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CANADIAN INTEGRATED PROGRAM FOR ANTIMICROBIAL RESISTANCE SURVEILLANCE (CIPARS) ANNUAL REPORT

CHAPTER 1.
DESIGN AND METHODS

Canada

TO PROMOTE AND PROTECT THE HEALTH OF CANADIANS THROUGH LEADERSHIP, PARTNERSHIP, INNOVATION AND ACTION IN PUBLIC HEALTH.

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1 DESIGN AND METHODS

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PREAMBLE

ABOUT CIPARS

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), created in 2002, is a national program dedicated to the collection, integration, analysis, and communication of trends in antimicrobial use (AMU) and resistance (AMR) in selected bacteria from humans, animals, and animal-derived food sources across Canada. This information supports (i) the creation of evidence-based policies for AMU in hospitals, communities, and food-animal production with the aim of prolonging the effectiveness of these drugs and (ii) the identification of appropriate measures to contain the emergence and spread of resistant bacteria among animals, food, and people.

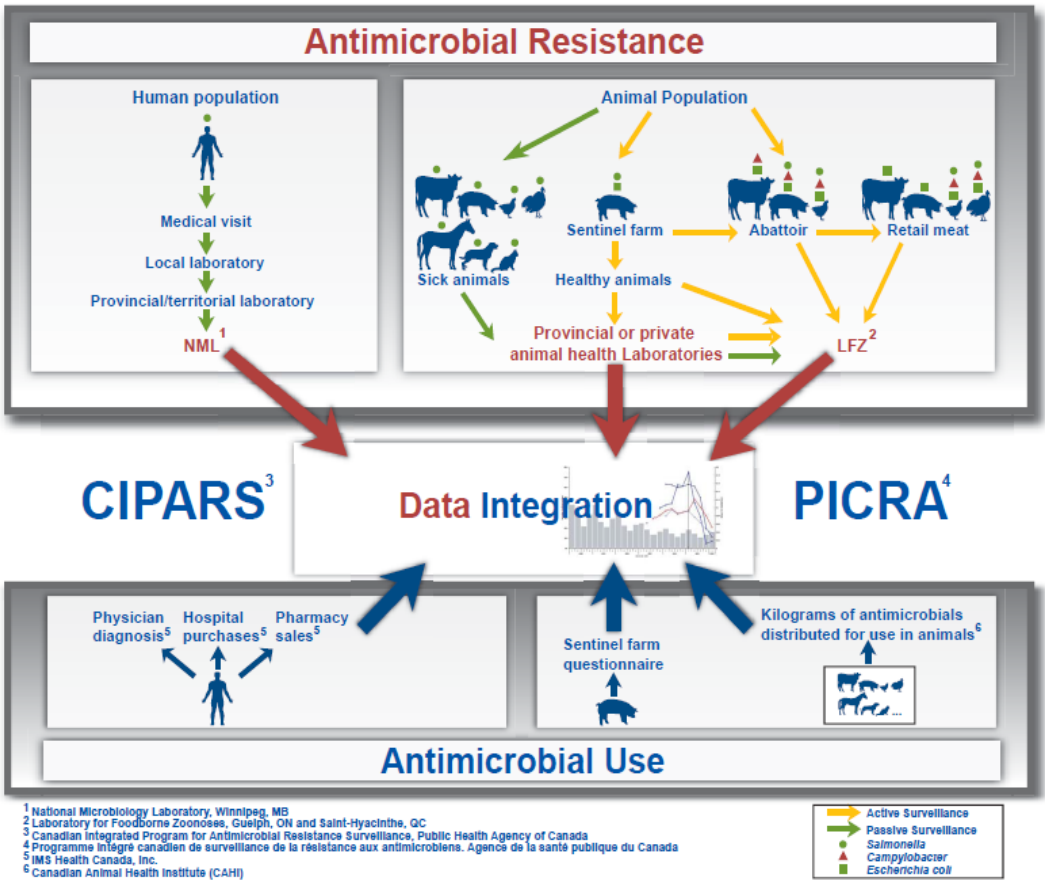
During 2012, CIPARS held discussions on new ways of analyzing and presenting the surveillance data, to adjust for different data closure dates and to maximize the integration of existing data. The Annual Report will now be released in a Chapter format to improve the timeliness of the data release where possible and consist of four chapters: Chapter 1 – Design and Methods, Chapter 2 – Antimicrobial Resistance, Chapter 3 – Antimicrobial Use, and Chapter 4 – Integrated Findings and Discussion. Chapter 1 includes detailed information on the design and methods used by CIPARS to obtain and analyze the AMR and AMU data, including two tables (AMR and AMU) describing changes that have been implemented since the beginning of the program. Chapter 2 and 3 present results for AMR and AMU, respectively, with each one including a section presenting the top key findings. Chapter 4 aims to bring together some of the results across surveillance components, over time and regions, and across host/bacterial species in an integrated manner and includes interpretation of this integration.

CIPARS OBJECTIVES

- Provide a unified approach to monitor trends in antimicrobial resistance and antimicrobial use in humans and animals.
- Facilitate assessment of the public health impact of antimicrobials used in humans and agricultural sectors.
- Allow accurate comparisons with data from other countries that use similar surveillance systems.

CIPARS SURVEILLANCE COMPONENTS

Figure 1. Diagram of CIPARS surveillance components in 2012



ANTIMICROBIAL RESISTANCE

WHAT'S NEW

- Bacterial culture and antimicrobial susceptibility testing of *Salmonella*, *Escherichia coli* and *Campylobacter* isolates from retail turkey was started in January 2012.
- Bacterial culture and antimicrobial susceptibility testing of *Campylobacter* isolates from pigs at the abattoir was started in January 2012.
- CIPARS adopted a new Enterobacteriaceae plate CMV2AGNF for *Salmonella* and *E. coli* in 2012. The changes to the Enterobacteriacae plate were: addition of azithromycin (Category II) and removal of amikacin (Category II).
- CIPARS adopted the new Clinical and Laboratory Standards Institute (CLSI) [1] resistance breakpoint of ≥ 1 µg/mL for ciprofloxacin in *Salmonella* and *E. coli* isolates in 2012. The decision by CIPARS to expand the breakpoint change to all *Salmonella* serovars and *E. coli* was based upon the desire to keep the breakpoints harmonized across the Enterobacteriaceae we monitor, due to their close biological similarities and ease of sharing of resistance genes. Specifically for *E. coli*, using the same breakpoint reinforces its role as a commensal indicator of the pool of resistance genes available for exchange with more pathogenic organisms. Furthermore, the ciprofloxacin resistance breakpoints were the same in the past for both genera (at ≥ 4 µg/mL); keeping the breakpoints the same maintains the precedent previously set. In this report, all ciprofloxacin data have been recalculated retrospectively using this new breakpoint. The impact of this change was minimal considering that most *Salmonella* and *E. coli* isolates tested have minimum inhibitory concentrations (MIC) for ciprofloxacin that are many dilutions below the resistance breakpoint.

[1] Clinical Laboratory Standards Institute (CLSI) M100-S22. For reporting *S*. Typhi and extraintestinal *Salmonella* spp. only.

HUMAN SURVEILLANCE

OBJECTIVE(S)

The objective of the *Surveillance of Human Clinical Isolates* component of CIPARS is to provide a representative and methodologically unified approach to monitor temporal variations in the prevalence of antimicrobial resistance in *Salmonella* isolated from humans.

SURVEILLANCE DESIGN

Hospital-based and private clinical laboratories culture human *Salmonella* isolates in Canada. Although reporting is mandatory through laboratory notification of reportable diseases to the National Notifiable Disease Reporting System, forwarding of *Salmonella* isolates to provincial reference laboratories is voluntary and passive. A high proportion (84% in 2001)[2] of *Salmonella* isolates are forwarded to Provincial Public Health Laboratories (PPHLs), but this proportion may vary among laboratories. The Yukon, Northwest Territories, and Nunavut, which do not have a PPHL counterpart, forward their isolates to one of the PPHLs.

Prior to 2002, PPHLs forwarded *Salmonella* isolates to the Enteric Diseases Program, National Microbiology Laboratory (NML), Public Health Agency of Canada (PHAC), Winnipeg, Manitoba for confirmation and subtype characterization. A letter of agreement by which provinces agreed to forward all or a subset of their *Salmonella* isolates to NML for CIPARS was signed in 2002 by the PPHLs and PHAC. This agreement officially launched the surveillance program.

To ensure a statistically valid sampling plan, all human *Salmonella* isolates (outbreak-associated and non-outbreak-associated) received passively by PPHLs in Saskatchewan, Manitoba, New Brunswick, Nova Scotia, Prince Edward Island, and Newfoundland and Labrador were forwarded to the NML. The PPHLs in more heavily populated provinces (British Columbia, Alberta, Ontario, and Québec) forwarded only the isolates received from the 1st to the 15th of each month. However, all human *S.* Newport and *S.* Typhi isolates were forwarded to the NML because of concerns of multidrug resistance and clinical importance, respectively.

The PPHLs were also asked to provide a defined set of data for each forwarded isolate, including serovar name, date collected, and patient age, sex, and province of residence.

[2] Report of the 2001 Canadian Laboratory Study, National Studies on Acute Gastrointestinal Illness, Division of Enteric, Foodborne and Waterborne Diseases, 2002.

RETAIL MEAT SURVEILLANCE

OBJECTIVE(S)

The objectives of CIPARS *Retail Meat Surveillance* component are to provide data on the prevalence of antimicrobial resistance and to monitor temporal variations in selected bacteria found in raw meat at the provincial/region level.

SURVEILLANCE DESIGN

Retail surveillance provides a measure of human exposure to antimicrobial-resistant bacteria via the consumption of undercooked meat. Retail food represents a logical sampling point for surveillance of antimicrobial resistance because it is the endpoint of food animal production. Through meat sample collection and testing, the retail surveillance provides a measure of human exposure to antimicrobial resistant bacteria through the consumption of meat products available for purchase by Canadian consumers. The scope of the surveillance framework can be modified as necessary (e.g. to evaluate different food commodities, bacteria, or geographic regions) and functions as a research platform for investigation of specific questions regarding antimicrobial resistance in the agri-food sector.

The unit of concern in *Retail Meat Surveillance* in 2012 was the bacterial isolate cultured from one of the commodities of interest. In this situation, the commodities were raw meat products commonly consumed by Canadians, which originated from the 3 animal species sampled in the *Abattoir Surveillance* component as well as turkey beginning in 2012. These raw meat products consisted of chicken (legs or wings [skin on]), turkey (ground), pork (chops), and beef (ground).

For ground beef, a systematic collection of extra-lean, lean, medium, and regular ground beef was performed to ensure representation of the heterogeneity of ground beef with respect to its origins (e.g. domestic vs. imported beef or raised beef cattle vs. culled dairy cattle". The meat cuts "legs or wings with skin on", "ground turkey", "pork chops", and "ground beef" were chosen on the basis of suspected high prevalences of the targeted bacterial species within and the low purchase prices of these commodities[3] and for comparability to other international retail surveillance programs .

Bacteria of interest in chicken and turkey were *Campylobacter*, *Salmonella*, and generic *E. coli*. In pork both *Salmonella* and *E. coli* were cultured, but only isolates of *E. coli* underwent antimicrobial susceptibility testing for routine surveillance. *Salmonella* was isolated from pork mainly to provide recovery estimates from this commodity for other Public Health Agency of Canada programs. Because the prevalence of *Salmonella* in pork is low, antimicrobial susceptibility results are not presented on an annual basis but are pooled and presented over a multi-year period in the interest of precision. Recovery of *Campylobacter* from pork was not

[3] Ravel A. Antimicrobial Surveillance in food at retail – Proposal for a pilot project. 2002. 13 pp.

attempted because of the low prevalence observed in the initial stages of *Retail Meat Surveillance*. In beef, only *E. coli* was cultured and then tested for antimicrobial susceptibility given the low prevalence of *Campylobacter* and *Salmonella* in these commodities at the retail level, as determined during the early phase of the program. In turkey, *Campylobacter*, *Salmonella*, and *E. coli* were isolated from retail samples.

SAMPLING METHODS

Generally, the sampling protocol was designed to evaluate antimicrobial resistance in certain bacterial species that contaminate retail meat and to which Canadian consumers may subsequently be exposed. In 2012, it primarily involved continuous weekly submission of samples of retail meat from randomly selected geographic areas (i.e. census divisions defined by Statistics Canada), weighted by population, in each participating province.

Retail meat samples were collected in British Columbia, Saskatchewan, Ontario, and Québec. In past years retail data have been presented for the Maritimes (a region including the provinces of New Brunswick, Nova Scotia, and Prince Edward Island). In 2012, due to unforeseeable delays with respect to resuming sampling, very few retail samples were collected and thus, data from the Maritimes region are not presented in the 2012 Annual Report. Retail data for this region will be presented again in the 2013 Annual Report.

