the epidemiology of the disease, and therefore refining our comprehension of the relative contribution of reservoirs, pathways, exposures and risk factors. It can provide a means of continually updating and refining our understanding of changes in reservoir attribution and epidemiology over time, which is of particular value when assessing the impact of interventions [48]. This requires an integrated approach as outlined below in section 8, and the development and further refinement of models. The disadvantages are the costs of sampling, isolation and genotyping of isolates which, if not already integrated within existing surveillance programmes may be prohibitive, and the lack of genetic discrimination between the *Campylobacter* populations found in hosts such as cattle and sheep.

4 Campylobacteriosis source attribution in New Zealand - what was done, and what has been achieved?

This section summarises the work done in New Zealand that has contributed to our understanding of the reservoirs and principal routes or pathways of infection for human campylobacteriosis. In this section we use the broad

categories described in [2], although it is recognised that there is still some debate about the use of the terms source, reservoir and pathway attribution, as outlined in the section 3.

4.1 Epidemiological observations and studies

Case control studies have been used in New Zealand to determine the most important risk factors for human clinical disease [25, 12, 4] and estimates of population attributable risk have been used for source attribution[12]. These have been summarised in other reports [28]. All case control studies identified poultry as a significant risk factor, although it is interesting to note the observation in the first study [25] that 81% of both cases and controls reported the consumption of poultry, highlighting the potential problem with conducting case-control studies of common infectious diseases. In [12] the PAR% was 7% for the consumption of raw milk compared to 13% for the consumption of chicken at a restaurant. In the same study the 'chicken-related' variables were combined to provide an esimated PAR% of >50% attributable to chicken.

Epidemiological data, such as surveillance data, have also been used in combination with other data and modelling approaches, including comparative exposure modelling, to inform pathway attribution [27]. This study was part of a larger programme of work funded by the Cross Departmental Research Pool summarised in a technical report [32]. Comparative exposure models were developed for food (predicting the number of human infections based on exposures to *Campylobacter* from consumption of chicken, beef, sheep meat, pork, offal, turkeys and ducks), drinking water, and recreational water (swimming in the natural environment). Finally a 'pathway model' brought together attribution using an analysis of notified campylobacteriosis cases, reservoir attribution modelling based on microbial subtyping in the Manawatu, and the exposure models. The analysis compares both pre- and post poultry intervention periods and estimates the total number of cases attributable to each pathway. Although the number of notified cases attributable to poultry via the food pathway was estimated to have declined from over 12,000 to under 4,000, this pathway/exposure was still the most important post-intervention [27].

4.2 Compilation of outbreak data

Outbreak data from New Zealand have been compiled and summarised to provide evidence for the relative contribution of different reservoirs, pathways, exposures and risk factors for cases linked to known outbreaks (i.e. non sporadic cases or raised notification rates that could not be linked to a particular event) [28, 54]. These analyses can be very useful when considered alongside other investigations of the epidemiology of campylobacteriosis, but it is important to bear in mid that the determinants of the relatively small number of cases associated with outbreaks may be very different to the determinants of the much greater number of sporadic cases. A summary of 189 outbreaks of campylobacteriosis reported to the national surveillance system between January 2000 and March 2004 were collated and reviewed [28]. Of these, poultry was implicated in just 63 (50 poultry meat and 13 poultry livers), whereas the remainder were associated with drinking water (22), animal contact (14), other meat (19), person to person contact (16) and unpasteurised milk (3). The number with laboratory confirmation of *Campylobacter* in the vehicle was very low (7), and presumably there were a large proportion with no identified source. The report concludes 'Consequently the strength of evidence of information on risk factors from notifications and reported outbreaks for indicating transmission routes is low (apart from some indication of the importance of overseas travel)' [28]. A systematic review of 13 published and 16 unpublished outbreak reports, in addition to surveillance data on 216 outbreaks is provided by Wilson [54]. This report also concluded that food was the predominant vehicle for outbreaks, and highlights issues concerning data quality and the completeness of information '....65% of outbreaks involved evidence from cases on exposure history, only 23% involved an environmental investigation, only 3% had laboratory evidence on the source, and only 2% involved a proper epidemiological study' [54].

4.3 Comparative exposure/risk assessment

A Monte Carlo simulation model was developed to compare the relative contribution of four different exposures to the risk of campylobacteriosis in New

Zealand [33]. These were: food (poultry and red meat), drinking water (high and low quality treated, and untreated), freshwater swimming and occupational contact with livestock. The model was developed from an earlier version that considered many more sources, pathways and exposures, with the aim of focusing on the more important issues and refining some of the methods used. Data for parameter estimation were acquired from surveys, published reports, surveillance and expert opinion. Importantly, this was a risk assessment rather than an exposure assessment because a dose-response model was used to estimate the relative number of human cases arising from each exposure. The study concluded that poultry, and to a lesser extent red meat, were likely to be the most important exposures, followed by occupational contact with livestock. Sensitivity analysis revealed that the model output was most sensitive to knowledge of the levels of contamination of poultry and red meat, and identified a number of other data gaps. Uncertainty arising from the dose-response model, including the effect of protective immunity and subtype variation, and the impact of different model configurations, were discussed.

Comparative exposure models for food and environmental pathways, using Monte Carlo simulation, were also provided as part of the integrated modelling approach described in section 4.1 and summarised in two reports [32, 27].

4.4 Expert opinion

Structured approaches to expert elicitation have been used to inform risk ranking for foodborne diseases in New Zealand, including campylobacteriosis [9, 8]. The consultation process carried out in 2005 provided minimum, most likely and maximum estimates of the relative contribution of food pathways and specific sources. Using the most likely values, 57.5% of campylobacteriosis cases were estimated to be foodborne and, of these, poultry was estimated to contribute 52.9%. Both figures are lower than later estimates provided by comparative risk modelling [33] and microbial subtyping [41, 39].

4.5 Methods based on microbial subtyping/molecular genotyping

In this section a brief outline of the use of molecular genotyping to inform campylobacteriosis reservoir attribution in New Zealand is provided; describing the approach taken and the data collected, and giving a summary of the data and model outputs used to inform decision making.

4.5.1 Manawatu sentinel surveillance site

In 2005 it was decided to focus on a 'representative' region of New Zealand in a smaller scale reservoir attribution pilot study, rather than planning a country-wide study, which would have been a more costly and potentially high-risk approach. The Manawatu sentinel surveillance site was initiated in 2005 and data collection has continued to the present day; extending the pilot to a more extensive longitudinal study. Two comparative studies were also carried out in Auckland and Christchurch, which were reported in 2008 [19]. Figure 3 shows the boundary of the sentinel site in lower North Island (this figure also illustrates the spatial epidemiology of campylobacteriosis in children and is taken from an earlier report [31]).

Using a sentinel-site approach, the aim was to sample *Campylobacter jejuni* from humans, food, animals and the environment concurrently in space and time. From the 1st March 2005, human faecal specimens submitted to MedLab Central, Palmerston North that were positive for *Campylobacter* by ELISA, were sent to Molecular Epidemiology and Public Health laboratory at Massey University. In addition, epidemiological data on the cases were acquired in collaboration with the MidCentral Public Health Services (MCPHS), working with ESR Ltd, via a link to the national disease database (EpiSurv). The epidemiological data collection was optimised as part of a separate NZFSA contract with MCPHS [50, 49].

Contemporaneous sampling of food and the environment was carried out to capture the likely strains of *C. jejuni* that the human population in the sentinel site was exposed to. Whole poultry carcases were sampled monthly from retail outlets in Palmerston North, representing the different poultry

suppliers. In addition, samples of fresh red meat and offal (pork, beef and lamb mince and lamb and beef liver) were sampled monthly from retail stores in Palmerston North. Six sites, identified as high use recreational swimming spots by Horizons Regional Council, were sampled every two weeks between January 2006 and April 2009, and cattle and sheep faeces from farms in the catchment of these river sources were sampled. These sites are shown in Figure 4, which was taken from a report describing the molecular epidemiology in multiple sources, including environmental water wildlife, and pets [15].

