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Challenges and opportunities for the implementation of the Three Rs in Canadian vaccine quality control

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Abstract

A case-study approach was used to identify opportunities and challenges to the implementation of the Three Rs in vaccine testing in Canada. Data was obtained through interviews with 16 Canadian stakeholders involved in the production, testing and evaluation of vaccines. Participants identified inconsistent regulatory testing requirements, the lack of biological functionality of some in vitro methods, the benchmarking of in vitro against in vivo assays, and high caution towards method changes as major challenges to implementation. Opportunities to implementation were identified as the desire for and steps taken towards harmonization of test methods between countries, collaborations on new method development, the poor performance of traditional animal methods, the domino effect of one regulatory authority accepting a method after another, and stakeholder concerns for the ethical care and use of animals used in vaccine testing. These results suggest that industry and the Canadian government are open to implementing the Three Rs in vaccine quality control, but methods adopted must be reliable and biologically relevant. Improving the harmonization of regulatory requirements will assist in furthering the implementation of alternative methods.

Highlights

► Identified factors to implementation of Three Rs in vaccine testing. ► Challenges: regulations, biological function, comparison to in vivo, high caution. ► Opportunities: harmonization, collaborations, performance and ethical concerns. ► All stakeholders want adopted methods to be reliable and biologically relevant. ► Further harmonization of regulations will assist in method implementation.

Keywords

- Vaccine;
- Three Rs;
- Regulatory testing;
- Canada;
- Test methods;
- Alternatives;
- Pertussis;

- Diphtheria;
- Tetanus

1. Introduction

Vaccines have been an essential tool for the protection of public health for over 200 years, since Dr. Edward Jenner successfully developed and administered the smallpox vaccine in England in 1796 (Baylor and Midthun, 2008). The introduction and continued use of immunization programs in Canada and elsewhere in North America has markedly decreased the incidence of many debilitating and fatal diseases, and in some cases (such as smallpox), eradicated them completely (Public Health Agency of Canada, 2006).

Vaccines are biological products composed of whole or components of microorganisms and as such, batches may have slight variations in composition. The complexity and variability of vaccines is further complicated by the fact that many are produced as combinations of antigens from different microorganisms (such as diphtheria, tetanus, pertussis, polio), and may include the addition of preservatives and adjuvants. While vaccines fall within the larger category of pharmaceuticals, their complex nature renders them unique in comparison to other pharmaceutical products. As a result, although newer vaccines tend to be well characterized, there are still gaps in our knowledge of the structure and activity of some of the older vaccine components. Therefore, there are still unknowns in extrapolating human responses from animal based tests typically used to determine their safety and efficacy ([Dellepiane et al., 2000], [Giezen et al., 2008] and [Hendriksen, 2002]). In addition, human variability can affect whether and how well a vaccine works, as individuals can experience different degrees of protection from the same immunization. There may be side effects in some subpopulations due to interactions with unforeseen genetic or environmental factors.

In order to minimize the potential risks to recipients, each batch of vaccine must undergo extensive quality control testing. Though a manufacturer will perform numerous tests at various stages throughout vaccine production, regulators usually require that the final formulation of every vaccine batch be tested for both safety and potency before the lots may be released onto the market. Safety tests are performed to detect contaminants or active toxins, which can cause adverse reactions after immunization, while potency tests are performed to evaluate the ability of vaccines to induce the same amount of protective immune response as was found in the initial batches of vaccine used in the clinical trials. Once a final formulation has passed manufacturer tests, vaccine batch samples and the data from manufacturer testing are submitted for review and in some cases, additional testing. In Canada, this review is conducted at the Biologics and Genetic Therapies Directorate (BGTD), a department within the regulatory agency of Health Canada.

Much of the quality control testing performed on final formulated vaccine products, as with other testing carried out for regulatory purposes, is performed in animals. A large number of these tests cause severe pain and distress, and in some cases result in the death of the test animals ([Council of Europe, 2008] and [Hendriksen, 2002]). For example, in much of the world including Canada, the US and Europe, acellular pertussis vaccines are tested for safety with the histamine sensitization test (HIST), in which mice may die after suffering from anaphylactic shock ([Council of Europe, 2008] and [Yuen et al., 2002]).

In Canada, standards for the ethical use and care of animals in science are set and overseen by the Canadian Council on Animal Care (CCAC). The fundamental policy statement on the ethics of animal investigation requires adherence to the principles of humane science: that animals be used only where no non-animal method exists; that animal use is reduced; and pain and distress minimized (refined) as far as possible (CCAC, 1989). These principles of humane science are commonly referred to as the Three Rs of replacement, reduction and refinement and are internationally recognized as an important part of the advancement of ethical, animal-based science, forming the basis of both legislated and non-legislated animal oversight systems around the world (OIE Standards for Terrestrial Animal Health, 2010).