Data from Statistics Canada were used to define strata. This was done by using cumulative population quartiles (or thirdtiles) from a list of census divisions in a province, sorted by population in ascending order. Generally, between 15 and 18 census divisions per province/region were then chosen by means of stratified random selection and weighted by population within each stratum. The number of sampling days allocated to each stratum was also weighted by population and is summarized as follows:

ONTARIO and QUÉBEC

- Stratum One: 10 divisions selected, with 2 sampling days per division per year
- Stratum Two: 4 divisions selected, with 5 sampling days per division per year
- Stratum Three: 2 divisions selected, with 10 sampling days per division per year
- Stratum Four 1 division selected, with 20 sampling days per year

SASKATCHEWAN

- Stratum One: 9 divisions selected, with 2 sampling days per division per year
- Stratum Two: 5 divisions selected, with 3 sampling days per division per year
- Stratum Three: 2 divisions selected, with 5 sampling days per division per year
- Stratum Four: 1 division selected, with 7 sampling days per year

BRITISH COLUMBIA

- Stratum One: 10 divisions selected, with 1 sampling day per division per year
- Stratum Two: 4 divisions selected, with 3 sampling days per division per year
- Stratum Three: 1 division selected, with 20 sampling days per year

MARITIMES PROVINCES

- For the 3 Maritimes provinces, results are aggregated and presented at the Maritimes region level; however, sampling activities for this region were proportional to the population within each province as indicated below. Furthermore, as with the other provinces sampled in the retail component, sampling within each province was proportional to the census division subpopulations and is summarized as follows:
- Nova Scotia
    - Stratum One: 5 divisions selected, with 1 sampling day per division per year (on average)
    - Stratum Two: 4 divisions selected, with 2 sampling days per division per year
    - Stratum Three: 1 division selected, with 10 sampling days per division per year
- New Brunswick
    - Stratum One: 5 divisions selected, with 1 sampling day per division per year (on average)
    - Stratum Two: 4 divisions selected, with 2 sampling days per division per year
    - Stratum Three: 2 divisions selected, with 4 sampling days per division per year (on average)
- Prince Edward Island
    - Stratum One: 1 division selected, with 1 sampling day per division per year
    - Stratum Two: 1 division selected, with 2 sampling days per division per year

Generally, field workers in Ontario and Québec conducted sampling on a weekly basis, and those in British Columbia, Saskatchewan, and Maritimes region (no retail data presented for this region in 2012) conducted sampling every other week. Sampling was less frequent in British Columbia, Saskatchewan, and the Maritimes region (very sparse number of samples for this region in 2012) because of funding constraints, limited laboratory capacity, and a desire to avoid over-sampling at particular stores. Samples were collected on Mondays or Tuesdays for submission to the laboratory by Wednesday. Samples submitted from outside Québec (with the exception of samples from the Maritimes region) were sent to the same laboratory via 24-hour courier. In the rare sampling weeks for the Maritimes region in 2012, samples from the whole Maritimes region were collected on Mondays or Tuesdays and submitted to a laboratory in Prince Edward Island within 24 hours.

In each province, 2 census divisions were sampled each sampling week. In each census division, 4 stores were selected prior to the sampling day, based on store type. Generally, 3 chain stores and 1 independent market or butcher shop were selected. An exception to this protocol was made in densely populated urban census divisions (e.g. Toronto or Montréal), where 2 chain stores and 2 independent markets or butcher shops were sampled to reflect the presumed shopping behaviour of that subpopulation. From each store type, 1 sample of each commodity of interest was attempted, for a desired total of 15 meat samples (4 chicken, 4 turkey, 4 pork, and 3 beef samples) per division per sampling day[4]. When possible, specific stores were sampled only once per sampling year. In some cases due to reduced availability of certain meats and store closures *etc*., the desired sample yield was not achieved.

Prevalence estimates were used to determine the numbers of samples to be collected, which were based on an expected yield of 100 isolates per commodity per province per year, plus 20% to account for lost or damaged samples. Because sampling was less frequent in British Columbia, Saskatchewan, and the Maritimes region than in Ontario and Québec, the target of 100 isolates per year may not have always been met in those provinces/region.

In 2012, personal digital assistants (PDAs) were used to capture the following store and sample data:

- Type of store
- Number of cash registers (surrogate measure of store volume)
- "Sell-by" or packaging date
- "May contain previously frozen meat" label – yes or no
- Final processing in store – yes, no, or unknown
- Air chilled – yes, no, or unknown (applied to chicken samples only)
- Organic – yes, no, or unknown
- Antimicrobial free – yes, no, or unknown
- Price per kilogram

Individual samples were packaged in sealed zipper-type bags and placed in 16-L thermal coolers for transport. The ambient environmental temperature was used to determine the number of ice packs placed in each cooler (i.e. 1 ice pack for temperatures below 20°C and 2 ice packs for temperatures 20°C or higher). In 1 or 2 coolers per sampling day, instruments for recording temperature data[5] were used to monitor temperatures to which samples were exposed.

[4] At 1 store in each division, the beef sample was not collected to minimize over-sampling of this commodity.
[5] Ertco Data Logger™, West Patterson, NJ, USA

ABATTOIR SURVEILLANCE

OBJECTIVE(S)

The objectives of the CIPARS *Abattoir Surveillance* component are to provide nationally representative, annual antimicrobial resistance data for bacteria isolated from animals entering the food chain, and to monitor temporal variations in the prevalence of antimicrobial resistance in these bacteria.

SURVEILLANCE DESIGN

Abattoir Surveillance only includes animals that originated from premises within Canada. Established in September 2002, this component initially targeted generic *Escherichia coli* and *Salmonella* within the food animal commodities associated with the highest per capita meat consumption: beef cattle, broiler chickens, and pigs. In 2003, the component was refined to discontinue *Salmonella* isolation from beef cattle because of the low prevalence of *Salmonella* in that population. *Campylobacter* surveillance was initiated in beef cattle in late 2005 in order to include a pathogen in beef cattle surveillance and to provide data on fluoroquinolone resistance, following the approval of a fluoroquinolone for use in cattle. *Campylobacter* surveillance was also initiated in chickens in 2010 and pigs in 2012.

In the *Abattoir Surveillance* component, the unit of concern (i.e. the subject of interest) was the bacterial isolate. The bacteria of interest were isolated from the caecal contents (not carcasses) of slaughtered food animals to avoid misinterpretation related to cross-contamination and to better reflect antimicrobial resistance in bacteria that originated on the farm.

Over 90% of all food-producing animals in Canada are slaughtered in federally inspected abattoirs annually[6]. The program is based on the voluntary participation of federally inspected slaughter plants from across Canada. The sampling method was designed with the goal that, across Canada, 150 isolates of each targeted bacterial species would be recovered from each of the 3 animal species over a 12-month period. The exception was *Campylobacter* in beef cattle, for which it was estimated that 100 isolates would be recovered over the same period. These numbers represented a balance between acceptable statistical precision and affordability[7]. The actual number of samples collected was determined for each food animal species on the basis of the expected caecal prevalence of the bacteria in that animal species. For example, if the expected bacterial prevalence was 10%, then 1,500 samples would need to be collected and submitted for bacterial isolation.

[6] Agriculture and Agri-Food Canada. Red meat market information. Available at: www.agr.gc.ca/redmeat-vianderouge/index_eng.htm. Accessed May 2013.

[7] Ravel A. Development of the Canadian antimicrobial resistance surveillance system (agri-food sector) – sampling design options. Presented to the National Steering Committee on Antimicrobial Resistance in Enterics, Canada, 2001. 79 pp.

The sampling design was based on a 2-stage sampling plan, with each commodity handled separately. The first stage consisted of random selection of federally inspected slaughterhouses. The probability of an abattoir being selected was proportional to its annual slaughter volume. The second stage involved systematic selection of animals on the slaughter line. The annual number of caecal samples collected at each abattoir was proportional to its slaughter volume.

SAMPLING METHODS

To minimize shipping costs and allow each abattoir to maintain efficiency, the annual total number of samples to be collected in each abattoir was divided by 5, resulting in the number of collection periods. For each collection period, 5 caecal samples were collected within 5 days, at the convenience of the slaughterhouse staff, provided the 5 animals and associated samples originated from different groups. Sampling from different groups of animals was important to maximize diversity and avoid bias attributable to overrepresentation of particular producers. The largest plants were scheduled to sample up to 7 animals from different groups over the 5 day collection period in order to achieve the required number of samples annually. Collection periods were uniformly distributed throughout the year, leading to an abattoir-specific schedule for collection of caecal contents. The uniform distribution of the collection periods helped to avoid any bias that may have resulted from seasonal variation in bacterial prevalence and antimicrobial susceptibility test results.

Thirty-seven federally inspected slaughter plants (4 beef cattle plants, 22 poultry plants, and 11 swine plants) from across Canada participated in the 2012 CIPARS *Abattoir Surveillance* component. Samples were obtained according to a predetermined protocol, with modifications to accommodate various production-line configurations in the different plants. Protocols were designed to avoid conflict with carcass inspection methods, plant-specific Food Safety Enhancement Programs, and Health and Safety requirements. They were also designed to avoid situations of potential cross-contamination. All samples were collected by industry personnel under the oversight of the Veterinarian-in-Charge of the Canadian Food Inspection Agency.

FARM SURVEILLANCE

OBJECTIVE(S)

The objectives of the CIPARS *Farm Surveillance* component are to provide data on antimicrobial use and resistance, to monitor temporal trends in the prevalence of antimicrobial resistance, to investigate associations between antimicrobial use and resistance on grower-finisher pig farms, and to provide data for human health risk assessments.

SURVEILLANCE DESIGN

The *Farm Surveillance* component was the third active surveillance component implemented by CIPARS. Taken together, with the Abattoir and Retail Surveillance components, these data validate the information collected at key points along the farm-to-fork food production chain. This initiative is built on a sentinel farm framework. Questionnaires are used to collect data on farm demographics, animal health and antimicrobial use. Composite pen fecal samples are collected and submitted to laboratories for bacterial isolation and antimicrobial susceptibility testing. The CIPARS *Farm Surveillance* component is administered and coordinated by the Laboratory for Foodborne Zoonoses.

CIPARS *Farm Surveillance* component was initiated in 2006 in the 5 major pork-producing provinces in Canada (Alberta, Saskatchewan, Manitoba, Ontario, and Québec). The swine industry was selected as the pilot commodity for development of the farm surveillance infrastructure because the Canadian Quality Assurance (CQA®) program had been extensively implemented by the industry and because, in 2006, unlike in the other major livestock commodities, there had not been a recent outbreak of foreign animal disease in pigs.

The *Farm Surveillance* component concentrates on grower-finisher hogs. Pigs in this stage of production were chosen because of their proximity to the consumer.

SAMPLING METHODS

Swine veterinarians recruited sentinel herds to participate in this voluntary national surveillance program. The number of sentinel herds allocated to each of the 5 participating provinces was proportional to the national total of grower-finisher units, except in Alberta, where 10 additional sentinel herds were included. Support for the 10 extra herds, and laboratory testing for all samples collected from the CIPARS sentinel herds in Alberta was provided by the Alberta Agriculture and Rural Development Agri-Food laboratory.

To preserve the anonymity of participating producers, herd veterinarians collected the samples and data and submitted coded information to the Public Health Agency of Canada. In the case of corporate herds, 2 noncorporate supervisory veterinarians ensured confidentiality by holding the key to corporate herd codes. This step was taken because knowing a corporate veterinarian's name could have identified the corporation associated with the herd, thereby breaking anonymity.

Veterinarians were purposively selected from the list of veterinarians practicing swine medicine in each province. Each veterinarian selected a predetermined number of sentinel farm sites by use of specific inclusion and exclusion criteria. To be included, herds were required to be CQA® validated, produce more than 2,000 market pigs per year, and be representative of the characteristics (i.e. similar production volumes and types of production systems) and geographic distribution of herds in the veterinarian's swine practice. Herds were excluded when they were regarded as organic with respect to animal husbandry, were fed edible residual material, or were raised on pasture. These criteria helped ensure that the herds enrolled were representative of most grower-finisher swine herds in Canada.

Sentinel grower-finisher herds were visited once per year for sample and data collection. Pooled fecal samples were collected from 6 pens of pigs that were close to market weight (i.e. more than 80 kg [175 lb]).

SURVEILLANCE OF ANIMAL CLINICAL ISOLATES

OBJECTIVE(S)

The objective of *Surveillance of Animal Clinical Isolates* is to detect emerging antimicrobial resistance patterns as well as new serovar/resistance pattern combinations in *Salmonella*.

SURVEILLANCE DESIGN

This component of CIPARS relies on samples that are typically collected and submitted to veterinary diagnostic laboratories by veterinarians and/or producers. Consequently, sample collection and submission as well as *Salmonella* isolation techniques varied among laboratories over the year.

Salmonella isolates were sent by provincial and private animal health laboratories from across the country to the *Salmonella* Typing Laboratory (STL) at the Laboratory for Foodborne Zoonoses, Guelph, Ontario (LFZ-Guelph) with the exception of Québec, where isolates from animal health laboratories were sent to the Laboratoire d'épidémiosurveillance animale du Québec, du ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec for serotyping. Isolates and serotyping results from Québec were then forwarded to the LFZ-Guelph to perform phage typing and antimicrobial resistance testing. Not all isolates received by provincial animal health laboratories were forwarded to the LFZ-Guelph, with the exception of isolates received by laboratories in British Columbia, Ontario, Québec, and Prince Edward Island. Therefore, coverage may have varied considerably among provinces.

Samples may also have been collected from animal feed, the animal's environment, or non-diseased animals from the same herd or flock. Reported here are results from chicken, turkey, cattle, pigs, and horses. Cattle isolates could have originated from dairy cattle, milk-fed or grain-fed veal, or beef cattle. Chicken isolates were largely from layer hens or broiler chickens, but could also have been from primary layer breeders or broiler breeder birds. A proportion of the turkey isolates might have been recovered from turkey-related environmental samples.

