Adventitious agents in viral vaccines: Lessons learned from 4 case studies

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Abstract

Since the earliest days of biological product manufacture, there have been a number of instances where laboratory studies provided evidence for the presence of adventitious agents in a marketed product. Lessons learned from such events can be used to strengthen regulatory preparedness for the future. We have therefore selected four instances where an adventitious agent, or a signal suggesting the presence of an agent, was found in a viral vaccine, and have developed a case study for each. The four cases are: a) SV40 in polio vaccines; b) bacteriophage in measles and polio vaccines; c) reverse transcriptase in measles and mumps vaccines; and d) porcine circovirus and porcine circovirus DNA sequences in rotavirus vaccines. The lessons learned from each event are discussed. Based in part on those experiences, certain scientific principles have been identified by WHO that should be considered in regulatory risk evaluation if an adventitious agent is found in a marketed vaccine in the future.

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1. Introduction

The discovery of an adventitious agent in an already licensed vaccine, or a signal suggesting an adventitious agent, has presented difficult issues for national regulatory authorities (NRAs), national control laboratories (NCLs), public health officials, manufacturers, and the general public since the mid-1900s. We have selected four instances where a contaminating agent, or a signal suggesting the presence of an adventitious agent, was found in a viral vaccine, and have developed a case study of how each was dealt with by selected NRAs, manufacturers, and WHO. Each case study is a summary of key events in a complex series of activities that involved multiple organizations. As such, they attempt to capture only the key interactions that occurred among individuals and organizations. The two most recent cases are presented in more detail only because more information was available, more studies were undertaken, and more interactions among organizations took place.

The same format is used for each of the four cases and includes: 1) the initial findings that suggested the presence of an adventitious agent; 2) background information on the agent or signal; 3) follow-up steps that were taken by relevant organizations; 4) scientific advice that was sought; 5) regulatory issues and actions that were taken; 6) vaccine supply implications; 7) public transparency and communication with other organizations; 8) public health and other issues; 9) the overall outcome of the event; and 10) lessons learned. The purpose of this review is to present a summary of the response to those four instances by manufacturers, selected regulatory authorities, and public health officials, because we believe they can be instructive to the biomedical community, in general, and to regulatory authorities in particular, as relevant background information that could help guide the response to future similar events, should they occur.

Although adherence to current Good Manufacturing Practices and Quality by Design principles, as well as the advent of increasingly sensitive technologies for detection of adventitious agents, should make these events increasingly rare in the future, regulatory preparedness will be key to addressing any potential future events. The specific activity that led to the development of these four case studies is the World Health Organization (WHO) draft document [1] that is intended to assist regulatory authorities in their...
assessment of risk when, in the future, a new adventitious agent is discovered. The recent revision of WHO Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks [2] provides principles for evaluation of adventitious agents, but it does not address the issue of risk assessment and related regulatory decisions/actions in such circumstances. For example, a clear and consistent evaluation strategy was not evident in all jurisdictions to support regulatory decision-making when the sequences of porcine circoviruses or infectious circovirus were reported in rotavirus vaccines (see case study 4 below).

Similar situations have occurred in the past, including finding SV40 in poliovirus vaccines in the 1960’s (see case study 1 below). In the 1970’s, findings of bacteriophage in live viral vaccines led to the need for regulatory actions (see case study 2 below). The development of the Product-Enhanced Reverse Transcriptase (PERT) and related PCR-based reverse transcriptase (RT) assays led to finding RT activity at levels not detectable by the conventional RT assay used in control of avian cell-derived vaccines in the mid-1990’s, which suggested the possible presence of a contaminating retrovirus (see case study 3 below).

The above examples illustrate that both conventional and new methods have led to the discovery of infectious agents, or the marker of a viral agent, in vaccines in the past. Recent advances in technology have the potential for other types of findings to be made that are suggestive of an adventitious agent. These might include the discovery of a structure similar to a viral particle by visualization technologies, such as enhanced electron microscopy; or discovering a partial nucleic acid sequence suggestive of an adventitious agent by modern (“next generation”) amplification or sequencing technologies. Therefore, regulators may be faced with making risk evaluations and decisions about the safety of licensed vaccines on the market in their country on the basis of incomplete data with regards to whether an actual adventitious agent is present or not, and its potential medical significance. Although these events are rare, regulators should be prepared if they were to occur in the future. Learning lessons from past experiences may assist in reaching a globally convergent approach to regulatory risk evaluation and regulatory decision-making.

The main pieces of information that should be considered in the risk evaluation performed by NRAs can be summarized in the responses to the following questions:

➢ Where was the signal detected?
➢ What exactly was detected?

The order in which the questions are answered is of no significance. Each time new data emerge, a new benefit/risk assessment might be necessary. In other words, the process is likely to be dynamic and new data for evaluation will continue to emerge during the process. This implies the need for transparent communication practices between the NRA/NCL and the manufacturer, and potentially among NRAs as well as other groups, and with relevant experts from the scientific community.

2. Case study no. 1: SV40 in polio vaccines

2.1. Initial findings

Studies by Dr. Bernice Eddy in 1959 at the Division of Biologics Standards (DBS), NIH (the NRA for biologicals in the USA at that time) showed that a factor in primary rhesus monkey kidney (RMK) cells caused tumors in hamsters. Independently, Dr. Maurice Hilleman and colleagues at Merck, a pharmaceutical company that manufactured vaccines, identified a new viral agent, SV40, in RMK cells in 1960.

2.2. Background

Dr. Eddy and Dr. Sarah Stewart at NIH discovered mouse polyoma virus in 1957 [3] and showed that it could induce tumors in hamsters and other small animals [4]. Dr. Eddy hypothesized that similar agents might be found in monkey kidney cells, which were being used to produce polio vaccines. In 1959, she inoculated RMK cell lysates into newborn hamsters, since she knew from her work with the mouse polyoma virus that hamsters were potentially sensitive hosts. Initial results showed that most of the animals inoculated with RMK cell lysates developed tumors.

In October 1960, Dr. Eddy gave a talk at the New York Cancer Society on mouse polyoma virus. Based on the results of her initial studies, she suggested that monkey kidney cells contained similar viruses [5].