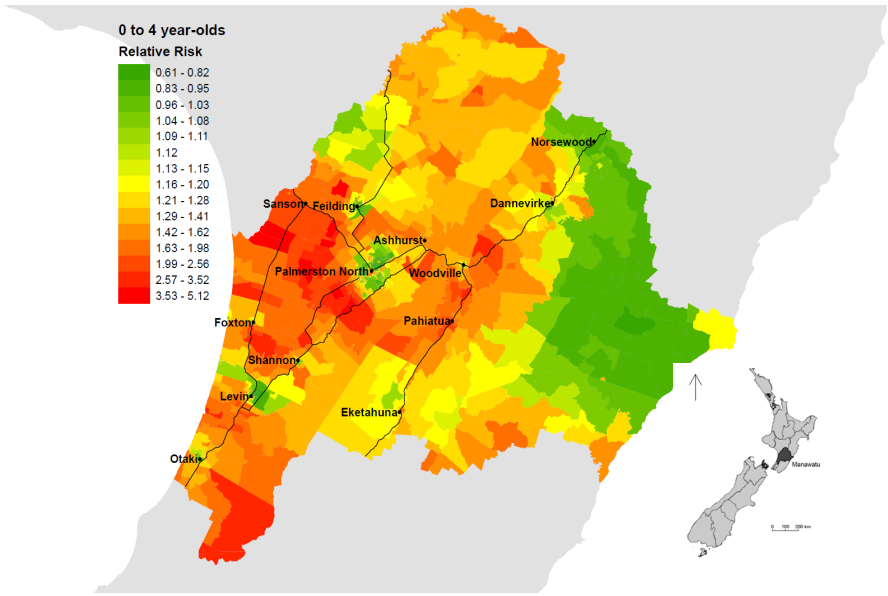


Figure 3: Map showing the boundary of the Manawatu sentinel surveillance site in North Island. The coloured polygons are meshblocks (the smallest unit of population aggregation in New Zealand) depicting the relative risk of campylobacteriosis notification in pre-school children.

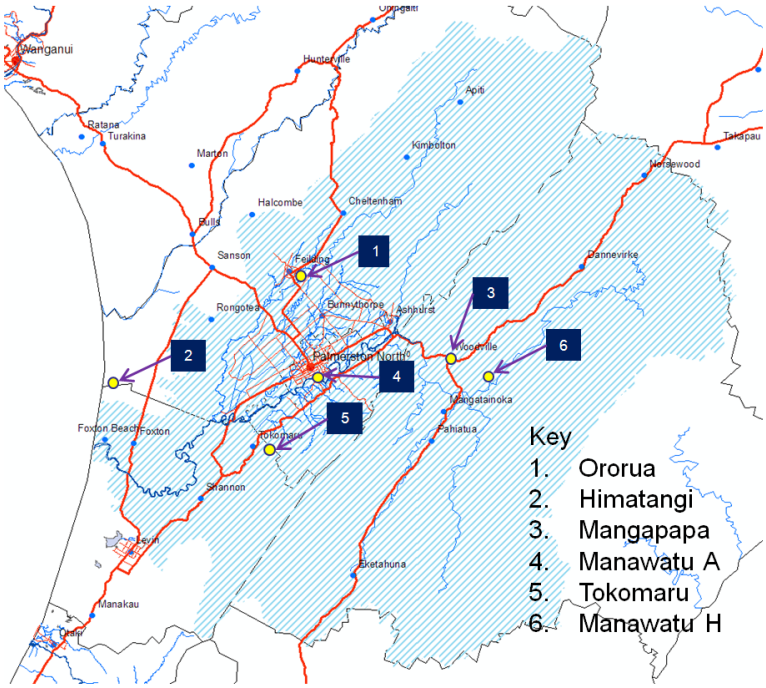


Figure 4: Map showing the Manawatu River catchment (blue shaded area) and the sampling sites for the study of recreational swimming water. Manawatu A refers to the Albert Street section of the Manawatu River, and Manawatu H refers to the Hopelands Picnic Reserve section of the Manawatu River.

The reasons for selecting and maintaining the Manawatu as a sentinel site include the following:

- The region has both urban and rural populations and contains the eighth largest city, in terms of population size, in New Zealand (Palmerston North).
- There was good cooperation between the local Medical Officer of Health and Public Health Unit, the diagnostic microbiology laboratory (MedLab Central) and the research team at Massey University.
- High quality data on human cases were available in the region (and this was further improved with an enhanced surveillance programme).
- The ^{m}EpiLab testing laboratory and research group was located in the area.

- The number of cases was deemed large enough to provide sufficient power for source attribution and epidemiological studies.
- An initial comparison of isolates from humans and poultry in the Manawatu and Auckland and Christchurch was reported in the first technical report in 2008 [19], and this showed a similar pattern of sequence types in human cases and in poultry.

Table 1 summarises the number of samples collected in each year from human cases and potential sources in the Manawatu. The sampling strategy was adjusted over time to make the most efficient use of resources. For example environmental water sampling was added to the programme in 2006 and withdrawn in 2009 because the pattern of genotypes showed little variation over time. The nesting of short term projects is also evident with the addition of non-chicken poultry sources in 2008/9 [20] and ruminant isolates in 2011 [18].

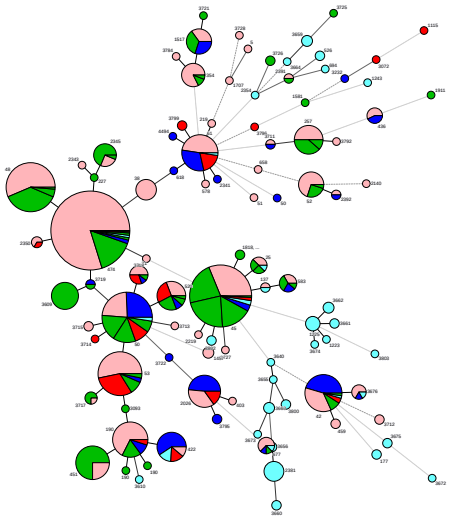
Table 1: The number of samples analysed for source attribution work funded directly by the NZFSA. Additional sampling funded from other sources contributed to the extended attribution work described in [15], but is not included in this table.

		Poultry						Mammalian livestock			Other	
		Chicken			Other poultry			Ruminants				
Year	Human	Supplier A	Supplier B	Supplier other	Turkey	Duck	Spent hen	Cattle	Sheep	Pig	Env. water	Total
2005	264	72	64	60				62	24	55	2	603
2006	395	75	45	24				234	225	179	150	1327
2007	365	79	72	41				255	258	181	156	1407
2008	187	77	95	44	15	15		260	259	177	174	1662
2009	204	41	55	27	48	60	42	77	86	66	101	807
2010	185	26	30	16				36	34			327
2011	187	22	35	15				229	115			603
2012	41	8	8	8								65
All years	1828	400	404	235	63	75	42	1153	1001	658	583	6442