FEED AND FEED INGREDIENTS

SAMPLING DESIGN

Data from the *Feed and Feed Ingredients* component of CIPARS were obtained from various sources, including monitoring programs of the Canadian Food Inspection Agency (CFIA) and a few isolates from provincial authorities. Information on specimen collection methods was only available for the CFIA monitoring programs.

The CFIA collects samples of animal feed under 2 different programs: Program 15A (Monitoring Inspection – *Salmonella*) and Program 15E (Directed Inspection – *Salmonella*). Under Program 15A, feeds produced at feed mills, rendering facilities, ingredient manufacturers, and on-farm facilities are sampled and tested for *Salmonella*. Although this program makes use of a random sampling process, extra attention is paid to feeds that are more likely to have a higher degree of *Salmonella* contamination, such as those that contain rendered animal products, oilseed meals, fish meals, grains, and mashes. Program 15E targets feeds or ingredients from establishments that (i) produce rendered animal products, other feeds containing ingredients in which Salmonella could be a concern (e.g. oilseed meal or fishmeal), or a significant volume of poultry feed; (ii) are known to have repeated problems with *Salmonella* contamination; or (iii) have identified a *Salmonella* serovar that is highly pathogenic (e.g. Typhimurium, Enteritidis, or Newport). Program 15E is a targeted program; samples are not randomly selected.

BACTERIAL ISOLATION METHODS

All samples were cultured by use of standard protocols as described below. All primary isolation of human *Salmonella* isolates was conducted by hospital-based or private clinical laboratories in participating provinces. Most primary isolation of *Escherichia coli, Salmonella*, and *Campylobacter* from agri-food samples was conducted at the Laboratory for Foodborne Zoonoses, Saint-Hyacinthe. Primary isolation for *Retail Meat Surveillance* in Prince Edward Island was conducted at the Atlantic Veterinary College, University of Prince Edward Island. Part of the primary isolation for *Farm Surveillance* was conducted at the Agri-Food Laboratory of the Alberta Agriculture and Rural Development. Samples from the CIPARS *Animal Clinical Isolates* component were cultured by various participating laboratories. Most primary bacterial isolation from *Feed and Feed Ingredients* samples was conducted by the CFIA – Laboratory Services Division (Calgary or Ottawa).

SALMONELLA

SURVEILLANCE OF HUMAN CLINICAL ISOLATES

Hospital-based and private clinical laboratories isolated and identified *Salmonella* from human samples according to approved methods[8,9,10,11].

SURVEILLANCE OF AGRI-FOOD ISOLATES (*Retail Meat Surveillance*, *Abattoir Surveillance* and *Farm Surveillance*)

The method used to isolate *Salmonella* was a modification of the MFLP-75 method[12]. This method allowed isolation of viable and motile *Salmonella* from fecal (*Farm Surveillance*), caecal (*Abattoir Surveillance*) content, and meat (*Retail Meat Surveillance*) from agri-food samples. It is based on the ability of *Salmonella* to multiply and be motile in modified semi-solid Rappaport Vassiliadis (MSRV) medium at 42°C.

***Retail Meat Surveillance*:** Depending on the sample type either 1 chicken leg[13], 1 pork chop or 25 g of ground turkey was added to 225 mL of Buffered Peptone Water (BPW). One hundred milliliters of the peptone rinse were kept for *Campylobacter* and/or *E. coli* isolation. Chicken and turkey samples were left in the remaining volume of peptone rinse and incubated at 35 ± 1°C for 24 hours. Afterward, a MSRV plate was inoculated with 0.1 mL of the rinse and incubated at 42 ± 1°C for 24 to 72 hours. Suspect colonies were screened for purity and used to inoculate triple-sugar-iron and urea agar slants. Presumptive *Salmonella* isolates were assessed using the indole test, and their identities were verified by means of slide agglutination with *Salmonella* Poly A-I and Vi antiserum.

***Abattoir Surveillance* and *Farm Surveillance*:** A 10-g portion of each pig cecal or fecal sample was mixed with 90 mL of BPW. Chicken caecal contents were weighed and mixed with BPW at a ratio of 1:10. Samples were incubated at 35 ± 1°C for 24 hours. Afterward, the method used was the same as the one described in the *Salmonella* – *Retail Meat Surveillance* section.

SURVEILLANCE OF ANIMAL CLINICAL ISOLATES

Salmonella was isolated according to standard procedures, which varied among laboratories. Most methods for detecting *Salmonella* in animal clinical isolates were similar in principle and

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[8] Kauffman F. The Bacteriology of Enterobacteriaceae. Baltimore: Williams and Wilkins Co, 1966.
[9] Ewing WH. Edwards and Ewing's Identification of Enterobacteriaceae. 4th ed. New York: Elsevier Science Publishing Co, 1986.
[10] Le Minor L. Guidelines for the preparation of *Salmonella* antisera. Paris, France: WHO Collaborating Centre for Reference and Research on *Salmonella*, Pasteur Institute, 2001.
[11] Murray PR, Baron EJ, Pfaller MA, et al, eds. Manual of Clinical Microbiology. 8th ed. Washington DC, ASM Press, 2005.
[12] Compendium of Analytical Methods, Health Protection Branch, Methods of Microbiological Analysis of Food, Government of Canada.
[13] When legs with skin on were not available, wings with skin on or other cuts were purchased instead.

involved pre-enrichment, selective enrichment, differential and selective plating, isolation, and biochemical and serological confirmation of the selected isolates.

FEED AND FEED INGREDIENTS

Under both Canadian Food Inspection Agency programs (15A and 15E), all samples were collected aseptically and submitted for bacterial culture and isolation. For *Salmonella* isolation, MSRV medium was used.

ESCHERICHIA COLI

RETAIL MEAT SURVEILLANCE

Fifty milliliters of the peptone rinse prepared as stated in the S*almonella – Retail Surveillance* section were mixed with 50 mL of double strength EC Broth and incubated at 45 ± 1°C for 24 hours. One loopful of the mixture was then streaked onto Eosin Methylene Blue agar and incubated at 35 ± 1°C for 24 hours. Suspect colonies were screened for purity and transferred onto trypticase soy agar with 5% sheep blood. Presumptive *E. coli* colonies were assessed using Simmons citrate and indole tests. The *E. coli* isolates with negative indole test results were confirmed using a bacterial identification test kit[14].

ABATTOIR AND FARM SURVEILLANCE

One drop of the peptone mixture prepared as stated in the *Surveillance of Agri-Food Isolates*/*Salmonella – Abattoir and Farm Surveillance* section was streaked onto MacConkey agar and incubated at 35°C for 18 to 24 hours. Suspect lactose-fermenting colonies were screened for purity and transferred onto Luria-Bertani agar. Presumptive *E. coli* colonies were assessed as in the *Retail Meat Surveillance* for *E. coli*.

CAMPYLOBACTER

RETAIL MEAT SURVEILLANCE

Fifty milliliters of the peptone rinse prepared as stated in the *Salmonella – Retail Surveillance* section were mixed with 50 mL of double-strength Bolton broth and incubated in a microaerophilic atmosphere at 42 ± 1°C for 44 to 48 hours. A loopful of broth was then streaked onto a modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) plate and incubated in a microaerophilic atmosphere at 42 ± 1°C for 24 to 72 hours. Suspect colonies were streaked onto a second mCCDA and on a Mueller Hinton agar plate. Both plates were incubated in a microaerophilic atmosphere at 42 ± 1°C for 24 to 48 hours. Presumptive *Campylobacter* colonies were identified using the following tests: Gram stain, oxidase, and catalase. A

[14] API® 20E system

multiplex PCR (mPCR)[15] was used to speciate colonies. Specific genomic targets (hippuricase in *C. jejuni* and aspartokinase in *C. coli*) were amplified by mPCR from bacterial lysates. Products were visualized on agarose gel and identified based on their specific molecular size. An internal universal control (16s rRNA) was incorporated into the PCR method. The priming oligonucleotides used in the PCR were highly specific for *C. jejuni* or *C. coli* and will not amplify DNA present in any other *Campylobacter* spp. or non-*Campylobacter* organisms. Unidentified species of *Campylobacter* are collectively referred to in the CIPARS reports as "other *Campylobacter* spp." However, when used alone, the term "*Campylobacter*" refers to all *Campylobacter* species.

ABATTOIR SURVEILLANCE

One milliliter of BPW mixture prepared as stated in the *Salmonella* – Abattoir and Farm section was mixed with 9 mL of Hunt's enrichment broth (HEB) and incubated in a microaerophilic atmosphere at 35 ± 1°C for 4 hours. After this first incubation, 36 µL of sterile cefoperazone were added to the HEB tubes which were then sent back to microaerophilic incubation, this time at 42 ± 1°C for 20 to 24 hours. A loopful of HEB was then used to inoculate a mCCDA plate which was incubated at 42 ± 1°C in microaerophilic conditions for 24-72 hours. Suspect colonies were assessed as in the *Campylobacter - Retail Meat Surveillance* section.

SEROTYPING AND PHAGE TYPING METHODS FOR *SALMONELLA*

SURVEILLANCE OF CLINICAL HUMAN ISOLATES

In general, clinical laboratories forwarded their *Salmonella* isolates to their Provincial Public Health Laboratory (PPHL) for identification and serotyping. The PPHL further forwarded *Salmonella* isolates to the National Microbiology Laboratory (NML) according to the predefined testing protocol. Isolate identities were confirmed by the NML when isolates received did not have a serovar name[16] or when inconclusive results arose during phage typing. The O or somatic antigens of the *Salmonella* isolates were serotyped by use of a slide agglutination method[17]. At the NML, *Salmonella* H or flagellar antigens were detected via slide and confirmatory tube agglutination methods. *Salmonella* isolates were maintained at room temperature (25° to 35°C) until typed.

[15] The multiplex PCR speciation of *Campylobacter jejuni* and *Campylobacter coli* was based on the following published method. Persson S, KE Olsen. Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejuni* from pure cultures and directly on stool samples. J Med Microbiol 2005; 54:1043–1047.

[16] Le Minor L, Popoff M. Antigenic formulas of the *Salmonella* serovars. 8th revision. Paris, France: WHO Collaborating Centre for Reference and Research on *Salmonella*, Pasteur Institute, 2001.

[17] Ewing WH. Edwards and Ewing's Identification of Enterobacteriaceae. 4th ed. New York: Elsevier Science Publishing Co, 1986.

Phage typing was performed at the NML for isolates of the following *Salmonella* serovars: Enteritidis, Heidelberg, Typhimurium, Hadar, Newport, Typhi, Paratyphi A, Paratyphi B[18], Paratyphi B var. L(+) tartrate (+), Infantis, Thompson, Oranienburg, Panama, I 4,[5],12:b:-, and I 4,[5],12:i:-. For phage typing the standard technique described by Anderson and Williams[19] was followed. Isolates were streaked onto nutrient agar plates and incubated at 37°C for 18 hours. One smooth colony was selected and used to inoculate 4.5 mL of phage broth[20], which was then incubated for 1.5 to 2 hours in a shaking water bath at 37°C to attain bacterial growth with a turbidity equivalent to 0.5-McFarland standard. Phage agar plates[21] were flooded with approximately 2 mL of culture medium, and the excess liquid was removed with a Pasteur pipette. Flooded plates were allowed to dry for 15 minutes at room temperature. Afterward, approximately 20 µL of each serovar-specific typing phage was used to inoculate the bacterial lawn by means of a multiple inoculating syringe method[22]. The plates were incubated at 37°C overnight, and lytic patterns were subsequently interpreted[23].

Salmonella Enteritidis strains were phage typed with typing phages obtained from the International Centre for Enteric Phage Typing (ICEPT), Central Public Health Laboratory, Colindale, UK[24]. The phage-typing protocol and phages for *Salmonella* Typhimurium, developed by Callow[25] and further extended by Anderson[26] and Anderson and colleagues[27] were obtained from the ICEPT. The *Salmonella* Heidelberg phage typing protocol and phages were supplied by the NML[28]. Isolates that reacted with the phages but did not conform to any recognized phage type were designated as atypical. Strains that did not react with any of the typing phages were designated as untypable.

The Identification and Serotyping and the Phage Typing units at the NML have attained International Standards Organization (ISO) 17025 accreditation by the Standards Council of Canada. These identification and Serotyping, Phage Typing, and Antimicrobial Resistance units participate in the annual Global Food-borne Infections Network (WHO-GFN), External Quality Assurance System of the World Health Organization, the Enter-net (a European network for the

[18] *Salmonella* Paratyphi B does not include *S*. Paratyphi B var. L (+) tartrate (+), formerly called *S*. Paratyphi var. Java. The biotype of *S*. Paratyphi B included here is tartrate (-) and associated with severe typhoid-like fever. *Salmonella* Paratyphi B var. L (+) tartrate (+) is commonly associated with gastrointestinal illness.

[19] Anderson E, Williams R. Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. J Clin Pathol 1956;9:94–127.

[20] Difco™ phage broth, Difco Laboratories, Baltimore, MD; pH, 6.8

[21] Difco™ phage agar, Difco Laboratories

[22] Farmer J, Hickman F, Sikes J. Automation of *Salmonella* typhi phage-typing. Lancet 1975;2(7939):787–790.

[23] Anderson E, Williams R. Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. J Clin Pathol 1956;9:94–127.