Also in 1960, Dr. Hilleman and colleagues at the Research and Development Department at Merck discovered a new viral contaminant of primary RMK cells that were being used to produce an adenovirus vaccine as well as polio vaccines [inactivated polio vaccine (IPV) which had been licensed by DBS and was on the market; and oral polio vaccine (OPV) which was still in clinical trials in the USA and elsewhere] [6]. At that time, RMK cells were used in
an in vitro quality control test to look for adventitious agents in the final bulk vaccine. Using that test system, there was no evidence for an adventitious agent. However, when African green monkey kidney (AGMK) cells were substituted for RMK cells in the quality control test, a cytopathic effect (vacuole formation) was noted. The agent responsible for the cytopathic effect initially was called the simian vacuolating agent, and later was re-named simian virus number 40 (SV40) — the 40th in a series of viruses that had been identified in nonhuman primates. In other words, RMK cells contaminated with SV40 does not cause a cytopathic effect, but when AGMK cells were used as the detection system, SV40 could be detected.

2.3. Follow-up steps

2.3.1. Merck

The adenovirus stocks were tested for SV40 and all were found to be positive. Subsequently, the polio virus seeds that had been used to produce experimental lots of OPV were tested, and were shown to be positive for SV40. Merck at that time was producing only IPV. Since IPV was inactivated with formaldehyde, studies were done to assess the effectiveness of formaldehyde to inactivate SV40. Those studies indicated that formaldehyde could destroy the infectivity of SV40. The results of those various studies were presented to DBS in a briefing by Merck [7]. Subsequently, a change from RMK to AGMK as the cell substrate for vaccine production was initiated by Merck since, unlike rhesus monkeys, SV40 was not an endogenous contaminant of African green monkeys.

Dr. Hilleman’s colleague, Dr. Girardi, initiated studies on the new viral agent, SV40, to assess its potential as an oncogenic agent. By June of 1961, results of his studies showed that SV40 could cause tumors in hamsters within six months of inoculation. Shortly after learning of the results, Dr. Hilleman informed the DBS and the already appointed NIH Technical Committee on Poliomyelitis Vaccine (TCPV) [8].

2.3.2. Wellcome research laboratories (WRL)

In March 1961, WRL published information to show that SV40 was, in fact, at least partially resistant to the inactivating effects of formaldehyde, and that antibodies to SV40 could be detected in recipients of IPV [9].

2.3.3. DBS

Human studies were conducted to assess the infectivity of SV40 and the results showed that when men were exposed to SV40 by the nasal and oral mucosal routes, live virus could be recovered 11 days after inoculation [10]. In addition, most of the human subjects developed antibodies within one month of inoculation. These data established that SV40 was infectious for humans.

In March of 1961, following the publication of the WRL data, tests were initiated at DBS to see if SV40 could be detected in 10 lots of IPV. All 10 were found to be positive for SV40, thus confirming the findings at WRL [11].

2.3.4. WHO

WHO had established an Expert Committee on Poliomyelitis (ECP) in the 1950s that was very active in various aspects of international efforts to control the disease as well as in keeping up to date on research and development programs. In its third report [12], taking into consideration the progress that had been made in OPV research and development including clinical studies, the ECP recommended that WHO convene a study group to undertake the drafting of international requirements for the production and testing of OPV. WHO agreed with that recommendation and established the Study Group on Requirements for Poliomyelitis Vaccine (oral) (SGPV). The SGPV, which included members from the USA, USSR, UK, and Canada, met on 7–12 November 1960. DBS participated in the WHO SGPV meeting and provided several working documents for consideration. The Requirements were annexed to the SGPV report, and included a section on adventitious agents in which the detection of simian agents such as SV40 is acknowledged and raises the problem of whether their presence is permissible and whether they can be removed. The SGPV considered it desirable that all seed lots should be free from adventitious agents. Section 3.6.2 of the WHO Requirements specified a test for SV40 that should be done on the final bulk.

2.4. Scientific advice

In May 1961, the NIH convened the TCPV to discuss the SV40 issue. The committee acknowledged that SV40 had been found in both IPV (licensed product) and OPV (investigational product), but they also said that there was no evidence that small amounts of SV40 are capable of producing disease in humans. The TCPV therefore stated that while efforts to remove SV40 from future lots of OPV were underway, the vaccination program then in progress should continue with vaccine then available (many lots of which contained SV40 as a contaminant). No vaccine on the market was recalled [13].

The TCPV was reconvened in June 1961 to discuss the Merck results showing that SV40 could cause tumors in hamsters. The committee took into consideration both the work of Dr. Eddy [14], and the report of Dr. Hilleman’s group. But the committee concluded that it was too early to draw any conclusions concerning the significance of the findings. The TCPV reaffirmed the position it took one month earlier, taking into consideration a number of factors such as benefit/risk in the midst of an epidemic, that polio vaccination programs with IPV should continue even though most of the vaccine lots were probably contaminated with SV40 [15].

2.5. Regulatory issues and actions

In August 1960, DBS convened a conference to discuss draft regulations for OPV, including how to handle the issue of SV40. The final regulations were issued in November 1960, and indicated that the vaccine must be free of agents that are viable and demonstrable [16]. The draft referred only to viable agents. Demonstrable depends on the length of time the control cell cultures are held as well as potentially other methodological factors. Some participants at the conference argued that the longer the cell cultures are held, the higher the probability of being able to demonstrate an adventitious agent present in low concentrations [17]. In other words, they suggested that a shorter observation period (2 weeks) vs a longer period (4 weeks) could mean that a low level of a viable agent such as SV40 might be missed [17]. The shorter observation period was included in the final regulations [16].

DBS issued a number of memos to manufacturers between April 1961 and August 1962 including: a) April 1961 — requested screening for SV40 using AGMK cells; b) June 1961 requirement to submit data showing that all final lots of vaccine are free of live SV40; and c) August 1962 — proposal to test virus pools for SV40 prior to formaldehyde inactivation. The regulations themselves were amended to accommodate the new requirements in 1963 [18].