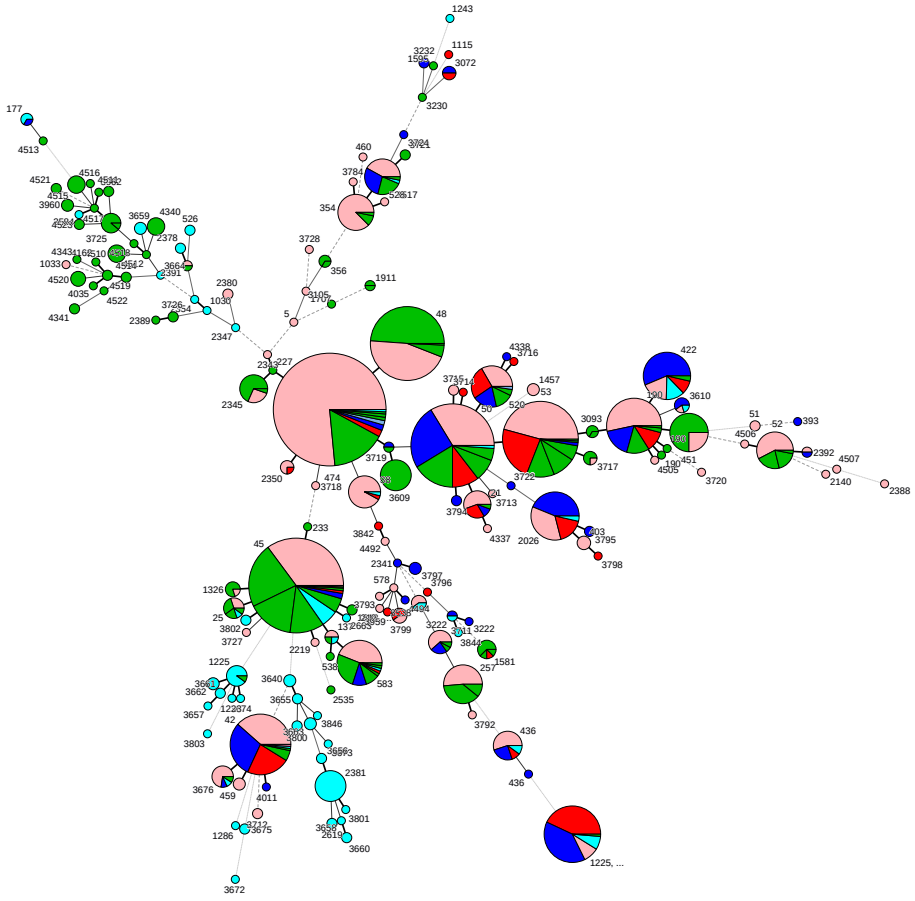
4.5.2 Microbial subtyping: trends in genotypes over time

Over time, the number of different genotypes expanded as the sample size from human cases and their potential sources in the Manawatu enlarged. Figure 5 shows how the number and diversity of genotypes increased from the initial period, when the first reservoir attribution estimates were made, to the entire period from 2005 to the present day. Each circle represents a genotype and the size is determined by the number of times that genotype was isolated. The pie chart within each circle shows the reservoirs from which the genotype was isolated, and the plot is arranged so that more closely related genotypes are closer together.

Figure 6 shows the trend in human cases caused by different genotypes. These plots demonstrate the dramatic reduction in the poultry associated genotypes ST-474 (associated with poultry Supplier A) and ST-48 (associated with Supplier B) following the intervention in the poultry industry. The other two plots show the different seasonal patterns in ruminant-associated genotypes, and highlight the evidence of seasonality in ST-45 - a feature that has been shown in other sources in the United Kingdom [22, 26, 51].



(a) 2005-7



(b) 2005-12

Figure 5: Minimum Spanning trees showing the number and diversity of genotypes from multiple sources in the Manawatu in the period 2005-7 (5a) and the period 2005-12 (5b). Each node is a genotype, the area of the circle is proportional to the number of isolates and the pie chart in each circle captures the proportion from each host where pink=human, red=cattle, dark blue=sheep, green=poultry, and light blue=environmental water. The solid lines connect genotypes that are related to each other.

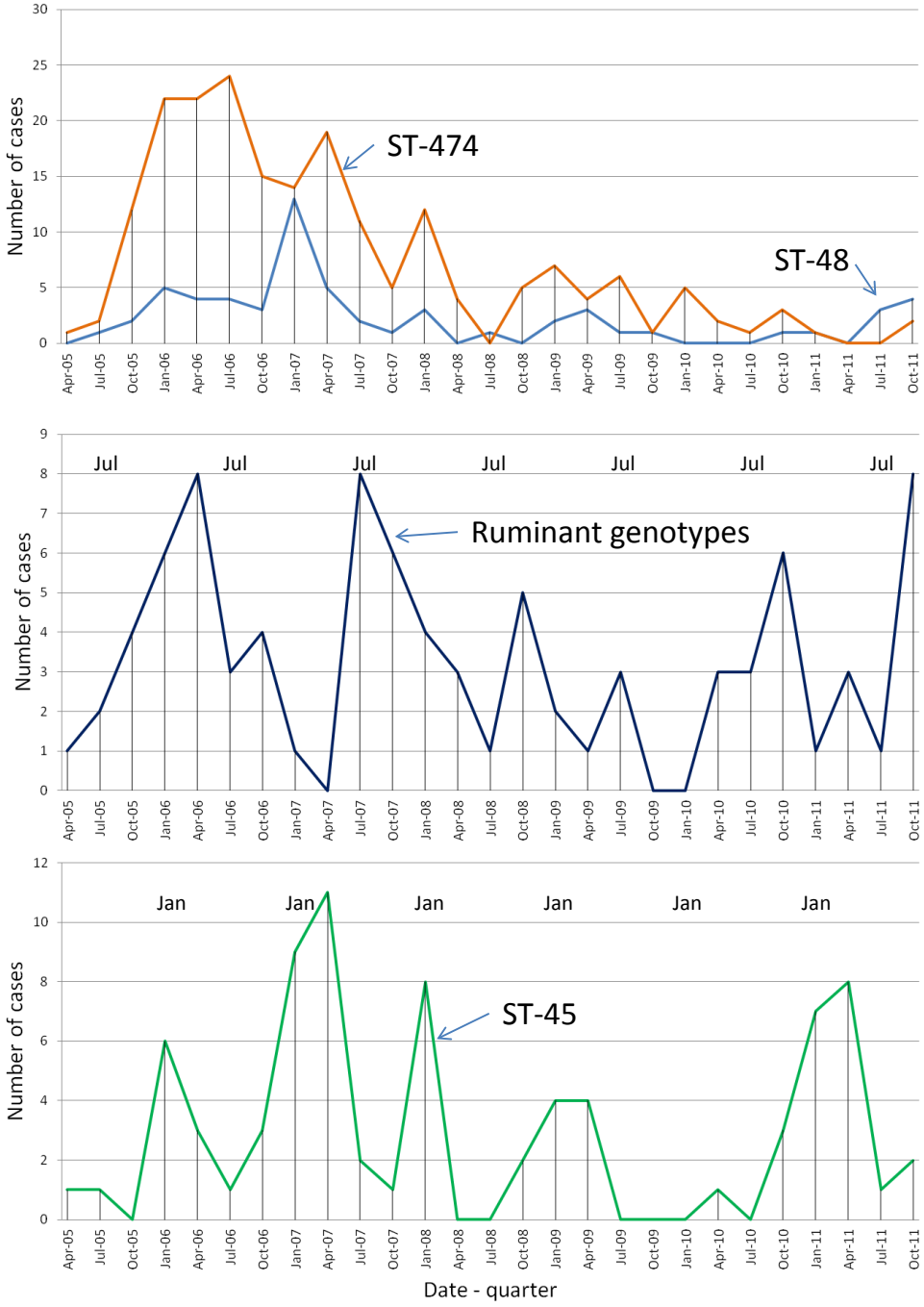


Figure 6: Changes in the number of human cases of campylobacteriosis caused by different genotypes over time. The top plot shows the time series of cases of two poultry-associated genotypes, ST-474 (associated with Supplier A) and ST-48 (associated with Supplier B). The middle plot shows the trend in cases of the genotypes associated with ruminants, ST-61,42, 422 and 53, highlighting peaks in winter months. The bottom plot shows the time series of cases of ST-45, which is associated with summer months in New Zealand and internationally.