[24] Ward L, de Sa J, Rowe B. A phage-typing scheme for *Salmonella* Enteritidis. Epidemiol Infect 1987;99:291–294.

[25] Callow B. A new phage typing scheme for *Salmonella* Typhimurium. J Hyg (Lond) 1959;57:346–359.

[26] Anderson E. The phagetyping of *Salmonella* other than *S*. Typhi. In: Van Oye E, ed. The World Problem of Salmonellosis. The Hague, The Netherlands: Dr W. Junk Publishers, 1964;89–100.

[27] Anderson E, Ward L, de Saxe M, et al. Bacteriophage-typing designations of *Salmonella* Typhimurium. J Hyg (Lond) 1977;78:297–300.

[28] Demczuk W, Soule G, Clark C, et al. Phage-based typing scheme for *Salmonella* enterica serovar Heidelberg, a causative agent of food poisonings in Canada. J Clin Microbiol 2003;41:4279–4284.

surveillance of human gastrointestinal infections) proficiency program for *Salmonella*, and a strain exchange with the Laboratory for Foodborne Zoonoses (*Salmonella* and *Escherichia coli*). The NML and the Centre for Foodborne, Environmental and Zoonotic Infectious Diseases have been a strategic planning members of the WHO-GFN program since 2002.

SURVEILLANCE OF AGRI-FOOD, ANIMAL CLINICAL AND FEED ISOLATES

Animal clinical *Salmonella* isolates from Québec were serotyped at the Laboratoire d'épidémiosurveillance animale du Québec, du ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec and were sent to the STL[29] for phage typing.

All *Salmonella* isolates from other provinces were submitted to the STL for serotyping and phage typing. The serotyping method detects O or somatic antigens of the *Salmonella* isolates via slide agglutination[30]. The H or flagellar antigens were identified with a microtitre plate well precipitation method[31]. The antigenic formulae of the *Salmonella* serovars as reported by Grimont and Weill[32] were used to identify and name the serovars.

For phage typing, the standard technique by Anderson and Williams[33] and described above was followed. The sources of the typing phages for *Salmonella* Enteritidis, Typhimurium and Heidelberg were the same as described above for *Surveillance of Human Clinical Isolates*.

Since 1995, the STL has participated in annual inter-laboratory exchange of serotyping panels with up to 3 other laboratories. The STL began external proficiency testing of the accuracy of phage typing in 2003. Every year, the STL participates successfully in phage typing proficiency panels from the Central Public Health Laboratory, Colindale, United Kingdom.

ANTIMICROBIAL SUSCEPTIBILITY TESTING METHODS

All *Salmonella* isolates of human origin were tested for antimicrobial susceptibility at the National Microbiology Laboratory (NML) and all isolates of agri-food or feed origin were tested for antimicrobial susceptibility at the Laboratory for Foodborne Zoonoses, Guelph, Ontario (LFZ-Guelph). The majority of *Campylobacter* and *Escherichia coli* isolates from all agri-food components were tested at the Laboratory for Foodborne Zoonoses, Saint-Hyacinthe, Québec

[29] Office Internationale des Épizooties (OIÉ); All World Organisation for Animal Health, Reference Laboratory for Salmonellosis, Guelph, Ontario.
[30] Ewing WH. Edwards and Ewing's Identification of Enterobacteriaceae. 4th ed. New York: Elsevier Science Publishing Co, 1986.
[31] Shipp C, Rowe B. A mechanised microtechnique for *Salmonella* serotyping. J Clin Pathol 1980;33:595–597.
[32] Grimont PAD, Weill FX. Antigenic Formulae of the *Salmonella* Serovars. 9th ed. Cedex, France: Collaborating Center for Reference and Research on *Salmonella*, Institut Pasteur, 2007.
[33] Anderson E, Williams R. Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. J Clin Pathol 1956;9:94–127.

(LFZ-Saint-Hyacinthe). In most instances, only 1 isolate per positive sample was submitted for antimicrobial susceptibility testing. In the case of *Farm Surveillance*, antimicrobial susceptibility testing was performed on 3 *E. coli* isolates, and 1 *Salmonella* isolate per sample. All *E. coli* isolates from *Retail Meat Surveillance* in Prince Edward Island were processed at the Atlantic Veterinary College, University of Prince Edward Island. Whereas a portion of *E. coli* isolates from *Farm Surveillance* in Alberta and Saskatchewan were processed by the Agri-Food Laboratory Branch, Alberta Agriculture and Rural Development.

The NML is a World Health Organization Collaboration Centre for Preparedness and Response to Enteric Pathogens and their Antimicrobial Resistance. The LFZ-Guelph and LFZ-Saint-Hyacinthe, the Alberta Agriculture and Rural Development, and Atlantic Veterinary College participate in external proficiency programs for antimicrobial susceptibility testing for *Salmonella* and *E. coli*. The LFZ-Guelph and LFZ-Saint-Hyacinthe participate in inter-agency proficiency programs for identification and antimicrobial susceptibility testing of *Salmonella*, *E. coli*, and *Campylobacter* with the National Antimicrobial Resistance Monitoring System, United States (NARMS). The LFZ-Guelph laboratory is ISO/IEC 17025-accredited for antimicrobial sensitivity testing.

SALMONELLA AND *ESCHERICHIA COLI*

The minimum inhibitory concentration (MIC) values for *Salmonella* and *E. coli* were determined by means of the broth microdilution method[34] by use of an automated system[35]. This automated incubation and reading system uses microtitre plates containing various concentrations of dehydrated antimicrobials. The CMV2AGNF plate[36] was designed by the NARMS and contains 15 antimicrobials (Table 1).

Isolates were streaked onto a Mueller Hinton or MacConkey agar plate and incubated at 36 ± 1°C for 18 to 24 hours to obtain isolated colonies. One colony was chosen from the plate and re-streaked onto agar plates for growth. The plates were incubated at 36 ± 1°C for 18 to 24 hours. A 0.5-McFarland suspension was prepared by transferring bacterial growth from the agar plates into 5.0 mL of sterile, demineralized water. Ten microliters of the water-bacteria suspension were transferred to 10 mL of Mueller Hinton broth (MHB). This suspension was dispensed onto CMV2AGNF testing plates at 50 µL per well and the plates were sealed with adhesive plastic sheets. After an 18 hour incubation at 36 ± 1°C the plates were read automatically with fluorometric plate reading system[37]. In accordance with standards set by the Clinical and Laboratory Standards Institute (CLSI)[38], *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212 were used for quality assurance purposes to ensure validity of the MIC values.

[34] Clinical and Laboratory Standards Institute (CLSI) M7-A8
[35] Sensititre™, Automated Microbiology System, Trek™ Diagnostic Systems Ltd, West Sussex, England
[36] Sensititre™, Trek™ Diagnostic Systems Ltd, West Sussex, England
[37] ARIS™, Trek™ Diagnostic Systems Ltd, West Sussex, England
[38] CLSI M100-S22

CAMPYLOBACTER

The MIC values for *Campylobacter* were determined by means of the broth microdilution method[39]. The CAMPY plates[36] designed by NARMS and containing 9 dehydrated antimicrobials were used (Table 2). Colonies were streaked onto Mueller Hinton agar plates with 5% sheep blood and incubated in a microaerophilic atmosphere at 42 ± 1°C for 24 hours. A 0.5-McFarland suspension of bacterial growth was prepared by transferring selected bacterial colonies into a tube containing 5 mL of MHB. Afterward, 10 µL of the MHB were transferred to 11 mL of MHB with laked horse blood. The mixture was dispensed onto CAMPY plates at 100 µL per well. The plates were sealed with perforated adhesive plastic sheets. After a 24 hour incubation in microaerophilic atmosphere at 42 ± 1°C, plates were read using the Sensititre Vizion System[40]. *Campylobacter jejuni* ATCC 33560 was used as quality control organism. The MIC values obtained were compared with those of CLSI standards[41].

ANTIMICROBIAL SUSCEPTIBILITY BREAKPOINTS

Table 1. Antimicrobial susceptibility breakpoints for *Salmonella* and *Escherichia coli*; CMV2AGNF plate

	Antimicrobial	Range tested (µg/mL)	Breakpoints[a] (µg/mL)		
			S	I	R
I	Amoxicillin-clavulanic acid	1.0/0.5 – 32/16	≤ 8/4	16/8	≥ 32/16
	Ceftiofur[b]	0.12 – 8	≤ 2	4	≥ 8
	Ceftriaxone	0.25 – 64	≤ 1	2	≥ 4
	Ciprofloxacin	0.015 – 4	≤ 0.06	0.12 – 0.5	≥ 1
II	Ampicillin	1 – 32	≤ 8	16	≥ 32
	Azithromycin[c]	0.12 – 16	≤ 16	N/A	≥ 32
	Cefoxitin	0.5 – 32	≤ 8	16	≥ 32
	Gentamicin	0.25 – 16	≤ 4	8	≥ 16
	Kanamycin	8 – 64	≤ 16	32	≥ 64
	Nalidixic acid	0.5 – 32	≤ 16	N/A	≥ 32
	Streptomycin[c]	32 – 64	≤ 32	N/A	≥ 64
	Trimethoprim-sulfamethoxazole	0.12/2.38 – 4/76	≤ 2/38	N/A	≥ 4/76
III	Chloramphenicol	2 – 32	≤ 8	16	≥ 32
	Sulfisoxazole	16 – 512	≤ 256	N/A	≥ 512
	Tetracycline	4 – 32	≤ 4	8	≥ 16
IV					

Roman numerals I to IV indicate the ranking of antimicrobials based on importance in human medicine as outlined by the Veterinary Drugs Directorate.

S = Susceptible. I = Intermediate susceptibility. R = Resistant. N/A = Not applicable.

[a] Unless otherwise specified, CLSI M100-S22 was the reference used for all antimicrobials in the panel.

[b] CLSI M31–A3.

[c] No Clinical and Laboratory Standards Institute interpretive criteria for Enterobacteriaceae were available for this antimicrobial. Breakpoints were based on the distribution of minimal inhibitory concentrations and were harmonized with those of the National Antimicrobial Resistance Monitoring System, United States.

[39] CLSI M45-A2

[40] Sensititre Vizion System™, Trek™ Diagnostic Systems Ltd, West Sussex, England

[41] CLSI M45-A2

Table 2. Antimicrobial susceptibility breakpoints for *Campylobacter*; CAMPY plate

	Antimicrobial	Range tested (μg/mL)	Breakpoints[a] (μg/mL)		
			S	I	R
I	Ciprofloxacin	0.015 – 64	≤ 1	2	≥ 4
	Telithromycin[b]	0.015 – 8	≤ 4	8	≥ 16
II	Azithromycin[b]	0.015 – 64	≤ 2	4	≥ 8
	Clindamycin[b]	0.03 – 16	≤ 2	4	≥ 8
	Erythromycin	0.03 – 64	≤ 8	16	≥ 32
	Gentamicin[b]	0.12 – 32	≤ 2	4	≥ 8
	Nalidixic acid[b]	4 – 64	≤ 16	32	≥ 64
III	Florfenicol[bc]	0.03 – 64	≤ 4	N/A	N/A
	Tetracycline	0.06 – 64	≤ 4	8	≥ 16
IV					

Roman numerals I to IV indicate the ranking of antimicrobials based on importance in human medicine as outlined by the Veterinary Drugs Directorate.
S = Susceptible. I = Intermediate susceptibility. R = Resistant. N/A = Not applicable.
[a] CLSI M45-A2.
[b] No Clinical and Laboratory Standards Institute interpretive criteria for *Campylobacter* were available for this antimicrobial. Breakpoints were based on the distribution of minimal inhibitory concentrations and were harmonized with those of the National Antimicrobial Resistance Monitoring System.
[c] For florfenicol, only a susceptible breakpoint has been established. In this report, we therefore only report the proportion of isolates non-susceptible.

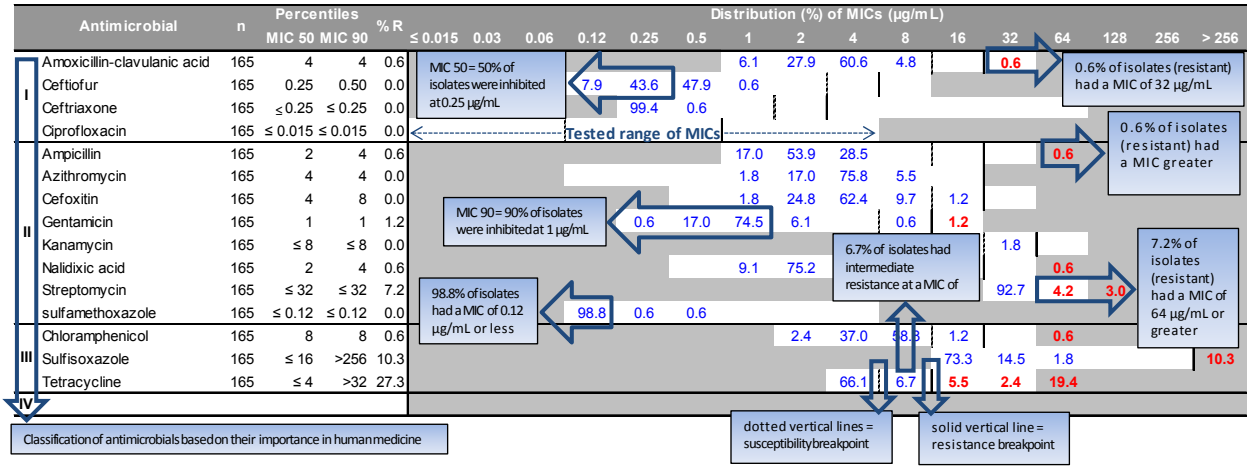
INTERPRETATION OF MINIMUM INHIBITORY CONCENTRATIONS (MICs)

The following information is important for the interpretation of tables presenting results on the distribution of MICs.