Animal use data collected by the CCAC shows that in Canada in 2007, over 85,000 animals were used for regulatory testing procedures and categorized within the CCAC's category of invasiveness E (CCAC, 2008). This number rose to just over 96,000 animals used in 2009 (CCAC, 2010). Following the CCAC's precautionary

approach, category of invasiveness E indicates that animals may suffer “severe pain near, at or above the pain tolerance threshold of unanesthetized conscious animals” (CCAC, 2008). For reasons of confidentiality the animal use numbers attributed to specific regulatory areas and specific institutions cannot be provided, but a considerable proportion of the numbers above represent animals used for the quality control testing of vaccines. Therefore, vaccine testing is an area where implementation of Three Rs principles is needed.

In recent years, methods that follow the Three Rs principles (sometimes referred to as “alternative” methods) have been, and continue to be, developed to reduce or replace animal use or to cause less pain and distress to test animals. In some cases these new methods may not only reduce animal suffering, but may also provide better data and in a reduced time frame (Centre and for the Validation of Alternative Methods (ECVAM), 2000 and [Gomez et al., 2006]). Even when this is the case, alternative methods to traditional animal-based tests may be slow to be accepted by regulatory agencies. For example, the serological assay for tetanus potency was developed in 1986, but it was not until 17 years later that it was validated and accepted as a recommended method in the European Pharmacopoeia (Hendriksen, 2007). In addition, there is no guarantee that a method with regulatory acceptance will also be implemented as a routine test by regulatory or industry laboratories.

This paper describes an interview-based study that was carried out to identify challenges and opportunities for implementing the Three Rs in vaccine quality control testing in Canada, with a view to facilitating the acceptance and use of scientifically sound alternative methods in vaccine quality control for both regulators and industry.

2. Methods

2.1. Case studies

In order to narrow the scope of the project, two human vaccine test case studies were used to address the research question. These vaccine tests were selected based on the vaccines involved being produced in Canada, and on the existence of Three Rs methods that had been implemented, or that were in the process of being accepted for use in Canadian regulation at the time of the study. These tests were also chosen due to having relatively recent activity in Three Rs implementation or consideration of such, and therefore provided the authors with an easily identifiable pool of knowledgeable stakeholders who could be approached for participation in this study. As few Three Rs methods have been implemented for safety and potency in Canada in recent years, there was a limited selection of tests which fit our criteria.

2.1.1. Case study 1: diphtheria/tetanus potency testing

The first case study looked at methods of potency testing, performed to evaluate the ability of a vaccine to induce immune protection. Canadian vaccine manufacturers are major producers of diphtheria and tetanus (D/T) vaccines for global distribution, which are typically administered as components of a combination vaccine. Historically, the standard potency test for diphtheria and tetanus performed by Canadian industry was a lethal challenge test in guinea pigs (Council of Europe, 2008), in which animals are either vaccinated with the test vaccine or injected with saline. After sufficient time to allow immunity to develop, both groups are challenged with either diphtheria or tetanus toxin, and animals with no or with insufficient antibodies to the vaccine die. In 2008, a serological method was accepted by BGTD and implemented as a replacement for the lethal challenge test by both industry and by the government for subsequent testing. In this serological method, blood is drawn from vaccinated animals and then tested in vitro for antibodies against diphtheria and tetanus antigens (Council of Europe, 2008). This test was used in the current interview-based study as an example of successful implementation of a method following Three Rs principles.

2.1.2. Case study 2: acellular pertussis safety testing

The second case study looked at methods of safety testing, performed to detect residual toxin or external contaminants in the vaccine, which can cause adverse reactions after immunization. Acellular pertussis vaccines are produced by combining various components of *Bordetella pertussis* bacteria, including detoxified pertussis toxin (toxoid), which in its active state, is thought to be responsible for much of the virulence of the pathogen (Gomez et al., 2006). The histamine sensitization test (HIST) is an internationally accepted assay for detecting residual pertussis toxin in pertussis vaccines. In this assay, mice are injected either with the vaccine batch being

tested, with a vehicle solution (such as saline) as a negative control, or with pertussis toxin as a positive control. Five days later, animals in all groups are challenged with a histamine injection and the number of animals that succumb are recorded. Pertussis toxin causes hypersensitivity in the mice to a histamine challenge that would not normally be lethal, which then leads to anaphylactic shock and death within 24 h (Council of Europe, 2008). A panel of three assays – an in vitro binding assay, an in vitro enzymatic assay and the Chinese Hamster Ovary (CHO) cell assay – is currently being assessed by BGTD as an alternative set of assays to the HIST. While the CHO and enzymatic assays have been available for some time, the binding assay has been recently developed to provide additional information in monitoring for residual active pertussis toxin in formulated vaccines. Samples of final lot vaccine are placed in wells containing antibodies to pertussis toxin, and detectable substrates are added to measure how much (if any) toxin exists in the samples (Gomez et al., 2006). The acellular pertussis binding assay was used in the current interview-based study as a case to identify factors involved in moving from an in vivo to an in vitro test system.