4.5.3 Model development and application

The approach taken at Massey University was to use comparative modelling of the strain-typing data from human clinical cases and the potential reservoirs, utilising models with different underlying assumptions. The rationale for this was that new models were being developed for reservoir attribution [24, 53] and a comparative approach was likely to be more informative about the nature of uncertainty in attribution estimates (both model and parameter uncertainty). This also provided an opportunity to work collaboratively with the model developers [24, 53], and improve the performance of an existing model previously developed for attributing salmonellosis cases in Denmark [39]. Four different approaches were considered and these are described in detail in reports, theses and journal articles (see [19, 37, 39] for examples). In summary four models were deployed. The first, the Proportional Similarity Index, simply assesses the area of overlap of the genotype distributions from each source with that of the human genotype distribution. The other three, the Dutch, Hald and Island models, estimate the number of human cases attributable to each reservoir using models based on different underlying assumptions:

Proportional Similarity Index The proportional similarity index (PS) is an objective and simple estimate of the area of intersection between two frequency distributions [46]. In this context, the PS estimates the similarity between the frequency distributions of STs of each source and the distribution of STs amongst human cases. The values for PS range from 1, for the highest possible similarity, to 0 for distributions with no common types.

Dutch model The Dutch method compares the number of reported human cases caused by a particular bacterial subtype with the relative occurrence of that subtype in each source. The number of reported cases per subtype and reservoir is estimated by:

$$\lambda_{ij} = \frac{p_{ij}}{\sum_j p_{ij}} x_i,$$

where p_{ij} = relative occurrence of bacterial subtype i in source j,
x_i = estimated number of human cases of type i per year,
λ_{ij} = expected no. of cases / year of type i from source j.

A summation across subtypes gives the total number of cases from source j, denoted by λ_j:

$$\lambda_j = \sum_i \lambda_{ij}.$$

We used the method of Garret et al. [21] and extended it to provide bootstrap confidence intervals for the Dutch model.

Modified Hald model We modified the Bayesian risk assessment model originally developed to quantify the contribution of different foods to the number of human cases of salmonellosis in Denmark [24]. The original model compares the number of human cases caused by different 'types' with their prevalence in different animal reservoirs and food products, weighted by the amount of food consumed. This model is a further development of the frequentist Dutch model described above and requires a heterogeneous distribution of some types among animal and food products. Like the Dutch model, this approach compares the number of human cases caused by different bacterial subtypes with their prevalence in different reservoirs and food products. However, by using a Bayesian approach, the Hald model can explicitly include and quantify the uncertainty surrounding each of the parameters. In our study the Hald model was adapted to overcome some of the problems associated overparameterisation and to incorporate uncertainty in the prevalence matrix. Further, the food consumption terms was removed to enable the inclusion of environmental pathways for campylobacteriosis. Other groups have attempted similar modifications of this model and applied the models to salmonellosis [23] and listeriosis [29].

Island model This method, first published September 2008 [53], is based on coalescent models, which are different from classical phylogenetic methods in their explicit considerations of the genealogical history of sampled alleles [47]. This population genetics approach is fundamentally different from the

Dutch and Hald models; the genealogy of all isolates is estimated, using their allelic profiles and taking into account the relatedness of STs.

Island models were first proposed by Wright, 1931 [55] and are models of gene flow derived from population genetics. The technique devised by Wilson et al 2008 [53] reconstructs the genealogical history of the isolates, based on their allelic profiles, and estimates mutation and recombination rates, as well as the 'migration' rates from each reservoir into the human 'Island'. It is these migration rates that are used to estimate the relative contribution from each reservoir. Importantly this technique has one major advantage over the other methods; it can assign human cases that have no identified animal reservoir.

4.5.4 Reservoir attribution estimates and their impact on policy

The first estimates of the relative contribution of different reservoirs to the burden of campylobacteriosis based on molecular genotyping were provided to NZFSA in 2006, but these were based on simple comparisons of genotype distributions in human cases and potential reservoirs. Model-based estimates became available in July 2007. Figure 7 was the first comparative modelling output produced, and this figure was presented to NZFSA and the poultry industry in August 2007. This strongly indicated a high proportion of cases were attributable to poultry, providing further evidence to support the implementation of the Risk Management Strategy.

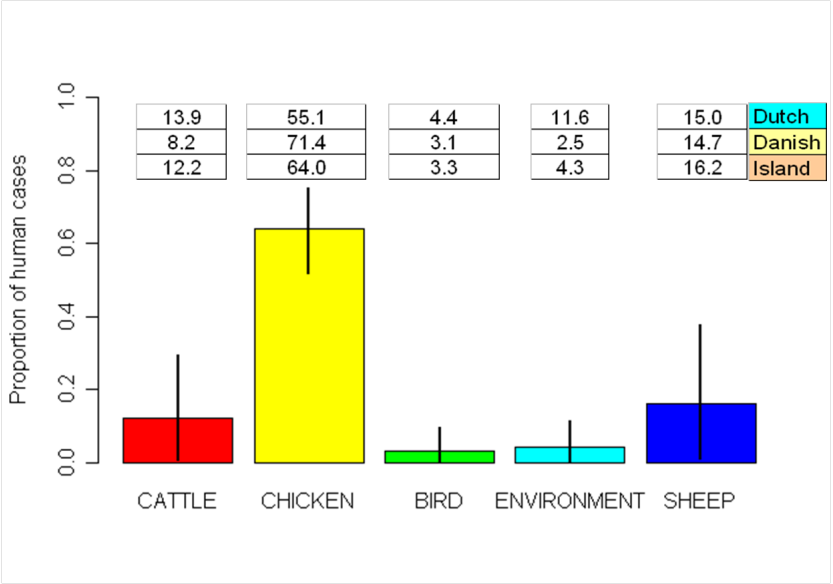


Figure 7: The first reservoir attribution model output provided to NZFSA in July 2007. The numbers at the top of each bar show the estimates from the Dutch, Hald (Danish) and island models and the bars show the estimates from the island model with 95% credible intervals.

Subsequently, models were updated and refined. Figures 8 and 9 summarise all reservoirs over the six-year period beginning on the 1st July 2005. The attribution estimates are summed across sources in Figure 8 to show the estimated attribution to all chicken, all ruminants and other reservoirs. The same information is presented as a stacked bar plot in Figure 9. Figure 10 shows how the attribution for each source changed yearly from 2005 to 2011.

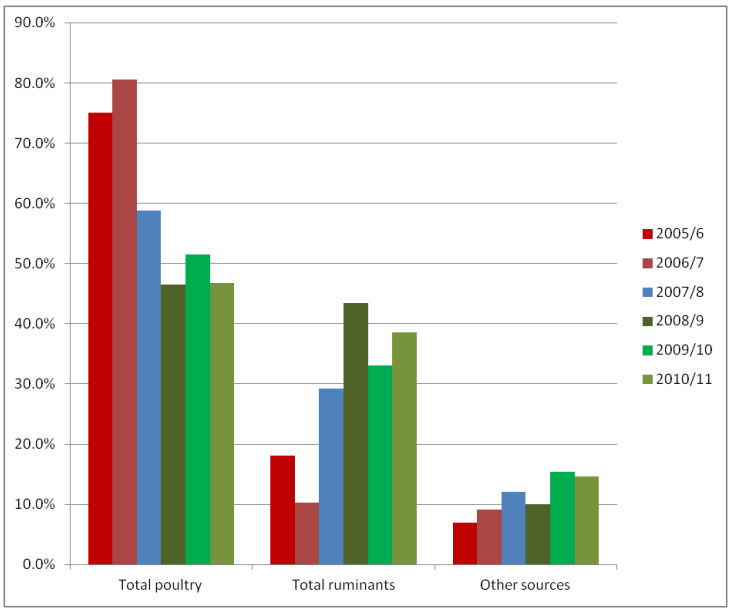


Figure 8: Poultry, ruminant and other reservoir attribution estimates for human cases in the Manawatu for 6 twelve monthly periods starting on the 1st July 2005 and ending on the 30th September 2011. The pre-intervention years are shaded red, the transition year blue and the post-intervention years are in green.