OPV vaccine produced in RMK of Chinese origin was approved for marketing in China in 1961. In the late 1970s and early 1980s, studies to detect SV40 were undertaken by OPV manufacturers. The 1979 edition of the Chinese Requirements for Biological Products stated that viral seed, primary RMK, and bulk vaccine should be screened for SV40 using an in vitro assay. In 1984, the Institute of Medical Biology developed a test to screen rhesus monkeys for SV40. Monkeys found to be positive were not permitted to be used for vaccine production (personal communication).
The 2000 edition of the Chinese Requirements for Biological Products required screening seed virus using a specific PCR assay as recommended by WHO. In 2001, OPV manufacturers were required to use the PCR assay to test all of the OPV seed viruses which were used before 2000 and not screened for SV40. SV40 was not detected. In 2004, the Institute of Medical Biology completed the PCR test for SV40 in 10 lots of seed virus, 140 lots of bulk and 128 lots of final products produced during the period 1997 to 2003, and SV40 was not detected in any of the samples (personal communication). Similar studies also were undertaken by FDA [19]. The negative results demonstrated the effectiveness of the measures undertaken to prevent SV40 contamination, after it was identified as an issue.

2.6. Vaccine supply implications

Because no action was taken to withdraw SV40-contaminated vaccine lots from the market, there was no impact on the supply of IPV. Similarly, since no action was taken to limit the use of experimental OPV, the field trials continued without interruption.

2.7. Public transparency and communication with other organizations

In June 1960, Dr. Hilleman announced at an international meeting on OPV, sponsored by the Pan American Health Organization and WHO, that a new monkey virus, SV40, had been identified as a widespread contaminant of RMK cells and that formaldehyde appeared to be an effective inactivating agent [20]. Dr. Eddy’s work on RMK cells was published in May 1961 [14].

The NIH TCPV’s conclusions and decisions on SV40 in May and June of 1961 were not made public. But as participants in those meetings, both DBS and Merck had access to the reports.

In June of 1961, Dr. Hilary Koprowski gave a talk at the annual meeting of the American Medical Association in which he drew attention to the SV40 contamination issue and the general problem of viral contamination of primary monkey kidney cell cultures [21]. He also pointed out that human diploid cell lines (e.g., WI-38) provided a cleaner alternative cell substrate.

Shortly thereafter, in July 1961, DBS issued a statement that essentially tracked the TCPV conclusions and recommendations.

2.8. Public health and other issues

The initial assessment, based on limited information on SV40, suggested that there was no risk to public health. The medical significance of human exposure to SV40 has been the subject of research for several decades. Although some studies have suggested that SV40 may have played a causative role in some human cancers [22], data are insufficient to show that the contaminating SV40 virus present in some vaccines in the 1950s and 1960s was the primary causative agent in human cancers. The potential cross-contamination of the PCR analyses cast doubt on the validity of some of the data claiming detection SV40 DNA in human samples, leading to inconclusive and sometimes contradictory results. Individuals who received the contaminated vaccine have the same overall cancer rates as those who were not inoculated. Nevertheless, some controversy still remains on the significance of human exposure to SV40.

The potential for the appearance and/or an actual conflict of interest in the assessment of risk is an important consideration. This is not limited to financial elements since personal and professional commitments to a program also may be, or appear to be, a potential conflict of interest. In order to maintain public confidence, conflict of interest in all forms must be avoided.

The SV40 contamination of vaccines caused some manufacturers to stop producing polio vaccines (e.g., Merck). In addition, some manufacturers became more interested in using human diploid cell lines as a substrate for vaccine production.

2.9. Overall outcome

Changes that were made in the manufacturing process for polio vaccines eliminated the risk of further SV40 contamination.

The unrecognized contamination of IPV, OPV, and adenovirus vaccine by SV40 in the 1950s and 1960s resulted in millions of people being exposed to SV40. The decision to allow SV40-contaminated IPV (licensed) and OPV (investigational) to continue to be used while efforts were being made to produce SV40-free virus seeds was likely to have been based in large part on benefit-risk considerations by the TCPV which concluded that continuing to administer the contaminated vaccine posed less of a health consequence for humans than discontinuing the use IPV and OPV, many lots of which were probably contaminated with SV40. Data available to date support that initial conclusion.

2.10. Lessons learned

There should be early public disclosure of findings that have potential public health consequences so that appropriate scientific and public health discussions can take place. However, it is important that the initial findings are confirmed before public disclosure in order to ensure the validity of the findings. In this case, no effort was made to replicate Dr. Eddy’s results either within DBS or externally. Her results were confirmed indirectly by studies of SV40 at Merck.

The sharing of information on the discovery of an adventitious agent in a licensed vaccine should take place as rapidly as possible so that it can be adequately discussed and further investigated in a coordinated manner. The evaluation of risks, benefits, and a consideration of various options for actions to be taken should be done in a transparent manner. Conflict of interest considerations should be taken into account in the context of public health decision-making. This is a current requisite consideration for many governmental body advisory committees and for WHO expert advisory committees.

3. Case study no. 2: bacteriophages in live viral vaccines

3.1. Initial finding

In 1973, live bacterial viruses (bacteriophages) were identified in several lots of live viral vaccines that had been submitted by Merck to the Bureau of Biologics (BB), Food and Drug Administration (FDA) for lot release.

3.2. Background

Bacteriophages were reported as a contaminant of bovine sera in 1972 [23]. Follow-up studies by BB confirmed and extended those initial results [24]. Since some lots of sera contained over 10⁴ pfu/ml of coliphage, it was reasonable to assume that bovine sera used in the manufacture of live viral vaccines might contain bacteriophages that could be carried through the manufacturing process into the final products.

Initial studies showed that the live viral vaccines from Merck contained bacteriophages at low concentrations (1–5 pfu/ml). The discovery was confirmed by an independent laboratory at BB using coded samples that included positive and negative controls along with samples of vaccine lots that had been reported positive in the initial experiments.
Soon after the results were confirmed by BB, Merck was contacted and asked to independently confirm the results using retention samples of the same lots of vaccines that were positive at BB. The manufacturer was initially unable to confirm the results. Discussions of the assay procedure did not reveal any differences that could explain the disparity in results. Personnel from BB went to the manufacturer's laboratory with all of their reagents and equipment in an attempt to replicate the results at the manufacturer's facility. Using the FDA procedure, the manufacturer's samples were shown to be positive for bacteriophages at the manufacturer's laboratory. The initial negative results had been due to the fact that the manufacturer's centrifugation step was different from that used at FDA, and it had not been sufficient to pellet the bacteriophages.