- Roman numerals I to IV indicate the ranking of antimicrobials based on importance in human medicine as outlined by the Veterinary Drugs Directorate, Health Canada.
- The unshaded fields indicate the range of concentrations tested for each antimicrobial in the test plate configuration.
- Red numbers indicate the percentage of isolates that were resistant to the antimicrobial according to the predefined resistance breakpoint.
- Numbers to the right of the highest concentration in the tested range (i.e. red numbers in shaded fields) represent the percentage of isolates with growth in all wells of the test plate within the tested range, indicating that the actual MICs were greater than the tested range of concentrations.
- Numbers at the lowest concentration in the tested range (i.e. blue numbers at the far left in unshaded fields) represent the percentage of isolates susceptible to the antimicrobial at the indicated or lower concentrations.
- Solid vertical lines represent resistance breakpoints.
- Dotted vertical lines represent susceptibility breakpoints.
- MIC 50 = MIC at which growth of 50% of isolates was inhibited by a specific antimicrobial.

- MIC 90 = MIC at which growth of 90% of isolates was inhibited by a specific antimicrobial.
- %R = Percentage of isolates that were resistant to a specific antimicrobial.

Table 3. Example on how to interpret minimum inhibitory concentration results



DATA ANALYSIS

HUMAN AND AGRI-FOOD SURVEILLANCE

DATA MANAGEMENT

Laboratory data from human and agri-food surveillance originated in 2 computer programs and were subsequently transferred to a central data repository using intermediary computer software[42]. Data were then transferred to a SAS® based harmonized database[43] called the Data Extraction and Analysis (DEXA) application. Additional antimicrobial resistance variables used for analysis are derived within the DEXA application; this application is also used as a central data access point. For the *Farm Surveillance* component of CIPARS, the bacterial species, serovar, and Minimum Inhibitory Concentration (MIC) data were maintained in a relational database[44].

[42] Oracle ®, Oracle Corp., Redwood Shores, CA, USA
[43] SAS® 9.3, SAS Institute Inc., Cary, NC, USA
[44] Microsoft® Access, Microsoft Corp., Redmond, WA, USA

DATA ANALYSIS

Data were analyzed with statistical softwares[45], and outputs were exported into a spreadsheet application[46]. All tables and figures were generated with the spreadsheet application[46].

For *Farm Surveillance*, statistical analyses were performed to account for clustering of antimicrobial resistance within swine herds through generalized estimating equations (GEE)[47]. All statistical models for pig farms included a binary outcome, logit-link function, and exchangeable correlation structure. Exact confidence intervals were computed by use of the BINOMIAL statement[48] and an alpha level of 0.05. When the prevalence was 0%, an alpha level of 0.1 was used instead. Null binomial response models were used to estimate the prevalence of resistance to each antimicrobial. From each null model, the intercept (β_0) and 95% confidence intervals were used to calculate population-averaged (i.e. GEE) prevalence estimates with the formula $[1 + \exp(-\beta_0)]^{-1}$.

RECOVERY RATE

For *Retail Meat Surveillance, Abattoir Surveillance,* and the *Farm Surveillance* components, recovery rate was defined as the number of positive culture results divided by the total number of samples submitted for culture.

RESISTANT ISOLATES

The percentage of isolates with resistance to antimicrobials was defined as the number of isolates resistant divided by the total number of isolates tested for each antimicrobial, multiplied by 100.

The breakpoints used for interpretation of antimicrobial susceptibility results are listed in Table 2 and Table 3. Intermediate MIC values were categorized as susceptible for all analyses. A new ceftriaxone breakpoint was officially adopted by the CLSI in January 2010. This breakpoint was applied to all data, including historical data, and was used when performing the analysis for the 2010 Annual Report. A new Enterobacteriaceae plate, CMV2AGNF, was utilized beginning in January 2011. Notable changes to the new plate included the removal of amikacin (Category II) and the inclusion of azithromycin (Category II). Additionally, in 2012, CIPARS decided to adopt a lower breakpoint (≥ 1 µg/mL) for ciprofloxacin than in past years (≥ 4 µg/mL) for both *Salmonella* and *E. coli*. Ciprofloxacin's new breakpoint was applied to all data, including historical data, and used for subsequent analysis. Resistance to ciprofloxacin is defined as having an MIC ≥ 1 µg/mL. All non-susceptible isolates (0.12-0.5 µg/mL) are interpreted as susceptible strains.

[45] SAS® 9.3; and Stata® 12 SE, Stata Corp., College Station, TX, USA
[46] Microsoft® Excel 2010, Microsoft Corp.
[47] PROC GENMOD, SAS® 9.3
[48] PROC FREQ, SAS® 9.3

RESISTANCE PATTERNS

The total number of antimicrobials in each resistance pattern was calculated by summing the number of antimicrobials to which each isolate was resistant. The most common resistance pattern may include patterns with only 1 antimicrobial. In this case, like for the most common patterns including 2 or more antimicrobials, the number of isolates reported includes only those resistant to this specific pattern (i.e. without any additional resistance to other antimicrobials).

PROVINCIAL INCIDENCE DATA IN HUMANS

For the provincial human incidence data, the number of *Salmonella* clinical cases in which a particular serovar was detected per 100,000 inhabitant-years was calculated by dividing the total number of isolates of each serovar received by CIPARS from that province by the provincial population (Statistics Canada post-census population estimates, January 1, 2005) and then multiplying by 100,000[49]. The national estimates for all serovars except *S*. Typhi and *S*. Newport were calculated as follows. In more heavily populated (or larger) provinces, the number of isolates resistant and the number of isolates submitted each month were multiplied by 2 as only isolates received in the first 15 days of the month were forwarded to CIPARS for testing. This provided us with an estimated total number of isolates resistant and estimated number of submissions for the larger provinces. Numbers of isolates resistant (estimated value in larger provinces or actual value in smaller provinces) for all provinces were summed to obtain the total estimated number of isolates resistant. Total numbers of isolates submitted (estimated value in larger provinces or actual value in smaller provinces) for all provinces were summed to obtain the total estimated number of submissions. Finally, the total estimated number of isolates resistant was divided by the total estimated number of submissions for each antimicrobial tested to obtain a national estimate of resistance for each antimicrobial and each serovar.

TEMPORAL ANALYSIS

Temporal analyses were performed for selected antimicrobials. Only 1 antimicrobial per antimicrobial class was selected among those antimicrobials commonly used in the agri-food and/or human sectors. Some antimicrobials were excluded from the temporal analyses for the following reasons:

- Resistance to the antimicrobial was absent or at a very low prevalence, or the breakpoint was debatable and other antimicrobials could be used to provide a surrogate measure of resistance or intermediate susceptibility (e.g. nalidixic acid for ciprofloxacin).
- The isolate was cross-resistant to another selected antimicrobial (e.g. amoxicillin-clavulanic acid and ceftiofur).

[49] Statistics Canada. Population by year, by province and by territory. Available at: www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/demo02a-eng.htm. Accessed May 2013.

- The antimicrobial has been banned for use in the agri-food sector, and resistance to this drug is maintained because of the use of another antimicrobial (e.g. chloramphenicol).

Logistic regression models (asymptotic or exact depending on prevalence of the outcome variable) were developed with year as an independent categorical variable. Data were analyzed with commercial software[50]. Analyses of *Farm Surveillance* data were adjusted for clustering at the herd level.

For all temporal analysis, the current proportion of isolates resistant to a specific antimicrobial has been compared to those observed during the first and the previous surveillance year. In a few specific instances, the first comparison year may vary to reflect the first year of surveillance as new regions were implemented (e.g. 2005 for retail data from Saskatchewan compared to 2003 for Ontario and Québec) or the implementation of new CIPARS components (e.g. 2006 for the *Farm Surveillance* component in pigs). For ampicillin and ceftiofur, special temporal analyses have been conducted in *E. coli* and *Salmonella* isolated from retail chicken or abattoir chickens to compare the current year's data with that of 2004 and 2006. This was due to a change in ceftiofur use practices by Québec chicken hatcheries in early 2005 and in 2007 (start and end of the voluntary period of withdrawal respectively). These special analyses were also conducted in human *Salmonella* Heidelberg isolates because this human serovar was suspected to originate from chicken. A value of $P \leq 0.05$ was considered significant for all temporal analyses.

[50] Stata ®12 SE

ANTIMICROBIAL USE

WHAT'S NEW

- The 2012 human antimicrobial use data are not included in this report but a summary of the 2012 and 2013 data will be presented in the *CIPARS 2013 Annual Report – Chapter 3. Antimicrobial Use*.
- The design or the methods of the CIPARS swine *Antimicrobial Use Farm Surveillance* component hasn't changed in 2012.
- The design or the methods of the *Surveillance of the antimicrobials distributed for sale for animals* (data provided by the Canadian Animal Health Institute – CAHI) hasn't changed in 2012.

HUMAN SURVEILLANCE

To be more timely with the analysis and distribution of the data, the 2012 human antimicrobial use data are not presented in this report but will be presented in the 2013 PHAC[51] report – Human Antimicrobial Use Annual Report and summarized in the *CIPARS 2013 Annual Report – Chapter 3. Antimicrobial Use*. The most recent information pertaining to the design and methods will be presented in the *CIPARS* report *Human Antimicrobial Use Report, 2011*.

FARM SURVEILLANCE

PIGS

FARM SWINE QUESTIONNAIRE

In the *Farm Surveillance* component of CIPARS, sentinel farm data were collected through questionnaires administered by the herd veterinarian (or designated staff) to the producer (or designated farm staff). The questionnaires collected data on antimicrobial use (AMU), herd demographics and animal health.

[51] Public Health Agency of Canada

Questions pertaining to the number of pigs in the population of interest differed by management system: continuous-flow or all-in-all-out. All-in-all-out management is a production system whereby animals are moved into and out of facilities in distinct groups. By preventing the commingling of groups, the hope is to reduce the spread of diseases. Facilities are normally cleaned and disinfected thoroughly between groups of animals. This type of management is generally by room or by barn. In continuous-flow operations, animals are continually being removed and added.

The AMU questionnaire was designed to collect data for herds of pigs in the grower-finisher production phase. No data on individual pigs were collected. Six pens representative of this population were selected for the collection of fecal specimens for bacterial culture and antimicrobial susceptibility testing. Thus, in herds with all-in-all-out management, the population of interest included all pigs that entered and exited the barn in the same group as the sampled pigs. The population of interest in herds with continuous-flow management was pigs that entered the grower-finisher unit with the sampled pigs.

Herd owners/managers were asked about AMU via feed, water, and injections. Data were collected on each diet fed to the specified group of pigs, including medicated and non-medicated feeds (non-medicated feeds did not contain antimicrobials). Information collected on each type of feed fed during the grow-finish period included the average number of weeks each ration was fed and the associated start and end pig weights. Additional information was collected for diets containing antimicrobials: active antimicrobial ingredient(s), their concentration(s) in the feed, and the primary reason(s) for that AMU (growth promotion, disease prevention, or treatment). Secondary AMU reasons were captured if the primary use was for disease prevention or treatment; secondary reasons included: respiratory disease, enteric disease, lameness or other diseases.

Data collected on exposure to antimicrobials through water or injection included active ingredient(s) in the drug(s) used, the reason(s) for use and the proportion of pigs exposed. The primary reasons for AMU in water included: disease prevention and disease treatment with associated secondary reasons for use being respiratory disease, enteric disease, lameness or other diseases. Only disease treatment reasons were collected for AMU administered by injection. The number of pigs exposed to AMU by water or injection were captured as categorical data with ranges of 1-25%, 26-50%, 51-75% or 76-100% of the pigs. No AMU data were collected for any production phase prior to the grower-finisher phase. Any data regarding AMU in pigs weighing less than 15 kg (33 lb) were excluded because this weight is considered below the industry standard for grower-finisher pigs.

DATA ANALYSIS

Data were entered into a PostGreSQL Database, and descriptive statistics were obtained with commercially available software[52].

[52] Microsoft Excel® 2003 and Microsoft Access® 2003, Microsoft Corp., Redmond, WA, USA; SAS® 9.1, SAS Institute Inc., Cary, NC, USA.

Antimicrobial exposures were summarized for each herd. An exposure was defined as any reported use of an active ingredient by a given route of administration in 2012. Data were reported as exposure to an active ingredient by a given route of administration, as well as by exposure to an active ingredient by any administration route. These exposures were summarized by antimicrobial class. It is important to note that antimicrobial exposures through feed tend to involve larger groups of pigs and longer durations of use than antimicrobial exposures via water. Alternatively, injectable antimicrobials are generally administered on an individual basis to a limited number of pigs[53].

Quantitative AMU data (dose and duration) were collected for antimicrobials administered through feed but not for antimicrobials administered through water or by injection. Table 9 summarizes the reported antimicrobial active ingredients and classes used by their *Categories of Importance to Human Medicine* and ATCvet codes. The amount of an antimicrobial consumed through feed was estimated from the concentration of the antimicrobial in a given ration multiplied by the cumulative tonnes consumed over the duration of exposure. The cumulative feed consumption was calculated using National Research Council feed intake estimates for average performing pigs for the weights indicated by the producer in the questionnaire for each specified ration[54]. Quantitative results for AMU through feed are reported as kilograms of active ingredient per 1,000 pig-days at risk, which standardizes the number of pigs and the duration of exposure for a given antimicrobial use.