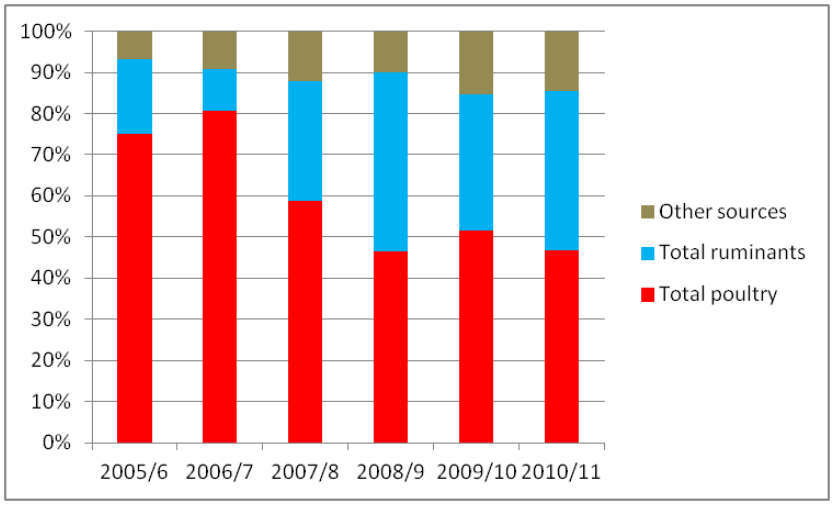
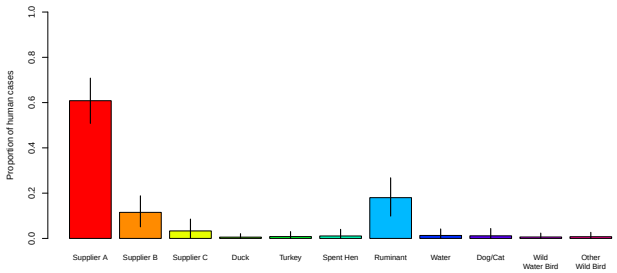
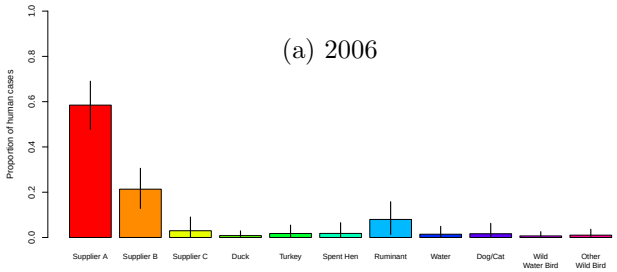


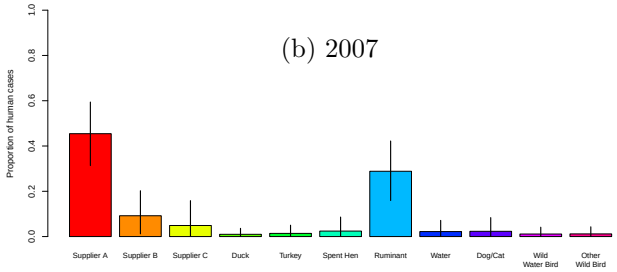
Figure 9: A stacked bar plot showing poultry, ruminant and other source attribution estimates for human cases in the Manawatu for 5 twelve month and one 15 month period starting on the 1st July 2005 and ending on the 30th September 2011.



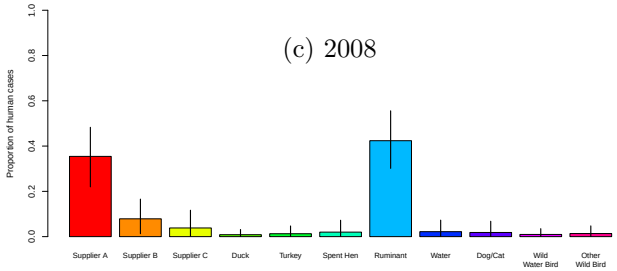
(a) 2006



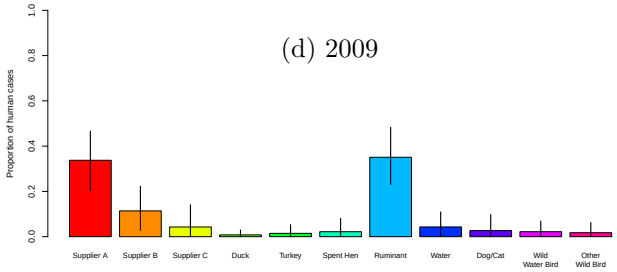
(b) 2007



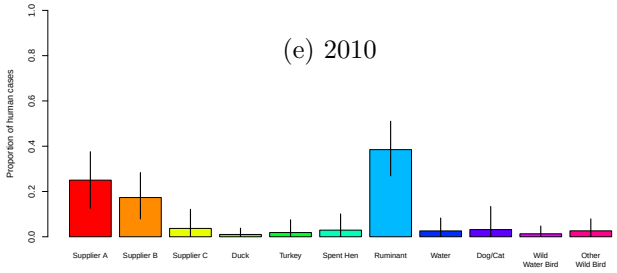
(c) 2008



(d) 2009



(e) 2010



(f) 2011

Figure 10: Attribution to poultry, ruminants and other reservoirs by year

5 Dynamic attribution

Dynamic modelling of the contribution of different reservoirs helps to identify, at a range of temporal scales, changes in the contribution of different reservoirs, and hence the impact of interventions. It also improves our understanding of the epidemiology of campylobacteriosis and could, with some modifications and more rapid typing, be used to identify changes in 'real time'. At a simple level, the repeated fitting of models on an annual basis as described in section 4.5.4 and Figure 10, provides an annual summary of how attribution changes over time. This can be done by using the genotyping data from reservoirs sampled only in the year in question or, under the assumption that the genotype patterns do not vary over time, by using all available genotyping data over all years. An understanding of how genotype patterns change over time in animal hosts is required, and this can help to decide which strategy to use, bearing in mind the loss of power if only one year of source data are considered. It is also important to take into account that, although the genotypes may vary in individual reservoirs over time, the signatures provided at the allelic level may not fluctuate as much.

5.1 Development and application of dynamic models in New Zealand

In an attempt to understand how reservoir attribution changes over a finer time scale, we developed a dynamic model [14, 34], based on the Hald model, that provides outputs with estimates of uncertainty (Bayesian credible intervals) in each quarter (Figure 11). The original intention was to develop an approach that could be used in real-time, but in practice this could not be achieved due to delays in reporting times and the acquisition of genotyping data for each case. The output does, however, provide a useful, albeit retrospective, indication of temporal changes in the major reservoirs and shows the continuing seasonality of poultry-associated cases. The striking reduction in poultry-associated cases after the intervention in 2007 is evident in Figure 12.

Figure 11 shows the output from the updated dynamic Hald model, display-

ing the attribution to poultry and ruminants over the six year period. With the exception of 2009/10, the summer peaks, most of which are attributed to poultry, can still be seen after the intervention, with the peak for summer 2008/2009 and 2010/11 approximately half the level of previous years. Cases attributed to ruminants account for around 10 to 20 cases per month. Figures 12 shows the number of cases attributed to poultry and cattle including 95% credible envelopes.