3.3. Follow-up steps

The initial findings were rapidly extended to other live viral vaccines and other manufacturers. The results showed that it was a general issue since 11 of 60 lots of live measles, mumps, rubella, and polio vaccines were positive for bacteriophages when tested using the C-3000 and K-12 strains of Escherichia coli as the host [25]. The host spectrum was expanded to include other bacterial strains, but no additional phages were detected. Only one phage, φV–1, was isolated from vaccines [26].

3.4. Scientific advice

An ad hoc advisory committee [27] that included experts in the fields of viral vaccines, public health, infectious diseases, and bacteriophages was convened to review the data to assess the potential risk to public health associated with bacteriophages in live viral vaccines. The committee concluded that the presence of bacteriophages in vaccines did not raise a substantial issue of safety insofar as could be determined from the then existing medical and scientific information.

3.5. Regulatory issues and actions

FDA regulations for purity in effect in 1973 stipulated that “viral vaccines shall not contain extraneous agents”. Therefore, after confirmation of the initial results within BB and at the manufacturer, no lots of live viral vaccines were released by BB for approximately two weeks.

Faced with the consequences of prohibiting the distribution of all live viral vaccines until phages could be eliminated from them, the FDA, after consultation with an ad hoc expert committee, published regulations that allowed phages to continue to be present in the vaccines for a limited time [28]. That decision was based on a benefit/risk assessment that weighed the theoretical risks of phages against the well-documented benefits and safety of the vaccines.

3.6. Vaccine supply implications

The short period of time (approximately 2 weeks) during which no lots of live viral vaccines were released by BB did not have a significant impact on vaccine availability.

3.7. Public transparency and communication with other organizations

In addition to the publication of FDA's regulatory action, a public workshop on the issue was held at the NIH to discuss the initial findings, other relevant studies, and the implications of the findings for public health. Articles appeared in the lay press and scientific journals and newsletters. The Bureau of Biologics (Canada) and WHO were notified of the findings and the actions that were being taken.

3.8. Public health implications and other issues

In addition to the regulatory issues raised by bacteriophages in live viral vaccines, there was a question of whether the presence of viable phages in vaccines constituted a threat to the public health. The theoretical risks included both indirect and direct effects of phages on humans: (a) the induction of a toxin by phages in appropriate bacterial hosts followed by a disease that is due to the toxin such as diphtheria caused by corynebacteria and its phages; and (b) the induction of changes in human cells which could then lead to any of a variety of diseases.

Although phages had been used as therapeutic agents and in a variety of clinical studies [29–31], no report could be found in which phages were injected into humans or animals with the main purpose of assessing in a prospective manner any adverse effects upon the health of the subjects because, by definition, phages were restricted in their host range to bacteria and were not expected to cause disease in humans.

Therefore bacteriophage φV–1, isolated from live virus vaccines in 1973, was evaluated with respect to its ability to induce disease in small laboratory animals and nonhuman primates [32,33]. Cytogenetic studies also were undertaken to evaluate the potential of φV–1 as a clastogen [32,34]. The phage caused neither an increased death rate nor more histopathologic lesions than were found in controls. Similarly, the chromosomal aberration rate in cell cultures inoculated with φV–1 was not different from controls. On the basis of these studies and the fact that no more than 20 PFU/ml were found in vaccines, it was concluded that it is unlikely that φV–1 posed a health hazard to vaccine recipients who had received it in the past. The low level of bacteriophage contamination in vaccines was most likely the result of dilution during the manufacturing process since the growth medium is removed from the cell culture either before or after inoculation of virus working seed, the cell cultures are rinsed, and the growth medium is replaced with serum-free maintenance medium. During the period 1974–1978, phages such as φV–1 were not detected in live virus vaccines released by BB.

3.9. Overall outcome

The possibility of φV–1 in vaccines having induced health abnormalities was considered to be remote based on the restricted host range of bacteriophages, the low levels of contamination, and the results of studies to assess risk [32–34].

The possibility that other phages may have been present in vaccines in the past could not be ruled out. However, on the basis of isolation results from sera and vaccines, the variety of phages that might have been inoculated into humans was not as broad as initially assumed [23–25].

3.10. Lessons learned

Better control of the quality of bovine sera used in vaccine production, and screening for phages in bovine sera before use in vaccine production eliminated detectable phages in vaccines. Although these measures can potentially add to the cost of vaccine production and possibly to the cost of the vaccines themselves, they are important steps that can minimize the risk of contamination.

When attempts to replicate results in an independent laboratory fail, it is essential to determine the basis of the problem and to
resolve the question of the validity of the initial results. In that regard, this case illustrates the benefit of an open and collaborative relationship between industry and the NRA on scientific issues. This case also points out that a more transparent process with due consideration for potential conflict of interest led to an outcome in which the public was made aware of the issue early in the process and was reassured of the continued safety and benefits of vaccines.

4. Case study no. 3: reverse transcriptase in muscles & other vaccines

4.1. Initial findings

The discovery in 1995 of reverse transcriptase (RT) activity in marketed measles, mumps and rubella (MMR) vaccine raised concerns that the vaccine was contaminated by an unrecognized avian retrovirus with unknown safety implications.

4.2. Background

The usual flow of genetic information is from DNA to RNA. However, the reverse of that process was discovered to be mediated by an RNA-dependent DNA polymerase (reverse transcriptase) that uses some RNA viruses, such as retroviruses, to use reverse-transcribe their RNA genomes into DNA. That viral DNA can then be integrated into the host genome and replicated, resulting in the production of more RNA virus. RT activity has therefore been used as a biochemical marker for the presence of retroviruses. However, the genes that encode RT are widely distributed in eukaryotic organisms and all reverse transcriptases are evolutionarily related. In addition, cellular DNA-directed DNA polymerases can exhibit some ability to use RNA as a template and reverse-transcribe as well. Thus, the detection of RT activity does not a priori constitute evidence of a replicating retrovirus.

