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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.

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; 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.

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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; 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; 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, 2011). 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 (The European Parliament and the Council of the European Union, 2010; World Organization for Animal Health, 2011).

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, 1991). 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 (European Centre for the Validation of Alternative Methods, 2000; 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.