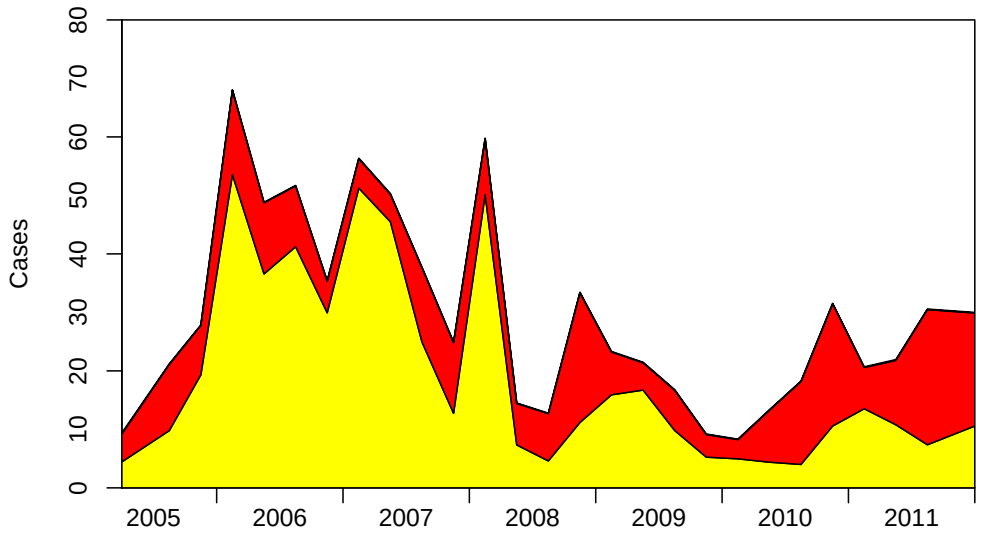
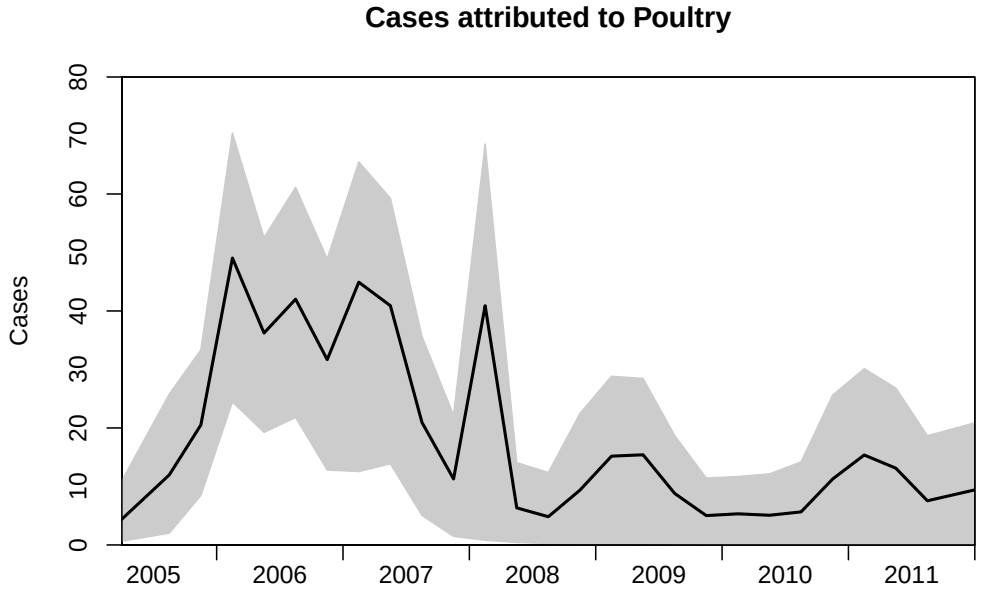
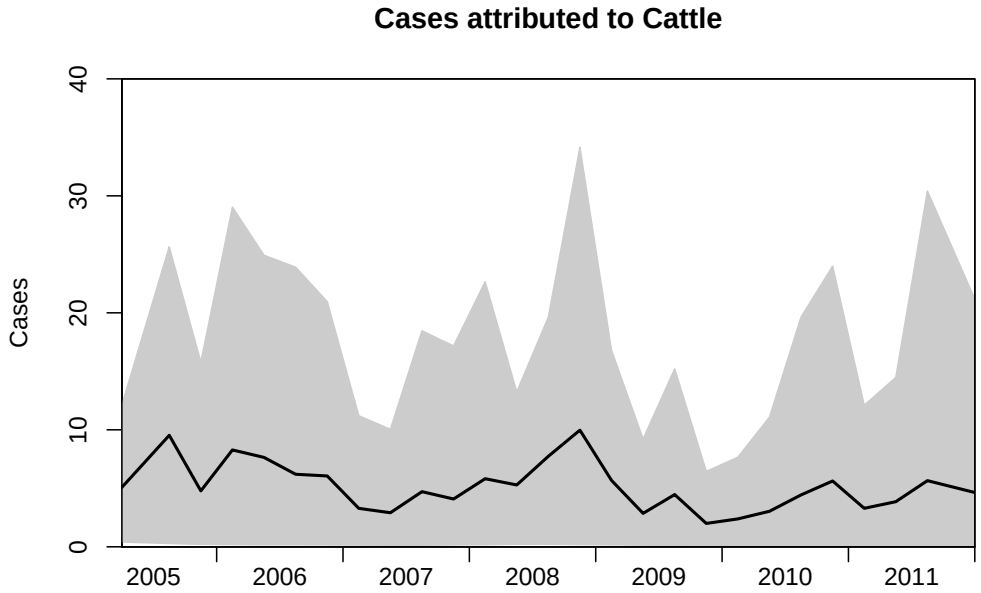


Figure 11: Estimated number of human cases per month attributed to each reservoir by the modified Hald model using three-monthly intervals from 1st March 2005 to September 2011. Colours indicate the reservoir the cases are attributed to: yellow (poultry) and ruminants (red). Other reservoirs contributed to fewer than one case per interval and are therefore not included in the graph.



(a) Poultry



(b) Cattle

Figure 12: Estimated number of human cases per month attributed to poultry and cattle by the modified Hald model with 95% credible intervals using three-monthly intervals from 1st March 2005 to September 2011.

5.2 Recommendations for improving dynamic attribution modelling

The current dynamic attribution model is based on the Hald model, a Bayesian model where sequence type information is used to associate human isolates in a particular time interval with reservoirs. There are a number of ways that this might be improved, and a recent publication from Finland has proposed a refinement of the dynamic Hald model that, although only utilised at the *Campylobacter* species level in the paper (*C. jejuni*, *C. coli* and other *Campylobacter* spp.), could be extended and applied to more refined subtyping data [45] and possibly incorporating National Microbiological Database information. Another improvement would be utilising a finer measure of dissimilarity between sequence types. The Hald model currently works directly with sequence type numbers, and thus any pair of isolates with different sequence types have the same dissimilarity measure. However, this doesn't take the sequence's allelic profile into account. Two sequence types that are single locus variants, for example, might be considered more similar than two sequence types that differ at 3 or more loci. Incorporating these differences may lead to improved attribution results for existing attributable isolates, and may also help attribute isolates that the Hald model cannot currently attribute to animal reservoirs (i.e. those isolates that have not previously been observed in the animal population).

6 Where are the data gaps and can they be filled?

There are a number of important areas that, if addressed, would improve the efficiency and utility of microbial-subtyping based source attribution for campylobacteriosis, and enable these techniques to be incorporated within a wider integrated surveillance programme based on sound science. Such an approach would facilitate surveillance to inform national control programmes, as well as outbreak detection and management, and could become part of a multi-pathogen programme extended to cover a range of infectious diseases. Improvements can be made in both data collection and analysis. The former

is dealt with in this section, in which issues related to sampling and genotyping are discussed, and in the section discussing the sentinel surveillance site (Section 7.1). The latter is addressed in the sections on dynamic attribution (Section 5), the use of epiclustR (Section 7.1) and options for the future (Section 8).

6.1 Data gaps

Ideally reservoir attribution and pathway attribution could be combined to provide information for policy makers on the contribution of individual animal amplifying host-pathway combinations. For example, it would be desirable to have separate estimates for the contribution from cattle-food, sheep-environmental and cattle-direct contact pathways, and even more refined estimates for particular host-pathway-exposure combinations, such as cattle-food-milk, and sheep-environmental-drinking water. This can be addressed to some extent through improvements in the integration of epidemiological approaches with reservoir and pathway attribution, but improvements in sampling strategies and genotyping could also help.