Regulatory requirements for measles and mumps vaccines in 1995 stipulated that chicken cells used in production should be free from infectious avian retroviruses. All licensed vaccines produced in chicken cells would therefore have been tested for the presence of avian retroviruses. The tests for retroviruses included standard assays for RT activity and a test to serologically screen the flocks from which the cells were derived (“COFAL”).

In 1994, researchers at the Swiss National Center for Retrovirus, University of Zurich, Switzerland reported the development of an ultrasensitive assay for RT, called Product Enhanced Reverse Transcriptase (PERT), which is at least a million-fold more sensitive than the standard assay [35]. The first PERT assay, and then variants of it developed independently by others, was used to examine vaccine cell substrates and vaccine samples for RT activity. Results showed the presence of low levels of RT activity in some vaccines derived from chicken cells, notably MMR vaccine produced by Merck, and yellow fever vaccine. No RT activity was detected in vaccines derived from defective endogenous avian retroviral pro-viruses in chicken cells, and no retrovirus replication and amplification could be demonstrated. There was evidence for the presence of both Endogenous Avian Virus (EAV) and Avian Leukosis Virus (ALV)-related sequences associated with the particles. Based on the then-current state of knowledge of avian retroviruses and endogenous retroviral genomes, together with epidemiological studies, it was concluded that the risk of vaccine-preventable disease was real and quantifiable, whereas the risk posed by the chicken-cell-derived particles was theoretical and remote.

There was also a broad recommendation that WHO should establish a task force to coordinate collaborative research relevant to the characterization, quality control and safety assessment of all cell substrates intended for use in vaccine production. In addition, it was suggested that further information on the characterization of the Endogenous Avian Virus (EAV) family of endogenous retroviral genomes should be obtained, including investigation of the EAV and Avian Leukosis Virus (ALV) endogenous genomes in flocks of chickens used for vaccine production. Even though there was lack of evidence for any real health concerns regarding human use of chicken-cell-derived live vaccines, theoretical concerns remained, such as the possibility of the endogenous retroviral particles in chicken cells forming pseudotypes with vaccine virus grown in chicken cells, and it was recommended that this aspect also should be investigated. Subsequent work showed that there was no evidence that this phenomenon occurred [38].

4.4. Scientific advice

In October 1995, the WHO called an informal consultation in Geneva to discuss the discovery of very low levels of RT activity in some vaccines produced in avian cells. The purpose of the meeting was to advise WHO regarding the interpretation of these observations and to recommend activities that may assist in clarifying the issues and any regulatory action that might be needed. Participants included some members of the WHO ECBS, invited vaccine manufacturers, National Control Authorities and independent experts. The report from this group was submitted immediately thereafter to the WHO ECBS for further deliberation. After consideration of all the data available at the time, it was concluded that there was no evidence to indicate that the RT activity found using the PERT assay had any medical significance for humans, or that it reflected the presence of a transmissible retrovirus in the vaccines [39,40].

The meeting participants heard that while RT is an essential component of the replication cycle of all infectious retroviruses, such enzyme activity also can be derived from other sources. Because the replication cycle of retroviruses involves a double-stranded DNA copy of the viral genome which integrates into the chromosome of the host cell, most animal and avian species contain evolutionary remnants of ancient infections as part of their normal genetic makeup. These are known as endogenous retroviral-like elements and generally no longer encode a functional viral genome. The presence of genes for RT in the absence of infectious virus had been reported in a variety of mammalian and avian cells. Furthermore, studies had shown that RT activity is not unique to retroviruses as it is encoded in various forms by other types of cellular genes, such as cellular DNA-directed DNA polymerases and other types of retro-elements (long interspersed nucleotide elements, etc.).

The WHO consultation concluded that the current methods for manufacturing and controlling vaccines produced in chicken cells were still appropriate and recommended that chicken-cell-derived vaccines, which have a major role in international immunization programs, should continue to be used. However, the group also recommended that further studies be undertaken urgently and internationally to put into perspective the very low levels of RT activity found in the vaccines.
4.5. Regulatory issues and actions

The detection of very low levels of RT activity in MMR vaccine prepared using chicken cells raised issues regarding the safety of all vaccines produced in chicken cells. No such RT activity was detected in measles vaccines produced in human diploid cells nor in inactivated vaccines derived from chicken eggs, such as influenza vaccines. The question arose as to whether live attenuated vaccines produced in chicken cells should be withdrawn from the market until the issue had been resolved.

At that time, MMR vaccines had been authorized in the European Union at the national level, and had not gone through the centralized procedure of the European Medicines Evaluation Agency (EMEA). Therefore the EMEA was not directly involved with this issue. However, the EMEA did facilitate scientific discussions of the issues among NRAs.

In 1996, CBER developed assay methods in-house to test vaccine cell substrates and viral vaccines by PCR-based RT assays. They also convened expert advisory committee meetings, at which the issue was discussed. A collaborative study between CBER, NIBSC, and the US Centers for Disease Control (CDC) was performed to inoculate various cell lines that could potentially be susceptible to avian retroviruses with samples that were PERT positive, without detection of infectivity. In addition, the CDC performed an analysis of serological specimens from vaccinated children who had received MMR vaccine and found no antibodies to avian retroviruses, supporting the contention that the vaccine recipients were not exposed to infectious and replicating retroviruses [41].

In 1998, FDA wrote to manufacturers concerning their new policy on the use of the PERT assay for detecting possible retroviral contamination of cell substrates used in viral vaccine production regardless of species of substrate because of the enhanced sensitivity of this method over conventional tests and because the conventional test was often subject to inhibition by the test materials, further limiting its sensitivity (letter from Dr Carolyn Harder, Director of the Office of Vaccines Research and Review, FDA). The FDA concluded that the benefits of the vaccine were known and quantifiable whereas any potential risk was theoretical and remote.

WHO recommended continued use of chicken-cell-derived vaccines. A subsequent version of the WHO Requirements for Cell Substrates Used for the Production of Biologicals referred to newer assays for the detection of possible retroviral contamination of cell substrates used for viral vaccine production as optional.

