

Sampling in Brazil affords representativeness for monitoring of persistent organochlorines in products of animal origin. The 2004-2013 experience

Moretti Ld^{1*}, Feijó, LD², Bonnet, M³, Olivares, I¹, Zaroni, MM, Pinhel, MFM¹, Mendonça, AO¹

¹Laboratório nacional agropecuário no estado de São Paulo (LANAGRO/SP. Rua Raul Ferrari, s/nº, Santa Marcelina. Caixa Postal 5538. CEP: 13100-105. Phone: (19) 3252-0155, Campinas/SP, Brazil;

*leandro.moretti@agricultura.gov.br

²Departamento de Inspeção de Produtos de Origem Animal (DIPOA). Esplanada dos Ministérios, Bloco D, Anexo - Ala A, 4º andar, Sala 401. CEP: 70043-900, Brasília/DF, Brazil

³Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA/CNPGL). Rua Eugênio do Nascimento, 610, Dom Bosco. CEP 36038-330, Juiz de Fora/MG, Brazil

Introduction

Surveys are count procedures applied to two complimentary processes. For binary events (positive/negative) they are conducted to estimate the frequency of positive results in populational basis (prevalence). Besides that they provide the foundation for the detection of at least one positive case⁵.

With epidemiologic surveys often is not operational/financially achievable to use census data. A survey sampling (n samples) is used to attain the rate of positives for a specified disease in the total number of individuals in the population (N individuals).

The term sample is used to indicate the set of n units withdrawn from the population (N) to calculate statistics that estimate population parameters.

Randomness is the basic assumption for sampling theory. The random selection of animals and count of the positive ones is an accurate picture of the population. In this case the frequency of event occurrence is expected being the same both in the sample and in the population. The randomization exerts a protection against the agglomerated selection of either positive or negative animals because those clumps of cases might distort the actual population proportion between positives and negatives, an event known as bias. Beyond randomness the reliability in sample representativeness depends upon sampling frame stratification, that is, the collection sites.

Sampling is essential to prospect disease occurrence in populations. By analogy the conformity or non-conformity (violation to maximum legal limits) can be evaluated in a binary format in the context of chemical residuals in food matrixes.

In Brazil the National Control Plan for Residues and Contaminants (PNCRC) is an initiative of the Ministry of Agriculture, Livestock and Food Supply (MAPA) that employs random sampling, stratified by size of slaughtering (meat, beef) or processing (milk, honey, eggs, fish). It applies to industries under federal inspection service (SIF) that represent the collecting sites out of the sampling lists referred by Thrusfield⁵ (p. 153). The more animals are slaughtered/processed the better the chances of the enterprise to be selected in that strata (for tissue collection and analysis). The chance of selection for any animal is proportional to the processing size of the industry. Therefore, independent of the size of the farm of origin of the animals. Weekly drawings are made by computerized internet system - SISRES (Residues and Contaminants Control System) up to the achievement of all analysis expected for annual program.

Materials and methods

PNCRC annual results were provided by official State publications of the Brazilian government by monitoring year [2004: Ordinance SDA nº 77/2005; 2005: Ordinance SDA nº 222/2006; 2006: Normative Instruction SDA nº 08/2007; 2007: Normative Instruction SDA nº 09/2008; 2008: Normative Instruction SDA nº 15/2009; 2009: Normative Instruction SDA nº 06/2010; 2010: Normative Instruction SDA nº 06/2011; 2011: Normative Instruction SDA nº 07/2012; 2012: Normative Instruction SDA nº 07/2013; 2013: Ordinance SDA nº 60/2014].

In this survey were used the eletronic records of total annual analysis for 2004-2013 related to organochlorine residues and contaminants (pesticides, polycyclic aromatic hydrocarbons – HPAs and polychlorinated biphenyls – PCBs).

Win Episcope 2.0⁶ was used for calculation of the maximum expected prevalence based on binomial probability distribution considering the known number of annually negative samples for organochlorides and $N \geq 10,000$ as the total size for the sampled population (indicating an infinite population).

Results and discussion

In Brazil, sampling was updated since 2007 for organochlorines (residues and contaminants) in products of animal origin. In the years 2004, 2005, 2006, respectively, the $n = 880, 1748, 1648$ (mean=1425.33; $\sigma=474.91$) decreased to a mean of 269.43 annual samples ($\sigma=143.04$) between 2007-2013, as the n was 371, 235, 152, 277, 536, 132, 183 in each of the seven years in the latter period. That reduction was chronologically associated with the budget increase for the laboratory network of US\$ 8 million to more than US\$ 21 million in 2006-2007 and with application of equipment, reagents and quality assurance procedures according ISO 17025⁴, as well the initiatives regarding the increase on the capability for detection thresholds in the context of the aims of a future advanced program for risk analysis.