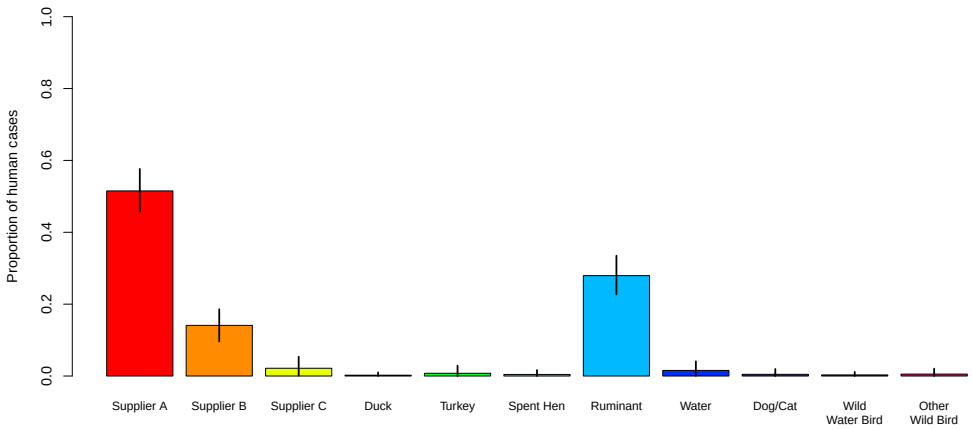
6.1.1 Improving genotyping tools

To date, the tool used to genotype isolates for source attribution in New Zealand has been primarily 7 gene multilocus sequence typing (MLST), as described by Dingle et al. in 2001 [10]. Whilst this has been valuable in distinguishing between reservoirs such as ruminants, wildlife and the different poultry suppliers, the similarity in the pattern of 7-gene MLST types in cattle and sheep has hampered efforts to provide precise estimates of the contribution of these reservoirs individually. This issue is discussed in detail in three reports [17, 44, 18], and illustrated below in Figure 13.

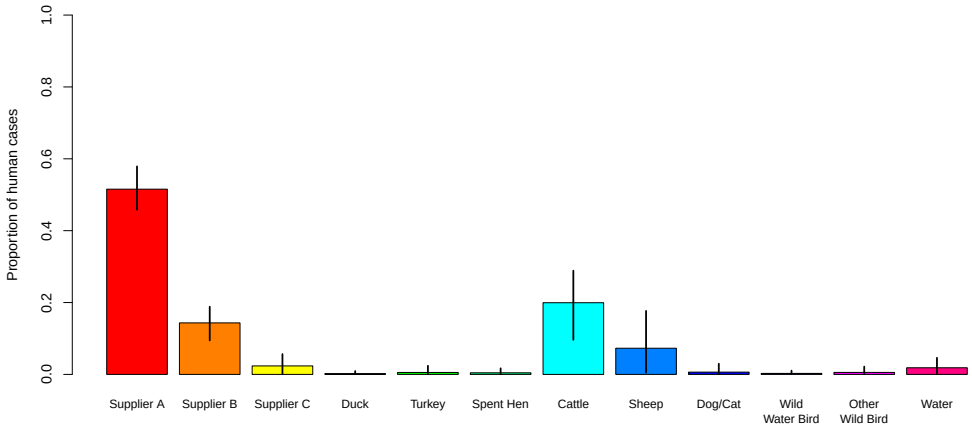
When all ruminant reservoirs are grouped into a single category (comprising all cattle and sheep from both food and the environment), we obtain narrow confidence intervals for both poultry and ruminants. When we combine food and environmental pathways by host species to provide two categories: cattle and sheep, there appears to be a greater contribution from cattle compared

to sheep, however, the lack of population differentiation results in wider and overlapping confidence intervals.

An ideal genotyping tool would be based on genomic markers that are not only indicative of the animal reservoir (i.e. genetic markers that are the basis of host association) but also of pathway (i.e. use genes that indicate susceptibility or resistance to 'stress' associated with the food production pathway, such as resistance to oxidative, osmotic, pH and temperature fluctuations). We have explored the use of an extended MLST scheme that includes more hypervariable genes, such as *flaA* and *porA*, to see if these are correlated with animal hosts and pathway [18, 44]. The addition of these two genes did provide some further evidence of population differentiation, but it was unclear what the impact of this would be on attribution estimates [18, 44]. High throughput sequencing technology, combined with phenotypic microarray systems offer considerable opportunities for the detection of such markers, and with the advent of indexing technology it may soon be cheaper to sequence full genomes rather than carry out MLST. New work ongoing in the Hopkirk Research Institute has lead to the detection of potential markers for ruminant association using full genome sequencing (e.g. the *ykgC* gene [7]) and work is underway to determine the genetic basis for phenotypic variation between a number of genome sequenced New Zealand *Campylobacter* spp. isolates, using the Omnilog system.



(a) Ruminants considered as single group



(b) Cattle and sheep considered separately

Figure 13: Reservoir attribution for all human cases in the Manawatu reported between 2005 and 2011. This plot illustrates the lack of precision when ruminants (13a) are divided into cattle and sheep (13b). Notice the large and overlapping error bars for cattle and sheep (95% credible intervals), in Figure 13b compared to the estimate for ruminants in Figure 13a

6.1.2 Sampling other hosts, sites and pathogen species

Although we have extended our approach to consider other non-chicken poultry, cats, dogs and wildlife [15, 20], we have not considered all possible reservoirs and our work has been restricted to the Manawatu sentinel site (the latter generalisability issue is addressed in section 7.1 below). For example we have not sampled deer, goats or other pet animals and our focus has been

entirely on *C. jejuni* and ignored the approximately 10% of cases caused by *C. coli* and other *Campylobacter* spp.

6.1.3 Sampling efficiency and the detection of genotype shifts

Periodic surveys are required to detect shifts in genotype patterns in particular reservoirs. This is currently being done for chicken sources on a quarterly basis in the Manawatu [17], as a result of evidence of the appearance and disappearance of new strains in the poultry supply [38], but similar, less frequent cross sectional studies of other reservoirs are required to ensure that any new genotypes that have emerged in a particular reservoir are detected. Potentially, a random selection of isolates associated with the National Microbiological Database testing could be used, as occurred in the Antimicrobial Resistance baseline Survey, to assess national shifts in poultry genotypes This could not be used for other species as poultry is the only species tested for *Campylobacter*.

6.1.4 Human case exposure data

In the Manawatu sentinel site, the data on human exposure captured in EpiSurv was enhanced, achieving high levels of completeness [49], and additional variables were added to gather information on specific exposures. However, the data for other DHBs is generally less complete and with lower coverage of exposures, and this represents a significant knowledge gap for the remainder of the country outside the sentinel site. However, there are others sources that could be accessed to provide some additional exposure data at the national level. For example the National Nutrition Survey[2] could provide useful data on food exposures, for example the consumption of goat meat at the population level, and there may be other data that could provide useful information on non-food exposures (e.g property rating databases for non-public water supplies to homes).

[2] http://www.health.govt.nz/nz-health-statistics/national-collections-and-surveys/surveys/current-recent-surveys/nutrition-survey

7 Optimising surveillance: current issues and recent developments of relevance to source attribution

7.1 Representativeness and utility of maintaining a sentinel site

The rationale for conducting source attribution studies in a single site, and the choice of the Manawatu as the site, is provided in section 4.5.1. However, changes in both the epidemiology of campylobacteriosis post-intervention, and in the patterns of livestock production and human demography in New Zealand, combined with the lack of any formal evaluation of the site, have lead to questions concerning the validity of using such a single site.

To address this a study is underway to compare the demographics of the human population of the Manawatu with other regions of New Zealand, examining variables that have been shown to be related to the incidence of campylobacteriosis including: the urban / rural mix, social deprivation index, population density, age structure and ethnicity. In addition, other variables related to the incidence and source attribution of campylobacteriosis will be compared including the poultry supply (which suppliers provide fresh poultry to each region), the density of farm animals and available epidemiological variables from case reports.

In addition, the distribution of multilocus genotypes will be compared between Manawatu and Christchurch (where an HRC-funded study of campylobacteriosis was conducted in 2010/11), comparing human cases and putative sources where available. This will include a consideration of how these distributions have changed over time. The objectives of this study are to:

- Compare and the demography of the human population in the Manawatu with other regions across New Zealand (using National Census data).
- Compare the distribution of variables of relevance to the epidemiology of campylobacteriosis in the Manawatu with other regions including

Canterbury, Capital and Coast and Auckland (e.g. animal densities derived from AgriBase, poultry suppliers market share, NMD data on contamination of poultry, drinking water supplies).