Some NRAs had considered suspending implicated vaccines, but decided not to do so in light of the WHO recommendations. Switzerland requested a revision of the package label to include a statement that low levels of RT had been detected in the product but that it was of no clinical significance, referring to the conclusions of the ECBS and WHO publications on the matter (letter from the Swiss Federal Office of Public Health to WHO, FDA and NIBSC, November 1998).

4.6. Vaccine supply implications

There was no interruption in vaccine supply due to this finding of RT in MMR.

4.7. Public transparency and communication with other organizations

The Swiss laboratory that discovered RT activity in MMR reported the fact to Merck, the manufacturer of the MMR made in chicken cells, and Merck confirmed the results. The FDA also was informed and confirmed the finding. The WHO, Health Canada, the EMEA, the Paul-Ehrlich Institute (PEI, Germany), and the NIBSC (UK) were advised of the issue.

The discovery of traces of RT activity in MMR vaccine quickly became public knowledge and articles appeared in newspapers [e.g., [42]].

The WHO also took on the responsibility of coordinating communication about the issue amongst the agencies, and made public the outcomes of all of its consultations and recommendations in a timely manner [36,37,39,40,43].

The medical community in the USA was kept informed of developments by FDA in a letter dated 4 Jan 1996. Such actions were shared beforehand with other agencies via WHO so that all interested parties were aware of developments.

4.8. Public health and other issues

Because early data suggested (and was later confirmed) that there was only a theoretical risk associated with the presence of low levels of RT activity, chicken-cell-derived vaccines were allowed to remain on the market. A regulatory decision to suspend production and use of chicken-cell-derived vaccines would have had huge implications for global immunization programs since there was insufficient diploid cell-derived vaccine available. This would have led to a significant increase in morbidity and mortality due to the infectious diseases the vaccines prevent.

However, the WHO consultation, as well as the ECBS, recommended that surveillance related to issues of the safety of viral vaccines should continue, including sero-epidemiological studies of vaccine recipients and studies for the presence of avian genetic material in vaccine recipients. It was also recommended that further studies on the evidence for cancer or other possible adverse effects, including the analysis of existing data should be undertaken. Epidemiological studies at that time had revealed no association between the use of chicken-cell-derived vaccines and an increased rate of detection of cancers, including those of childhood.

It was concluded that the presence of RT activity, especially at the very low levels detected by the novel ultrasensitive assays involving PCR was insufficient to prove contamination by an infectious avian retrovirus. Furthermore, human exposure to avian retroviruses through food and food processing was expected to be a common occurrence and there were no known adverse outcomes from such exposures. Limited studies of the sera of vaccines following several doses of measles vaccine had not revealed any antibodies to avian retroviral antigens and no retroviral genome sequences were detected in their peripheral blood mononuclear cells. In contrast, it had been reported that poultry workers, who are potentially exposed to infectious avian retroviruses, do have some antibodies to avian retroviral antigens in their blood, but with no observable ill effects.

Merck identified a line of chickens that had been bred at the Canadian Centre for Food and Animal Research (CCFAR) in Ottawa for their ability to lay larger eggs. As a coincidence, those chickens had lost one of the two known retrovirus genome families. Merck purchased that line from CCFAR, which was scheduled for closure, and established multiple flocks in isolation at several locations. Subsequently, Merck converted production to eggs from that source.

4.9. Overall outcomes

No regulatory action was taken to suspend the production or use of chicken-cell-derived vaccines, and they continue to play a major role in immunization programs worldwide today. They have a long history of safe usage and efficacy.
5. Case study no. 4: PCV in rotavirus vaccines

5.1. Initial findings

Academic researchers, led by Dr. Delwart at the Blood Systems Research Institute and the University of California, San Francisco used microarray and high-throughput sequencing to characterize eight marketed live viral vaccines (trivalent oral poliovirus (OPV), rubella, measles, yellow fever, varicella-zoster, multivalent Measles/Mumps/Rubella, and two rotavirus live vaccines) for viral diversity and to determine if other viral sequences were present in the vaccines. The researchers detected DNA fragments of porcine circovirus-1 (PCV-1) in two lots of the Rotarix® vaccine manufactured by GlaxoSmithKline (GSK). Their results were published online ahead of print on 7 April 2010 [44].

5.2. Background

PCV-1 and PCV-2 are small (<20 nm), non-enveloped, single-stranded DNA viruses, that infect some mammalian cells. PCV-1 can persist without causing any visible cell changes, but PCV-2-transfected cells show cytopathogenic effects. Existing evidence supports the view that PCV-1 is not pathogenic for humans. PCV-2, in contrast to PCV-1, is associated with disease in pigs (e.g. post-weaning multi-systemic wasting syndrome, PMWS), but existing evidence suggests that PCV-2 also is not pathogenic in humans. It should be noted that the rotavirus vaccines are both oral vaccines, delivered in the same manner by which most humans might be exposed to porcine viruses, i.e., through ingestion of food.

The development of advanced technology such as microarray and high-throughput sequencing provided an opportunity to re-examine live attenuated viral vaccines for sequence changes, minority variants, as well as for potential contaminants that might have been introduced during the attenuation process, from the cell substrate used, and/or from the animal sera or other biological starting materials often used in cell cultures [44].

5.3. Follow-up steps

5.3.1. GSK

GSK initiated extensive experiments to confirm the results reported to them by Delwart et al. and to investigate those findings further. The studies included a series of assessments on the same two lots of the finished vaccine tested in the Delwart laboratory [44], as well as on Rotarix® vaccine materials at different stages of the production process. These follow-up tests confirmed the presence of DNA from PCV-1 in Rotarix® final containers, manufacturing process intermediates, the master cell bank, the working cell bank, and the master and working viral seeds from which the vaccine was derived.

GSK generated data on the infectivity potential of the PCV-1 found in Rotarix® and found that the PCV-1 sequences in Rotarix® bulks and final containers were infectious. The results of this experiment confirmed the early studies of Delwart’s laboratory [44] and those done subsequently by FDA (see FDA below) [45].

As part of their investigation, GSK tested all of their live viral vaccines for the presence of PCV-1 DNA, and found that, except for Rotarix®, all final containers were negative for PCV-1 DNA.