SURVEILLANCE OF ANTIMICROBIALS DISTRIBUTED FOR SALE FOR USE IN ANIMALS

QUANTITIES OF ANTIMICROBIALS DISTRIBUTED FOR SALE FOR USE IN ANIMALS

As an estimate of antimicrobials used in animals, data on active ingredients distributed for sale were aggregated and provided to the Public Health Agency of Canada by the Canadian Animal Health Institute (CAHI). CAHI is the trade association representing the companies that manufacture and distribute drugs for administration to food (including fish), sporting, and companion animals in Canada. The association estimates that its members' sales represent over 90% of all sales of licensed animal pharmaceutical products in Canada[55]. CAHI coordinates electronic collection of data from its members on the total kilograms of antimicrobials distributed for sale. Data collection and analysis are performed by a third party, Impact Vet[56] . The CAHI data include information from 15 companies that manufacture antimicrobials products for use in animals in Canada, and 5 major wholesalers/distributors. The CAHI data on the distribution of antimicrobials for use in animals provide a context to interpret other data on

[53] Version April, 2009. Available at: www.hc-sc.gc.ca/dhp-mps/vet/antimicrob/amr_ram_hum-med-rev-eng.php. Accessed May 2013.
[54] Nutrient Requirements of Swine, Animals Nutrition Series, National Research Council of the National Academies, National Academies Press, Washington, DC. 2012.
[55] Canadian Animal Health Institute. Available at: www.cahi-icsa.ca/about
[56] Division of AgData Ltd. Available at: www.impactvet.com. Accessed May 2013.

antimicrobial use in animals generated through research and farm data collection. They also provide a means to estimate gross temporal changes in antimicrobials used in animals.

The level in the distribution chain that kilograms of active ingredients are reported to CIPARS is at the feed manufacturer/veterinary clinic/over-the-counter outlet feed mill. Antimicrobial use was assigned to either production animal (inclusive of horses) or companion animal by the manufacturers according to label claim, and in the situation where mixed species was indicated on the label, the manufacturer assigned (estimated) the species as either companion animal or production animal based on the veterinary clinic practice profile.

These data do not represent actual antimicrobial use in a given year; rather, they reflect the volume of antimicrobials distributed by manufacturers and wholesalers. Distribution values should approximate amounts used, particularly when data from more than one year are included. However, when data from only one year are included, distribution values may vary from amounts actually used because of the time lag between distribution and actual use, as well as stockpiling of antimicrobials at various points in the distribution system. The sales data also do not account for drug wastage due to drug expiry.

The data do not include antimicrobials imported for personal use (own use importation - OUI) under the personal-use provision of the federal Food and Drugs Act and its Regulations, nor do they include imported active pharmaceutical ingredients (API), which are drugs imported in non-dosage form and compounded by a licensed pharmacist or veterinarian. These data also do not include prescriptions for antimicrobials used in companion animals could be filled at human pharmacies. Hence, the CAHI data are currently an underestimate of the true volume of antimicrobials used in animals in Canada. Also, as the CAHI data represent manufacture and distribution-level data, these data do not capture what happens to the drugs after purchase; hence this data cannot provide information the actual antimicrobial use practices, such as dose, duration, reason for use, detailed species-specific information, or extra-label use.

The CAHI data also include medicines sold directly to pharmacists that have a focus on dispensing for production medicine. It does not include antimicrobial agents moved from veterinarians to pharmacies and then subsequently dispensed by pharmacies. The latter distribution is captured with the veterinary clinic-level data.

CAHI provides the information in categories, with some antimicrobials not independently reported. This is based on a “3 company accounting rule” established by CAHI to comply with the European Union and the United States’ anti-competition regulations. CAHI added in some cases a “90% rule” to be sure not to infringe the regulations in the United States. These accounting rules can result in changes to the categorization of specific antimicrobials over time. For 2012, the antimicrobials are categorized as per Table 4.

Table 4. Canadian Animal Health Institute's aggregation of data on antimicrobial distributed for sale for use in animals, 2012

Antimicrobial class	Active ingredient included in the antimicrobial class
Aminoglycosides	Amikacin, apramycin, dihydrostreptomycin, gentamicin, neomycin, spectinomycin, streptomycin
Cephalosporins	Cefaclor, cefadroxil, cefovecin, ceftiofur, cephapirin
Chemical coccidiostats, arsenicals	Amprolium, arsanilic acid, arsenilate, clopidol, decoquinate, diclazuril, pyrimethamine, robenidine, zoalene
Fluoroquinolones	Enrofloxacin, danofloxacin, difloxacin, marbofloxacin, orbifloxacin
Ionophore coccidiostats	Lasalocid, maduramicin, monensin, narasin, nicarbazin, salinomycin
Lincosamides	Clindamycin, lincomycin, pirlimycin
Macrolides	Erythromycin, gamithromycin, tildipirosin, tilmicosan, tulathromycin, tylosin
Others	Bacitracin, bambermycin, florfenicol, nitrofurantoin, nitrofurazone, novobiocin, ormethoprim, polymixin, tiamulin, virginiamycin
β-Lactams / penicillin	Amoxicillin, ampicillin, clavulanic acid, cloxicillin, penicillin
Sulfonamides	Sulfabenzamide, sulfacetamide, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfaguanidine, sulfamerazine, sulfamethazine, sulfanilamide, sulfaquinoxaline, sulfathiazole, trimethoprim
Tetracyclines	Chlortetracycline, oxytetracycline, tetracycline

POPULATION CORRECTION UNIT

Changes in overall sales/distribution of antimicrobials over time may reflect several things: 1) true change in use practices, 2) a change in the numbers or types of animals in the population (requiring antimicrobials), 3) changes in disease prevalence necessitating antimicrobial use, and 4) changes in the types of antimicrobials administered (with different potencies). As one way to adjust the sales data for the changing animal populations over time, a denominator accounting for the number of animals and their standardized weights (animal biomass) was applied. This denominator was based on the methodology currently in use by the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC)[57].

ESVAC adjusts the sales data by a population correction unit (PCU)[57]; in which a PCU is a proxy for the animal biomass that is at risk of being treated with antimicrobials. The PCU has been described as "currently the best approximation of use, extrapolated from sales data, for changes within a country over time and comparison between countries"[58]. It is a technical measurement only; where 1 PCU = 1 kg of different categories of livestock and slaughtered animals. ESVAC methodology was applied to the greatest extent possible, however population information collected by Statistics Canada and Agriculture and Agri-Food Canada is different in

[57] Sales of veterinary antimicrobial agents in 25 EU/EEA countries in 2011 (EMA/236501/2013). European Medicines Agency. European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). Available at: www.ema.europa.eu/docs/en_GB/document_library/Report/2013/10/WC500152311.pdf. Accessed March 2014.

[58] 2012. UK Veterinary Antibiotic Resistance and Sales Surveillance Report. Veterinary Medicines Directorate - Government Department for the Environment, Food and Rural Affairs. UK-VARSS. Available at: www.vmd.defra.gov.uk/pdf/VARSS.pdf. Accessed March 2014.

structure somewhat from the data collected by Eurostat and TRACES, hence direct comparisons of PCU's or mg/PCU with ESVAC participating country data should only be made with due caution.

The PCU is calculated by multiplying the numbers of livestock and slaughtered animals in each species/production state (n) by the theoretical (standardized) weight at the most likely time of treatment[57,59].

$$\text{PCU (kg)} = \text{n} * \text{weight(kg)}$$

$$\text{AMU} = \frac{\text{Antimicrobials distributed (mg)}}{\text{PCU (kg)}}$$

National denominator data regarding the number of livestock and slaughtered animals for 2006 to 2012 were obtained from Statistics Canada[60], Agriculture and Agri-Food Canada[61], and Fisheries and Oceans Canada[62], and Equine Canada[63] websites. Validation of the data accessed for the animal populations is currently underway, the PCU measures as provided in this report should be considered provisional.

The average weights at treatment used in these calculations, as per ESVAC, can be found in Table 5. Canadian average weights were not used for this surveillance reporting period, as there is current on-going discussion with industry stakeholders to determine appropriate weights in the Canadian context. However, the intention is that future reporting of the CAHI data will additionally include average weights of treatment/average weights of the production stage more specific to the Canadian context. There may also be alterations in the production categories included in a Canadian PCU denominator; hence future reports using this metric will vary depending upon the outcomes of these discussions. Future reports will articulate a clear distinction in the results/methods as to which denominator is applied.

[59] Trends in the sales of veterinary antimicrobial agents in nine European countries - Reporting period: 2005-2009. European Medicines Agency. European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). Available at: www.ema.europa.eu/docs/en_GB/document_library/Report/2011/09/WC500112309.pdf. Accessed February 2014.

[60] Government of Canada. Statistics Canada. Available at: www5.statcan.gc.ca/subject-sujet/subtheme-sous theme.action?pid=920&id=2553&lang=eng&more=0. Updated 08/02/2104. Accessed March, 2014.

[61] Government of Canada. Agriculture and Agri-Food Canada. Available at: www.agr.gc.ca/index_e.php. Updated 07/03/2014. Accessed March 2014.

[62] Government of Canada. Fisheries and Oceans Canada. Statistics. Available at: www.dfo-mpo.gc.ca/stats/stats-eng.htm. Accessed February 14, 2014.

[63] Equine Canada, Industry Studies. 2010 Canadian Horse Industry Profile Study. Available at: www.equinecanada.ca/industry/index.php?option=com_content&view=section&id=103&Itemid=559&lang=en. Accessed April 2014.

Table 5. Animal production average weights at treatment used in calculation of the population correction unit

Animal species	Animal category	Type of data	Average weight at treatment (kg)[a]
Cattle			
Cattle	Cattle and calves	Slaughter[b]	425
Beef	Cattle and calves	Import for slaughter	425
Beef	Cattle and Calves	Export for slaughter	425
Beef	Cattle and calves	Import for fattening	140
Beef	Cattle and calves	Export for fattening	140
Beef[c]	Cows	Living, on-farm	425
Dairy	Cows	Living, on-farm	425
Pigs			
Swine	Finisher pigs	Slaughter	65
Swine		Import for fattening or slaughter[d]	65
Swine		Export for fattening or slaughter[d]	65
Swine	Sows and bred gilts	Living; on-farm	240
Poultry			
Chicken	Broiler	Slaughter	1
Turkey	Turkey	Slaughter	6.5
Poultry		Import	1
Poultry		Export	1
Poultry (< 185 g)		Live, export/import[e]	0.2
Poultry (> 185 g)		Live, export/import[e]	2
Sheep and Goats			
Sheep		Slaughter	20
Goats		Slaughter	20
Sheep		Import for fattening or slaughter[f]	20
Sheep		Export for fattening or slaughter[f]	20
Sheep	Ewes	Living; on-farm	75
Horses			
Horses		Living; on-farm	400
Fish			
Fish (shellfish and finfish)		Production data provided as tonnes	Not applicable
Rabbit			
Rabbit		Slaughter	1.4

[a] All average weights at treatment are per ESVAC, unless otherwise specified[64].

[b] The data provided in the Canadian cattle statistics cannot distinguish between slaughtered cows, calves (veal), heifers, or steers. The average weight at treatment chosen reflected a decision to use the ESVAC weight for slaughtered bullocks/bulls and import for slaughter (425 kg).

[c] ESVAC does not include beef cows.

[d] The data provided in the Canadian swine statistics cannot distinguish between import for slaughter versus import for fattening. The average weight at treatment chosen reflected a decision to use the ESVAC weight for import for slaughter.

[e] These import/export weights approximate (arbitrary assignation) the weights captured by the Statistics Canada data (<185 g and 185 g). These data are only available from 2009 onwards.

[f] The data provided cannot distinguish between import/export for fattening or slaughter.

[64] Trends in the sales of veterinary antimicrobial agents in nine European countries – Reporting period: 2005-2009. European Medicines Agency. European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). Available at: www.ema.europa.eu/docs/en_GB/document_library/Report/2011/09/WC500112309.pdf. Accessed February 2014.

Detailed inclusions and exclusions for the PCU denominator: As per ESVAC, exported animals were added to the PCU, whereas imported animals were subtracted, based on the ESVAC assumption that animals are treated in their country of origin. However, it was noted that in the Canadian context, this would vary depending upon the production stage that is crossing the border. For the purposes of calculating the PCU, production animal species with the largest populations were included, using the same production classes as ESVAC, with the exception that we additionally included beef cows (not included by ESVAC). Species currently excluded from our PCU calculations include game animals (e.g., moose), "pocket" companion animals (e.g., hamsters, guinea pigs, pet birds), reptiles, and amphibians. Import and export data for poultry are included in a different structure before and after 2009, based on the data available from Statistics Canada. For cattle, international export data was not stratified by the type of cattle (i.e., dairy versus beef cattle), as this stratification was not available in the data accessed. The total number of cattle slaughtered per year as provided/accessed was not stratified by type of cattle (beef versus cull dairy); hence it was assumed that the total slaughtered includes all cattle types (including cull dairy).