- Compare the genotype distributions in human cases and animal reservoirs in the Manawatu with the distributions of genotypes in Canterbury (and, if available, other regions).
- Compare the epidemiological features of campylobacteriosis cases in the Manawatu with Canterbury and, if available, other regions using primarily EpiSurv data (age, seasonality, occupation etc.).
- Combine the above into a detailed multivariate analysis using techniques such as Principal Components Analysis, Multi-dimensional Scaling and Multiple Joint Correspondence Analysis, to determine the interrelationships between the above variables and their relationship with geographical region, and the degree of discrimination between the epidemiology and source attribution in the Manawatu with other sites across New Zealand (note some comparisons can only be made with Canterbury).

7.2 Combining surveillance tools such as epiclustR with other models to create a toolbox to inform both national surveillance (e.g. reservoir attribution) and local outbreak investigation

In a recent review of surveillance systems, Baker et al. [5] make the distinction between control-focused surveillance and strategy-focused surveillance. The former would be used to inform, for example, outbreak investigations or other events that require a specific response. The latter informs prevention strategies, such as the use of reservoir attribution modelling and case-control studies, to improve population health [3] . In this paper, they make the im-

[3]These are defined in the paper as follows: '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 support delivery of an effective intervention. For example, a single case of polio, a common-source salmonellosis outbreak, a shipment of contaminated produce, or an un-immunised child. The purpose of strategy-focused

portant point that 'Control-focused surveillance usually provides information that can also be used for strategy-focused surveillance, so these purposes are often combined within the same surveillance activity. By contrast, strategy-focused surveillance cannot generally support control-focused surveillance.'

epiclustR [52] was developed to aid the detection of localised clusters of disease in space and time, and if combined with (rapid) appropriate microbial subtyping could be used to identify the source of outbreaks. The data collected and used in epiclustR, particularly if it is combined with subtyping of human cases and putative reservoirs, would also be valuable for source attribution studies - this would be an example of control-focused surveillance supporting strategy-focused surveillance.

The integration of epiclustR with a continued sampling programme for reservoir attribution modelling would potentially provide an efficient system for both control and strategy-focused surveillance that maximises the use of routinely collected surveillance data and microbial subtyping data. The addition of a standard control set measuring relevant exposures and risk factors (i.e. a sample of the population that was asked a set of questions that could be used to compare with cases for which similar risk factor data were gathered routinely through EpiSurv), would make it possible to examine the relative contribution of different reservoirs, pathways, exposures and risk factors - further enhancing strategy-based surveillance. This is discussed further in section 8 below.

8 Options for future

Suggestions for improving the current approach to source attribution have been introduced in earlier sections, for example: validation studies (Section 7.1) and model development (Section 5.2) and the integration of reservoir attribution into a wider programme of surveillance (Section 7.2). This section develops some of these ideas further.

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.'

8.1 Developing source attribution modelling

Further improvements to dynamic reservoir attribution are described in section 5.2. There is also considerable scope for modifying and developing new models for source attribution (i.e. including reservoir and pathway attribution and exposure assessment). Essentially, reservoir attribution is a classification problem: Given genetic information from an isolate, classify the isolate to the most likely reservoir. The model is trained on the reservoir data, where the reservoir is known, and then applied to the human data where no reservoir is known. Investigating other classification models, such as naive bayes, nearest neighbour, or neural networks would be useful. In addition, the basic classification model is an application of Bayes theorem, where the key probabilities to be estimated are the distribution of sequence types given the reservoir. Modelling these probabilities using evolutionary models (such as the coalescent model used in the Island model) may provide more precise estimates of attribution.

The current island model considers multiple animal reservoirs and 'environmental water'. Although environmental water is not an amplifying host, but an environmental pathway leading to multiple exposures, this approach has been justified by considering this 'island' to be representative of wildlife hosts that would not otherwise be captured by animal sampling. However, it is evident that farm animals also contribute to environmental water *C. jejuni*, albeit at a lower rate than wildlife [15]. Extending the current island model to consider explicitly the pathway from livestock and wildlife to water could help to refine estimates of the contribution of these amplifying hosts, and provide better estimates of the contribution made via the contamination of surface water.

8.2 More efficient use of existing data: the melding of source attribution, epidemiological modelling and outbreak detection

The current surveillance system could be redesigned to meet the needs of control and strategy-focused surveillance by integrating source attribution

with epidemiological studies of sporadic cases, and outbreak detection - and this could be extended to multiple pathogens. The current system could be augmented by: improving the quality and coverage of national EpiSurv data (possibly by focusing on a small number of key risk factors or a national sample of cases); initiating the routine annual sampling of a standardised control set (similar to the one developed for the ongoing case-control study on STEC); integrating *Campylobacter* data from the National Microbiological Database, more informed sampling of sentinel sites for reservoir attribution; and the introduction epiclustR-informed targeted sampling of case clusters. If the data were collated in a single repository, models could be run on a weekly basis for outbreak detection and annually for reservoir and pathway attribution and epidemiological risk factor determination.

8.2.1 Incorporation of typing information and evolutionary modelling into outbreak investigation and epidemiological studies

Outbreak investigation: Whilst sequence type information would be difficult to incorporate into epiclustR directly - the main difficulty being that the number of cases per sequence type per region would be very much lower, requiring a higher level of spatial aggregation - it would be possible to combine spatio-temporal information with sequence type information and use this to determine which cases may cluster together. A key requirement would be determining a suitable measure of dissimilarity that would incorporate both the genetic difference between isolates and the geographic distance between cases. Investigation of the appropriate weighting of both measures would be required, as would the investigation of suitable techniques for cluster location.

Case-control and other epidemiological studies: There is considerable interest internationally in improving the use of genotyping data in epidemiological studies. A simple approach is often taken whereby the genotyping data is used to refine the case definition, and separate models are built for individual genotypes (e.g. instead of cases of campylobacteriosis being considered in a case control study, cases of *C. jejuni* ST-474 are used as the outcome variable). However, there is scope for applying source attribution

modelling approaches, such as the island model, to provide a probabilistic source-assignment to each genotype, further refining this approach. These alternative outcome variables can be used in more sophisticated models of the determinants of, for example, poultry-associated cases and ruminant-associated cases. The first steps towards this are described in a case-case study by Mullner et al. [40], and in case-control studies by Tam et al. (unpublished, presented at the CHRO meeting in Vancouver in 2011) and Mughini-Gras et al. [36].

This approach, combining molecular genotyping, epidemiological and evolutionary modelling, and embedding these within a case-control study, could be particularly valuable for understanding the contribution of ruminants to the burden of campylobacteriosis, and identifying the precise pathways and exposures that contribute to disease. This may be efficiently achieved by piggy-backing on other ongoing case-control studies, using generic case and control questionnnaires delivered to standardised and updated sets of controls.

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A Appendix: Summary of publications arising from source attribution work based on *Campylobacter* genotyping in New Zealand.

The work on campylobacteriosis source (mainly reservoir) attribution using molecular genotyping in New Zealand is summarised in nine technical reports [17, 44, 14, 20, 19, 18, 16, 15, 31], two theses [37, 50, 44] and seven peer-reviewed journal articles [40, 35, 34, 48, 41, 39, 38]. These documents describe in detail the progress made with developing the science of source attribution, its application to inform policy for the control of campylobacteriosis in New Zealand, and the subsequent monitoring of the impact of control measures on notification rates. Hyperlinks to websites containing the earlier technical reports and theses are provided in the reference list at the end of this document.

The earlier reports and publications (2008/9) describe the setting up of the Manawatu sentinel surveillance site [19] and the development of modelling methods [39, 41, 14, 19]. The later reports and publications (2010-12) describe projects that extended the scope of the attribution work by: examining more reservoirs [18, 20, 15, 44]; describing the molecular epidemiology in humans and animal hosts [40, 35, 34, 38, 31]; and providing updates of the impact of interventions on reservoir attribution over time [48, 17, 16].

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