GSK expanded their investigation to include their inactivated poliovirus vaccine (IPV), since it is manufactured from a related cell bank to that used to produce Rotarix®. The harvest tested positive for the presence of PCV-1 DNA, whereas the purified bulks and final containers tested negative. Data from other studies indicated that the final container test was negative for PCV-1 because the PCV-1 DNA was eliminated during the purification of the IPV harvest. No infectious virus was detected in IPV bulks.

Contaminated (non-irradiated) trypsin, used in the mid-1990s to manufacture the Vero Cell Banks, the cell substrate used to produce Rotarix®, is considered the most likely source of the PCV-1 contamination. The use of irradiated trypsin and synthetic amino acids were introduced after preparation of the Vero master cell banks. Coincidently, Vero is one of the few cell lines that support the propagation of PCV-1 in culture.

GSK began working with the trypsin manufacturer to ensure that the existing inactivation methods are adequate to protect against new risks and that appropriate testing methods are applied to demonstrate absence of infectious PCV in trypsin lots.

5.3.2. Merck

While initial studies by Victoria et al. [44] showed no evidence of PCV DNA in RotaTeq®, subsequent studies conducted by Merck detected low level fragments of PCV DNA, subsequently confirmed to be primarily PCV-2, in RotaTeq®, via commercially available qPCR analysis. Merck developed an analytical test plan to assess the presence of PCV in the Master and Working Vero cell banks, the Master and Working rotavirus seeds, >30 rotavirus bulk lots (some tied to clinical trial lots), and gamma-irradiated trypsin. PCV DNA was not detected or was below the qPCR assay limit of detection for the Cell Banks and Seeds. Endpoint PCR for longer PCV amplicons confirmed the absence of long PCV DNA fragments; additionally, infectivity testing confirmed the absence of infectious PCV in Cell Banks and Seeds.

Low levels of small fragments of PCV-2 DNA were detected in some of the rotavirus bulk lots and the gamma-irradiated trypsin; endpoint PCR on 11 of the bulk lots confirmed the presence of longer PCV DNA fragments, albeit at the limit of the detection for the assay. Infectivity testing on the bulks and gamma-irradiated trypsin confirmed the absence of infectious PCV.

Merck performed a risk assessment and identified gamma-irradiated trypsin, the only porcine-derived raw material used in rotavirus bulk manufacturing, as the most likely source of the small fragments of PCV DNA detected in RotaTeq®. It seems likely that the residual fragments of PCV DNA were from porcine viral contaminants that may have been present in the porcine pancreas used to manufacture trypsin and may have been effectively destroyed by the methods used to inactivate adventitious agents in trypsin, leaving only remnants behind.

Merck further established that PCV-2 DNA detected in rotavirus bulks was from trypsin and not associated with infectious virus by demonstrating the mass balance of PCV DNA through the rotavirus bulk manufacturing process, confirming there was no amplification...
of PCV-2 during Merck's rotavirus bulk manufacturing and that trypsin is the source of the small PCV-2 DNA fragments.

Merck began working with the trypsin manufacturer to ensure the existing inactivation methods are adequate to protect against new risks and appropriate testing methods are applied to demonstrate absence of infectious PCV in trypsin lots.

5.3.3. EMA

The European Medicines Agency (EMA) began the consultation with its Vaccines and Biologics Working Parties for the assessment of a procedure under Article 20 of Regulation (EC) No 726/2004. The data provided indicated that PCV-1 DNA replicates in the initial phases of the Rotarix® manufacturing process but not in the subsequent steps. Results from infectivity studies indicated that PCV-1 infection of human cells is non-productive.

Collaboration with the Official Medicines Control Laboratories network was sought. The PEI (Paul-Ehrlich Institut, Germany) and Robert-Koch Institut initiated experiments to confirm results reported by GSK [46]. Using real-time PCR for PCV-1, titers of PCV-1 DNA in several batches of Rotarix® were obtained. Real-time PCR suggested that the porcine viral DNA was present in the vaccine in an encapsidated form. The PCV infectivity assay developed was not able to detect infectious PCV in Rotarix®. A more sensitive infectious virus assay needed to be developed.

The experimental findings suggested that the high amount of PCV-1 DNA present in Rotarix® does not reflect a corresponding proportion of biologically active virus particles, but rather points to only a small portion of the PCV-1 DNA being present in the vaccine as infectious virions.

With regards to RotaTeq®, experiments were performed at several stages of the manufacturing process. Quantitative PCR (qPCR)-based assays were used to screen for the presence of relatively small fragments of PCV DNA in RotaTeq® itself, vaccine bulk lots (the individual drug substance lots used to formulate RotaTeq®), cell banks, viral seeds, and porcine trypsin used as manufacturing inputs. Cell culture infectivity testing using permissive cell lines was then initiated, in particular in those samples that tested positive for long PCV DNA sequences.

The analyses submitted confirmed the absence of detectable PCV-1 DNA, indicating that this virus is not present in the vaccine. The amount of PCV-2 DNA found in the vaccine bulk lots can be accounted for by the PCV-2 DNA present in the trypsin (the sole raw material of porcine origin) used in one of the steps of the vaccine manufacturing process.

No infectious viral particles of either PCV-1 or PCV-2 were present in any of the cell banks, viral seeds, clinical bulk lots or vaccine bulk lots tested, or in the porcine trypsin used during manufacture of RotaTeq®. It was concluded that the presence of small fragments of PCV-2 DNA does not raise any safety concern.

5.3.4. FDA

FDA began to review the evidence and initiate its own testing as soon as the finding of PCV DNA in rotavirus vaccines was reported to the Agency. FDA staff consulted with experts, contacted other public health officials in the U.S., and communicated with international partners.

FDA initiated experiments to confirm results reported by GSK and extended the studies to conduct its own investigation. FDA confirmed the presence of PCV DNA in Rotarix®, and found that the PCV DNA was associated with particles (i.e., was resistant to nuclease digestion and could be pelleted by ultracentrifugation) and that these particle-associated PCV-1 sequences included entire PCV-1 genomes [45]. Cell culture infectivity tests were developed by FDA to determine whether the PCV in the vaccines was capable of replication. Study results demonstrated that PCV-1 in Rotarix® (but not in IPV) was able to replicate in porcine cells and to be passaged to fresh cultures.