The D/T and pertussis test methods represent cases of human vaccine testing in general. While it is possible that these particular methods have qualities and involve circumstances which make them distinct from other human vaccines, the inclusion of three different vaccine components and two different test types should provide sufficient variation to analytically generalize any theories which have arisen from this study ([Pal, 2005] and [Stake, 2000]). While the authors have explored implementation factors for the Three Rs in human vaccines in Canada, the challenges and opportunities identified here may be relevant to Three Rs implementation for veterinary vaccines as well. It is likely that there would be some differences however, due to the fact that veterinary vaccines are produced against different (and far more) pathogens than humans, and may be regulated, tested and administered in different ways than human vaccines.

2.2. Interviews

Factors involved in supporting or challenging the implementation of Three Rs test methods were identified through interviews with vaccine stakeholders to gain their perspectives. Sixteen in-depth interviews with stakeholders were performed between July and December 2009, with six participants from industry (5 research scientists, 1 regulatory affairs personnel) and 10 participants from government (Health Canada: 1 research scientist, 7 regulatory scientists and 2 animal care personnel). Participants were asked a series of open-ended questions on the case study methods and about safety and potency testing in general. The questions probed their vaccine and regulatory background and current area of responsibility, the importance of vaccine quality control testing, their knowledge of methods used for the case study tests, benefits and drawbacks of known methods, motivation for new test method development, how methods are identified for revision, the effect of international vaccine policies on Canadian policy development, and whether their organization's mandate affected their personal decision-making. The open-ended question style of the interviews was used to allow participants to expound on each question for as long as they wished, to allow for new questions prompted by points brought up by the participant, and to encourage honest dialog by allowing flexibility in the flow of the discussion. Participants provided responses specific to the case study methods, as well as more generally about traditional animal test methods, in vitro methods and other Three Rs methods, through prompting by the interviewer or brought up spontaneously by themselves. As participants were encouraged to respond at length, interview durations were dependent on participant responses and ranged between 26 and 77 min. All interviews were audio recorded for later transcription and analysis. Potential bias in participant responses was minimized through careful development and testing of the interview questions on volunteers prior to the study, as well as by the conscious efforts of the interviewer to maintain neutrality with each participant during interviews.

2.3. Participant selection

Participants were selected through purposive sampling (Palys and Atchison, 2007), based on the fulfillment of certain criteria: that they were either currently or previously involved in the production, testing or release of vaccines, either through direct involvement with the vaccines or with the animals used in one of these processes, and preferably that they had knowledge of or expertise with at least one of the case study vaccines. Initial

participants were identified through background research and informational interviews, after which the participant pool was expanded through referrals from interviewees (snowball sampling; Palys and Atchison, 2007).

2.4. Analysis

Analysis of participant responses began with the identification of key concepts from the transcribed interviews and the organization of these concepts into categories and themes, a process known as coding (Coffey and Atkinson, 1996). Factors affecting Three Rs method acceptance/implementation were identified within the coded sections, which were then analyzed to identify the most commonly and most strongly cited factors, both for vaccine test methods in general, as well as specifically for the case study test methods.

2.5. Consent and confidentiality

Prior to the initiation of the study, the materials and protocols for this study received ethical review and approval from Institutional Review Board (IRB) Services. All participants willingly signed consent forms and confidentiality agreements before taking part in their interview. Interviews were conducted under strict anonymity – only an assigned number identified participants in study documents.

3. Results and discussion

While a wide range of factors was identified as affecting the implementation of the Three Rs in vaccine quality control in Canada, only the most frequently cited factors among participants are described.

Factors are categorized as either challenges or opportunities to implementation. The challenges describe reasons for caution or the use of certain established procedures that inhibit method changes, while the opportunities describe motivations for adopting alternative methods/getting rid of traditional animal methods, or activities taking place which support Three Rs implementation. These challenges and opportunity factors are displayed in the figures as total participant responses (Fig. 1 and Fig. 3, respectively), as well as responses by organization (Fig. 2 and Fig. 4, respectively).