Between 2004-2013 the annual results of national monitoring showed that there was zero violations for organochlorine compounds in Brazilian control plan (PNCRC). Taking into account the variation in the theoretically possible prevalences in 2004, 2005, 2006, calculated by Win Episcope 2.0 with the binomial probability distribution, the observed prevalences for violations were at most of 0.33%; 0.16%; 0.17% (mean=0.22%, $\sigma=0.10\%$), respectively, with 95% confidence and of 0.50%; 0.25%; 0.26% (mean=0.34%, $\sigma =0.14\%$) with 99% certainty. In 2007-2013 the mean 269.43 for the annual sample size of the seven years in the period indicated the maximum prevalence of 1.35% ($\sigma =0.61\%$) violations, with 95% confidence, and in the same time 2.07% ($\sigma =0.93\%$), with 99% confidence.

The results afforded by the sampling in the period 2004-2006 indicated maximum average prevalences of 0.22% (with 95% certainty) and of 0.34% (with 99% certainty)

For period 2007-2013 the maximum average prevalences of 1.35% (with 95% certainty) and of 2.07% (with 99% certainty) pointed out to a bigger risk to the occurrence of violation regarding organochlorine compounds, despite being compatible with a suitable program for surveillance. It means that the sample size n in period 2007-2013 was sufficient to detect one single violation in the Brazil's animal population (considering the theoretical presence of at least one violation). As no violations occurred is possible to infer occurrence levels lower than 1.35-2.07%. Those levels are compatible with a controlled productive system even though with greater prevalences compared to period 2004-2006.

Since 2006, efforts to employment of modern essay methods using ISO 17025¹ framework at the official laboratory network have been progressively increasing. This is an analytical quality approach to improve the traceability and detection of pesticide and contaminant residues of organic/persistent nature (considering the application of different tools as validation, uncertainty, quality controls and others), therefore enhancing the reliability of Brazilian sample survey related to prohibition of pesticide use and to the screening of environmental pollutants in the organochlorine group.

Normative Instruction n°42² presents conceptual/operational information of the functional structure for PNCRC/MAPA. The sampling procedures are supported by the binomial probability distribution. The normative n°70 of *Codex Alimentarius* Commission (CAC)³ brings the statistical appraisal for population size that n is based on binomial distribution and will be always equal or greater that of the hypergeometric distribution. At the same time assures the solely application of hypergeometric distribution to finite populations. Between binomial and hypergeometric probability distributions the former requires greater n and therefore is more prone to detect violations, particularly at low prevalences. That argument along with the binomial distribution indication to infinite populations ($N > 10,000$ individuals) comes to strengthen the technical virtue of the Brazilian official sampling program.

Acknowledgements

This work is the summary of some of the control activities for residues/contaminants sponsored by Brazilian Ministry of Agriculture, Livestock and Food Supply. The authors wish to express gratitude to the work of every person at Ministry regarding contribution in each of their roles about sampling approach in the history of idealization and implementation of PNCRC.

References

1. ABNT (2005) General requirements for the competence of testing and calibration laboratories. NBR ISO/IEC 17025.
2. Brasil (1999). Ministry of Agriculture and Supply. Normative Instruction n°42, of december 22 of 1999. To amend the National Residue Control Plan of Animal Origin Products – PNCR and the Meat Residue Control Programmes – PCRC, Honey – PCRM, Milk – PCRL and Fish - PCR. Brasília.
3. FAO (2009). FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS. *Guidelines for the design and implementation of national regulatory food safety assurance programme associated with the use of veterinary drugs in food producing animals*. Codex Alimentarius Commission Guidelines. Rome: FAO-CAC/GL, 2009, n. 71.
4. Mauricio, A. Q., Lins, E. S., Alvarenga, M. B. (2009) A National residue control plan from the analytical perspective: the brazilian case. *Analytica Chimica Acta*, v. 637, n. 1-2, p. 333-336.
5. Thrusfield, M (1986). Surveys. In *Veterinary epidemiology*, pp 153-165, Butterworths, London.
6. Thrusfield, M., Ortega, C., De Blas, I., Noordhuizen, J. P., Frankena, K. (2001) WIN EPISCOPE 2.0: improved epidemiological software for veterinary medicine. *Veterinary Record*, v. 148, n. 18, p.567-572.