PROVINCIAL STRATIFICATION OF THE NUMERATOR AND DENOMINATOR

There may be subsequent distribution of antimicrobials across provincial borders after being distributed to the veterinary clinics (in particular the movement of medicated feed - for example, anecdotal information is that New Brunswick has a negligible feed-mill industry, they generally purchase their medicated feed from Québec), hence caution should be applied when interpreting the quantities of antimicrobials distributed for sale within each province. An effort was made to calculate a PCU at the provincial-level, however there is ongoing discussion with industry stakeholders regarding the inter-provincial movement of animals. As inter-provincial export data is not available for all species in all provinces, provincial calculations of PCU will be postponed pending further discussion.

OVERALL DISCUSSION OF STRENGTHS AND LIMITATIONS

CIPARS currently has farm-level surveillance in the swine and poultry sectors. The CAHI data provides a rough measure of antimicrobials distributed for sale for all animal species, including those not covered by CIPARS farm-level surveillance (with appropriate caveats regarding OUI/API). With respect to the PCU, as stated in the United Kingdom's surveillance report on antimicrobials sold for use in animals[65], the population is an important denominator, as the greater the number of animals, the greater the potential need for antimicrobial therapy. The PCU metric currently does not take into account the lifespan of the animal, which may affect the interpretation of the quantities of antimicrobials administered to animals. Also, use of a static standard weight may not reflect an industry shift in production affecting the average weights of animals treated, related to weather, trade, or other reasons. Measures of

[65] 2012. UK Veterinary Antibiotic Resistance and Sales Surveillance Report. Veterinary Medicines Directorate – Government Department for the Environment, Food and Rural Affairs. UK-VARSS. Available at: www.vmd.defra.gov.uk/pdf/VARSS.pdf. Accessed March 2014.

antimicrobial use as reported by broad categories and by a PCU denominator do not account for the individual potencies of the drugs that make up the category. For example, a decrease in the mg/PCU reported for a given year could potentially reflect a switch to using a more potent drug, as opposed to reflecting a decrease in the actual exposure of animals to antimicrobials. The CAHI data should be interpreted as one measure describing antimicrobials used in animals, strong caution should be applied with making inferences to any use practice for a particular animal species. CIPARS continues to work to improve this measure and other appropriate measures, to best reflect antimicrobial use in the Canadian context.

ANTIMICROBIAL CLASSIFICATION

CATEGORIZATION OF ANTIMICROBIALS

Categories of antimicrobials used in this report were taken from the document Categorization of Antimicrobial Drugs Based on Importance in Human Medicine[66] by Health Canada's Veterinary Drugs Directorate (Table 6). Antimicrobials are considered to be of Very High Importance in Human Medicine (Category I) when they are essential for the treatment of serious bacterial infections and there is no or limited availability of alternative antimicrobials for effective treatment. These antimicrobials include amoxicillin-clavulanic acid, ceftiofur[67], ceftriaxone, ciprofloxacin, and telithromycin. Antimicrobials of High Importance in Human Medicine (Category II) consist of those that can be used to treat a variety of infections, including serious infections, and for which alternatives are generally available. Bacteria resistant to antimicrobials of this category are generally susceptible to Category I antimicrobials, which could be used as alternatives. Antimicrobials of Medium Importance in Human Medicine (Category III) are used in the treatment of bacterial infections for which alternatives are generally available. Infections caused by bacteria resistant to these antimicrobials can, in general, be treated with Category II or I antimicrobials. Antimicrobials of Low Importance in Human Medicine (Category IV) are currently not used in human medicine.

[66] Version April, 2009. Available at: www.hc-sc.gc.ca/dhp-mps/vet/antimicrob/amr_ram_hum-med-rev-eng.php. Accessed May 2013.

[67] Ceftiofur is licensed for use in animals only. Resistance to ceftiofur is generally detected in combination with resistance to amoxicillin-clavulanic acid, cefoxitin, ampicillin and ceftriaxone (A2C-AMP-CRO resistance pattern).

Table 6. Categorization of antimicrobial drugs based on importance in human medicine

Category of importance in human medicine		Antimicrobial class
I	Very High Importance	Carbapenems
		Cephalosporins – the 3rd and 4th generations
		Fluoroquinolones
		Glycopeptides
		Glycylcyclines
		Ketolides
		Lipopeptides
		Monobactams
		Nitroimidazoles (metronidazole)
		Oxazolidinones
		Penicillin-β-lactamase inhibitor combinations
		Polymyxins (colistin)
		Therapeutic agents for tuberculosis (e.g. ethambutol, isoniazid, pyrazinamide, and rifampin)
II	High Importance	Aminoglycosides (except topical agents)
		Cephalosporins – the first and second generations (including cephamycins)
		Fusidic acid
		Lincosamides
		Macrolides
		Penicillins
		Quinolones (except fluoroquinolones)
		Streptogramins
		Trimethoprim-sulfamethoxazole
III	Medium Importance	Aminocyclitols
		Aminoglycosides (topical agents)
		Bacitracins
		Fosfomycin
		Nitrofurans
		Phenicols
		Sulfonamides
		Tetracyclines
		Trimethoprim
IV	Low Importance	Flavophospholipols
		Ionophores

Table 7. List of antimicrobials from the Farm Swine questionnaire database for each ATCvet[68] class

	ATCvet Class	Antimicrobial
I	Third Generation Cephalosporins (QJ01DD)	Ceftiofur (QJ01DD90)
	Fluoroquinolones	Enrofloxacin (QJ01MA90)
II	Amphenicols (QJ01BA)	Florfenicol (QJ01BA90)
	Penicillins with extended spectrum (QJ01CA)	Ampicillin (QJ01CA01)
		Amoxicillin (QJ01CA04)
	Beta-lactamse sensitive penicilliins (QJ01CE)	Penicillin Potassium (QJ01CE01)
	Combination of sulphadoxine and trimethoprim (QJ01EW)	Trimethoprim-sulphadoxine (QJ01EW13)
	Macrolides (QJ01FA)	Erythromycin (QJ01FA01)
		Tylosin (QJ01FA90)
		Tilmicosin (QJ01FA91)
		Tulathromycin (QJ01FA94)
	Lincosamides (QJ01FF)	Lincomycin (QJ01FF02)
	Streptogramins (QJ01FG)	Virginiamycin (QJ01FG90)
	Other aminoglycosides (QJ01GB)	Neomycin (QJ01GB05)
	Combinations with other antibacterials (QJ01RA)	Penicillin G Potassium-Streptomycin (QJ01RA01)
		Chlortetracycline-Sulfamethazine-Penicillin (QJ01RA90)
		Oxytetracycline-Neomycin (QJ01RA90)
		Tetracycline-Neomycin (QJ01RA90)
		Lincomycin-Spectinomycin (QJ01RA94)
	Other Antibacterials (QJ01XX)	Spectinomycin (QJ01XX04)
III	Tetracyclines (QJ01AA)	Chlortetracycline (QJ01AA03)
		Oxytetracycline (QJ01AA06)
		Tetracycline (QJ01AA07)
		Chlortetracycline, combinations (QJ01AA53)
	Sulfonamides (QJ01EQ)	Sulfa-Drugs (QJ01EQ30)
	Pleuromutilins (QJ01XQ)	Tiamulin (QJ01XQ01)
	Other Antibacterials (QJ01XX)	Bacitracin (QJO1XX10)
IV	No ATCvet Code	Bambermycin (No ATCvet Code)
	Pyranes and hydropyranes (QP51AH)	Salinomycin (QP51AH01)

[68] World Health Organization Collaborating Center for Drug Statistics Methodology. Available at: www.whocc.no/atcddd. Accessed May 2013.

SUMMARY OF DESIGN AND METHODS CHANGES

Table 8. Changes implemented since the beginning of the Antimicrobial Resistance (AMR) Surveillance program

Year	Component	Province / region	Species	Selected bacteria				Design	Methods
				Escherichia coli	*Salmonella*	*Campylobacter*	*Enterococcus*		
2012	*Surveillance of Human Clinical Isolates*	Across provinces	Humans		✓				Adoption of a lower breakpoint for ciprofloxacin (≥ 1 µg/mL; CLSI M100-S22) than in past years (≥ 4 µg/mL) for both *Salmonella* and *E. coli*. Ciprofloxacin's new breakpoint was applied to all data, including historical data.Then, the term "reduced susceptibility to ciprofloxacin" was dropped.
	Retail Surveillance	British Columbia Saskatchewan Ontario Québec Maritimes	Beef	✓					
			Chicken	✓	✓	✓			
			Pork	✓					
			Turkey	✓	✓	✓		Surveillance of *Salmonella, E. coli* and *Campylobacter* isolates in retail turkey was started in January.	
	Abattoir Surveillance	Across provinces	Beef cattle	✓		✓		Surveillance of *Campylobacter* in pigs at the abattoir was started in January.	
			Chickens	✓	✓	✓			
			Pigs	✓	✓	✓			
	Farm Surveillance	Alberta Saskatchewan Manitoba Ontario Québec	Pigs	✓	✓				
	Surveillance of animal clinical Isolates	Across provinces	Bovine		✓				
			Chickens		✓				
			Pigs		✓				
			Turkeys		✓				
	Feed and Feed Ingredients	Across provinces			✓				
2011	*Surveillance of Human Clinical Isolates*	Across provinces	Humans		✓			Human serovars : Newport added as a separate category	The CMV2AGNF susceptibility testing plate has replaced the CMV1AGNF plate for *Salmonella* and *E. coli*. Amikacin was removed and azithromycin was included in the panel.
	Farm Surveillance	Alberta Saskatchewan Manitoba Ontario Québec	Pigs				✗	Bacterial culture and antimicrobial susceptibility testing of *Enterococcus* isolates from pigs were discontinued as of January.	
2010	*Surveillance of Human Clinical Isolates*	Across provinces	Humans		✓			Isolates classified as "Other serovars" category were not tested or reported, but stored for future AMR testing.	Half of the *Salmonella* Enteritidis submitted by the most populated provinces (British Columbia, Alberta, Ontario, and Québec) during the first 15 days of the month were tested.
	Retail Surveillance	British Columbia Saskatchewan Ontario Québec Maritimes	Beef	✓				Bacterial culture and antimicrobial susceptibility testing of *Enterococcus* in chicken isolates discontinued as of January (no vancomycin resistance was detected since the program began in 2003).	A new ceftriaxone breakpoint was officially adopted by the CLSI in January 2010. It was applied to all data, including historical data. A new genus- and species-specific multiplex PCR method was used in replacement of the standard method (biochemical tests) to perform identification and speciation of *Campylobacter*.
			Chicken	✓	✓	✓	✗		
			Pork	✓					
	Abattoir Surveillance	Across provinces	Beef cattle	✓		✓		Bacterial culture and antimicrobial susceptibility testing of *Campylobacter* isolates from abattoir chickens was initiated in January.	
			Chickens	✓	✓	✓			
			Pigs	✓	✓				

Table 8. Changes implemented since the beginning of the Antimicrobial Resistance (AMR) Surveillance program (cont'd)

Year	Component	Province / region	Species	Selected bacteria				Design	Methods
				Escherichia coli	*Salmonella*	*Campylobacter*	*Enterococcus*		
	Surveillance of Human Clinical Isolates	Across provinces	Humans		✓			Human serovars: Newport not presented as a separate category; now included with the "other serovars"	
2009	*Retail Surveillance*	British Columbia Saskatchewan Ontario Québec Maritimes	Beef	✓				First full surveillance year in the Maritimes.	The CMV3AGPF susceptibility testing plate has replaced the CMV2AGPF plate for all *Enterococcus* isolates.
			Chicken	✓	✓	✓	✓		
			Pork	✓					
	Farm Surveillance	Alberta Saskatchewan Manitoba Ontario Québec	Pigs	✓	✓		✓	Sample collection from pigs on entry to the Grower-Finisher unit was terminated. Changed from 3 herd visits per year to 1 annual visit to collect fecal samples from close-to-market pigs.	
	Surveillance of Human Clinical Isolates	Across provinces	Humans		✓			Human serovars: Paratyphi A and B reported as a separate category along with Enteritidis, Heidelberg, Newport, Typhi, Typhimurium, and Other Serovars.	The ceftriaxone resistance breakpoint was changed to ≥ 4 µg/mL (CLSI M100-S20) for all *Salmonella* and *Escherichia coli* isolates. Quinupristin-dalfopristin was reclassified as Category II antimicrobial (High Importance in Human Medicine, Veterinary Drugs Directorate, Health Canada) for all *Enterococcus* isolates. Application of a more sensitive *Campylobacter* recovery method in abattoir beef cattle isolates. Quinupristin-dalfopristin reclassified as category II for all *Enterococcus* isolates.
2008	*Retail Surveillance*	British Columbia Saskatchewan Ontario Québec Maritimes (pilot)	Beef	✓				First surveillance year in British Columbia. Pilot surveillance also began in the Maritimes region in September 2008.	
			Chicken	✓	✓	✓	✓		
			Pork	✓					
2007	*Retail Surveillance*	British Columbia (pilot) Saskatchewan Ontario Québec	Beef	✓				Implementation of pilot retail surveillance in British Columbia.	Retail surveillance: Enhancement to the *Salmonella* recovery method yielded higher recovery rates than in prior years. For antimicrobial susceptibility testing of *Enterococcus,* bacitracin was removed and tigecycline removed from the panel. New resistance breakpoints were adopted for lincomycin (from ≥ 32 to ≥ 8 µg/mL) and kanamycin (from ≥ 512 to ≥ 1,024 µg/mL).
			Chicken	✓	✓	✓	✓		
			Pork	✓					
	Surveillance of animal clinical Isolates	Across provinces	Bovine		✓			Publication of surveillance findings from clinical isolates from horses.	
			Chickens		✓				
			Pigs		✓				
			Turkeys		✓				
			Horses		✓				
	Feed and Feed Ingredients	Across provinces	Not available		✓			*Feed and Feed Ingredients* presented as a separate surveillance component.	

Table 8. Changes implemented since the beginning of the Antimicrobial Resistance (AMR) Surveillance program (cont'd)

Year	Component	Province / region	Species	Selected bacteria				Design	Methods
				Escherichia coli	*Salmonella*	*Campylobacter*	*Enterococcus*		
2006	*Retail Surveillance*	Saskatchewan Ontario Québec	Beef	✓					The NARMS CAMPY plate has replaced the disk diffusion method (Etest) for antimicrobial susceptibility testing of Campylobacter.
			Chicken	✓	✓	✓	✓		
			Pork	✓	✓				
	Abattoir Surveillance	Across provinces	Beef cattle	✓		✓		Abattoir surveillance of *Campylobacter* from beef cattle was started in January	
			Chickens	✓	✓				
			Pigs	✓	✓				
	Farm Surveillance	Alberta Saskatchewan Manitoba Ontario Québec	Pigs	✓	✓		✓	Implementation of the CIPARS farm component in grower-finisher pigs of the 5 major pork producing provinces.	
2005	*Retail Surveillance*	Saskatchewan Ontario Québec	Beef	✓				Addition of Saskatchewan to the retail component.	Antimicrobial susceptibility testing of *Salmonella* and *E. coli* was fully performed by the NARMS CMV1AGNF plate in January.
			Chicken	✓	✓	✓	✓		
			Pork	✓	✓				
	Abattoir Surveillance	Across provinces	Beef cattle	✓		✓		Pilot surveillance of *Campylobacter* from beef cattle started in late 2005.	
			Chickens	✓	✓				
			Pigs	✓	✓				
2004	*Surveillance of Human Clinical Isolates*	Across provinces	Humans		✓				Antimicrobial susceptibility testing of human *Salmonella* was performed by the NARMS CMV7CNCD from January to April and the CMV1AGNF from April to December.
	Abattoir Surveillance	Across provinces	Beef cattle	✓	✗			*Salmonella* isolation discontinued because of its low prevalence in beef cattle.	
			Chickens	✓	✓				
			Pigs	✓	✓				
	Retail Surveillance	Ontario Québec	Beef	✓				There is a systematic rotational selection of extra lean, lean, regular, and medium ground beef.	
			Chicken	✓	✓	✓	✓		
			Pork	✓					
2003	*Surveillance of Human Clinical Isolates*	Across provinces	Humans		✓			Implementation of the CIPARS human component. Antimicrobial susceptibility testing done on all serovars but they were classified and reported into the following categories: Enteritidis, Heidelberg, Newport, Typhi, Typhimurium, and Other Serovars.	Susceptibility testing of *Campylobacter* and *Enterococcus* was performed with the disk diffusion method using the ETest® methodology (AB Biodisk, Solna, Sweden) and the NARMS CMV5ACDC plate respectively.
	Retail Surveillance	Ontario Québec	Beef	✓				Implementation of the CIPARS *Retail Surveillance* component in Ontario and Québec.	
			Chicken	✓	✓	✓	✓		
			Pork	✓					

Table 8. Changes implemented since the beginning of the Antimicrobial Resistance (AMR) Surveillance program (cont'd)

Year	Component	Province / region	Species	Selected bacteria				Design	Methods
				Escherichia coli	*Salmonella*	*Campylobacter*	*Enterococcus*		
2002	*Surveillance of Human Clinical Isolates*	Across provinces	Humans					Agreement signed with the Provinces to send all (or a subset) of *Salmonella* isolates to CIPARS. Data were not available for reporting that year.	
	Abattoir Surveillance	Across provinces	Beef cattle	✓	✓			Implementation of the first active suveillance component of CIPARS.	Antimicrobial susceptibility testing of *Salmonella* and *E. coli* was performed by the CMV7CNCD plate (Sensititre™), NARMS, United States.
			Chickens	✓	✓				
			Pigs	✓	✓				
	Surveillance of animal clinical Isolates	Across provinces	Cattle		✓			Implementation of the first passive suveillance components of CIPARS.	
			Chickens		✓				
			Pigs		✓				
			Turkeys		✓				
			Feed and Feed Ingredients		✓				

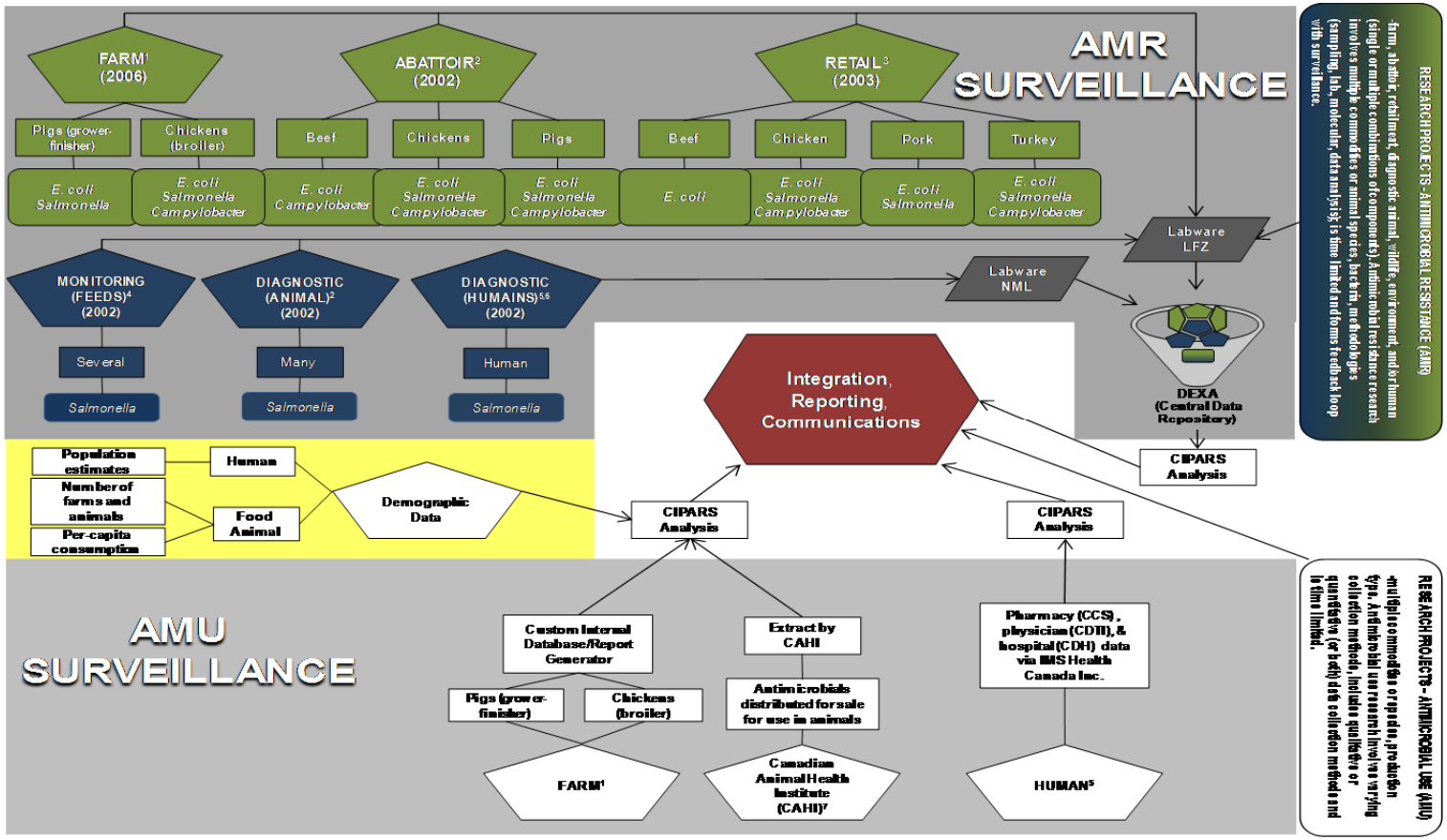
Table 9. Changes implemented since the beginning of the Antimicrobial Use (AMU) Surveillance program

Year	Component	Province / region	Population exposed	Reporting metrics	Dosage information	Design	Methods
2011	*Human antimicrobial use surveillance - Physician diagnosis data*	National Provincial Regional	Canadians	1) Total diagnoses/10,000 inhabitants 2) Total antimicrobial recommendations/10,000 inhabitants 3) Percentage diagnoses with antimicrobial recommendations		Enhancement of the *Human antimicrobial use surveillance* component. The design is based on a sample of physicians providing antimicrobial recommendation information for every patient in a 48-hour period four times a year.	Analysis based on the Canadian Disease and Therapeutic Index (CDTI) purchased from IMS Health Canada Inc.
	Human antimicrobial use surveillance - Hospital purchases	National Provincial	Canadians	1) Defined Daily Doses (DDD)/1,000 inhabitant-days 2) Total cost/1,000 inhabitant-days 3) Total cost per unit of antimicrobials 4) Total active ingredient (kg)		Enhancement of the *Human antimicrobial use surveillance* component. The design is based on a purchasing information for a number of Canadian hospitals extrapolated to all hospitals in Canada.	Analysis based on the Canadian Drugstore and Hospital Purchases Audit (CDH) purchased from IMS Health Canada Inc.
	Surveillance of the antimicrobials distributed for sale for animals	National	A national animal biomass denominator was calculated as per the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC)	1) Total of active ingredients (kg) (national and provincial; production animal, and companion animal); 2) mg/PCU (where PCU=population correction unit, a measure of animal biomass)			Stratification of CAHI data into production & companion animal; stratification by province; extraction of cephalosporins back into separate category; application of biomass denominator to national-level data.
2009	*Farm AMU surveillance in Pigs*	Alberta Saskatchewan Manitoba Ontario Québec	Number of grower-finisher pigs at start and end of grow, mortalities and culls	Farm count data for antimicrobial use by class, category of importance to human medicine, and reason for use	Inclusion rate in feed (g/tonne)	Annual and Sampling Day questionnaires were complied into a single Sampling Day Questionnaire which is applied once/herd/year.	Inclusion rate in feed ONLY; no dosage information collected for water or injections
2008	*Surveillance of the antimicrobials distributed for sale for animals*	National	Not applicable				CAHI has a "3 company accounting rule" to comply with the EU & the US' anti-competition regulations. CAHI added in some cases a "90% rule" to be sure not to infringe upon the regulations in the US. These accounting rules can result in changes to the categorization of specific antimicrobials over time.
2007	*Human antimicrobial use surveillance - Pharmacy sale*	National Provincial	Canadians	1) Prescriptions/1,000 inhabitants 2) Defined daily doses (DDDs)/1,000 inhabitant-days 3) Total cost/1,000 inhabitant-days 4) Total active ingredients (kg)			Data are now available separately for Newfoundland & Labrador and Prince Edward Island.
	Farm AMU surveillance in Pigs	Alberta Saskatchewan Manitoba Ontario Québec	Number of grower-finisher pigs at start and end of grow, mortalities and culls	Farm count data for AMU by class, category of importance to human medicine, and reason for use	Inclusion rate in feed and water (not collected for injections)		Questionnaire was refined to improve data quality and compliance.

Table 9. Changes implemented since the beginning of the Antimicrobial Use (AMU) Surveillance program (cont'd)

Year	Component	Province / region	Population exposed	Reporting metrics	Dosage information	Design	Methods
	Farm AMU surveillance in Pigs	Alberta Saskatchewan Manitoba Ontario Québec	Number of grower-finisher pigs at start and end of grow, mortalities and culls	Farm count data for AMU by class, category of importance to human medicine, and reason for use	Inclusion rate in feed and water (not collected for injections)	Implementation of the CIPARS farm component in grower-finisher pigs of the 5 major porc producing provinces.	Antimicrobial use in feed, water, and injection information was collected through 1 annual and 3 sampling day questionnaires/ herd/year.
2006	*Surveillance of the antimicrobials distributed for sale for animals*	National	Not applicable	1) Total of active ingredients (kg)	Not available	Implementation of surveillance of manufacturer and distributor-level data for antimicrobials used in animals as provided by the Canadian Animal Health Institute (CAHI)	
2003	*Human antimicrobial use surveillance - Pharmacy sale*	National	Canadians	1) Prescriptions/1,000 inhabitants 2) Defined daily doses (DDDs)/1,000 inhabitant-days 3) Total cost/1,000 inhabitant-days 4) Total active ingredients (kg)		Implementation of the *Human antimicrobial use surveillance* component. The design is based on a number of canadian pharmacies dispensing oral prescriptions extrapolated to all pharmacies in Canada.	Analysis based on the Canadian CompuScript (CCS) purchased from IMS Health Canada Inc.

SUMMARY OF CIPARS SAMPLES AND DATA FLOW



= Active surveillance; primary data, primarily for prevalence estimation = Passive surveillance ; secondary data, primarily for AMR detection

LFZ: Laboratory for Foodborne Zoonoses **NML**: National Microbiology Laboratory

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