FDA also evaluated RotaTeq® for PCV sequences. Although initial tests using less sensitive primer pairs were negative, relatively low copy numbers of PCV-1 and PCV-2 DNA fragments were identified in RotaTeq® by PCR performed at FDA using more sensitive primer pairs. Full-length or particle-associated PCV DNA was not detected in RotaTeq®. FDA also performed infectivity assays on RotaTeq® final container and bulks, and both short-term assays (identical to those in which Rotarix® was positive for PCV-1) and long-term assays (designed to detect even smaller amounts of virus) were negative for PCV-1 and PCV-2.

5.4. Scientific advice

5.4.1. EMA

The Committee for Medicinal Products for Human Use (CHMP) is the European Union body that is responsible for preparing the scientific opinions on questions concerning medicines for human use that are handled by the EMA, among them Rotarix® and RotaTeq®. After being informed of the presence of PCV DNA in rotavirus vaccines during the spring of 2010, both the European Commission and the EMA tasked the CHMP with reassessing the benefit/risk balance of the vaccines. In addition, a formal risk evaluation was undertaken by at least one European regulatory agency. The CHMP considered that the PCV findings did not present a threat to public health and consequently, that there was no need to restrict the use of the vaccines. This was confirmed during the review process, with final conclusions issued in September 2010 and January 2011 for Rotarix® and RotaTeq®, respectively. The CHMP ultimately concluded that the benefit/risk balance of both vaccines remained positive. To reach its opinion, the CHMP relied on the views of the Biologics Working Party (BWP), a CHMP Standing Working Party dealing with the quality aspects of biological medicinal products for human use, as well as on those of an ad hoc expert meeting convened in September 2010 at the EMA. Interaction with the European Pharmacopoeia and international partners, including the U.S. FDA and the WHO also was sought.

5.4.2. FDA

On 7 May 2010, FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC) convened to discuss the findings of PCV and PCV DNA in rotavirus vaccines. The meeting included a discussion of FDA's evaluation of laboratory results from the manufacturers of Rotarix® and RotaTeq® rotavirus vaccines and results from FDA's own testing to characterize the agent, as well as other information related to the safety of the rotavirus vaccines. Particularly, the clinical safety database from the pre-marketing clinical studies and post-marketing experience of Rotarix®, which had been re-evaluated, were also reviewed in this forum. The committee advised the FDA that the benefit/risk considerations remained in favor of continued use of both rotavirus vaccines despite the adventitious agents or sequences thereof, and that they should remain on the market in the USA, but that the companies should work towards establishing (in the case of Rotarix®) and maintaining (in the case of RotaTeq®) vaccines free from PCV as rapidly as feasible and prudently possible.

5.4.3. WHO

In order to evaluate in real time the product-specific issues generated by regular reports of new data received from the manufacturers, WHO convened: (a) an ad hoc prequalification advisory committee; and (b) a sub-committee of the Global Advisory Committee on Vaccine Safety (GACVS).
To evaluate the broader issues of the new testing methodologies, the already established WHO Working Group on Adventitious Agents in cell substrates considered the issues and provided advice to WHO. This advice was considered by the ECBS in 2010.

On 25 March 2010, WHO convened the GACVS by teleconference [47].

On 13–15 April 2010, WHO’s Strategic Advisory Group of Experts (SAGE) on Immunization also reviewed data relating to the finding of DNA fragments of PCV-1 in Rotarix® [48].

5.5. Regulatory issues and actions

As a consequence of the initial follow-up steps that were taken (see Section 5.3.), the EMA concluded that the PCV findings did not present a threat to public health and consequently, that there was no need to restrict the use of the vaccines. Neither vaccine was removed from the European market, and the use of the vaccines was not suspended during the investigation and risk assessment process.

However, it should be emphasized that whereas the European Community position was to maintain the vaccines on the market, some European national regulatory authorities took a more conservative approach by recommending that rotavirus vaccination be temporarily avoided pending the final outcome of the investigation. On 22 March 2010, FDA recommended that clinicians and public health professionals in the United States temporarily suspend the use of Rotarix® while the agency and manufacturer investigated the finding of DNA from PCV-1 in the vaccine. Because at that time there was no evidence of PCV contamination of Rotarix® vaccine, FDA’s recommendations were restricted to Rotarix®.

On 14 May 2010, FDA recommended resumption of the use of Rotarix® and the continued use of RotaTeq®. FDA reached its decision based on a careful evaluation of information from laboratory results from the manufacturers and the FDA’s own laboratories, a thorough review of the scientific literature, and input from scientific and public health experts, including members of the FDA’s Vaccines and Related Biological Products Advisory Committee that convened on 7 May 2010 to discuss these vaccines.

Both EMA and FDA considered the following in their decisions:

(a) both vaccines have strong safety records, including clinical trials involving tens of thousands of human subjects as well as post-marketing clinical experience with millions of vaccine recipients; and
(b) there was no evidence that PCV-1 or PCV-2 pose a safety risk in humans, and neither is known to cause infection or illness in humans.

EMA and FDA both concluded that the benefits of the vaccines are substantial, and include prevention of death and hospitalization for severe rotavirus disease. These benefits outweigh the risk, which is theoretical.

The finding that PCV-1 replicates in Vero cells emphasizes some of the shortcomings of the existing techniques for the detection of adventitious viruses in both virus seed stocks, as well as in cell banks. However, there is no evidence that PCV-1 replicates in humans. There may be similar cases in the future where an unexplained virus replicates in cell cultures with no apparent cytopathic effects, and is later found to be present in vaccine seed stocks prepared in the past.

The European authorities initially considered the advisability of introducing new technologies such as massively parallel sequencing, microarrays and PCR/mass spectrometry as complementary approaches for the detection of adventitious agents. However, given that these techniques have limitations in differentiating between viable virus and DNA fragments, the regulatory priority was focused on the improving the quality of trypsin used in vaccine production. Indeed, since the probable origin of the PCV contamination was the trypsin used in the manufacture of the vaccines or the cell banks, a new EMA guideline on the quality of porcine trypsin was drafted and has been submitted for public consultation.

The EMA recommended the revision of conditions and requirements of the marketing authorization for Rotarix® vaccine to include information about findings related to PCV. For RotaTeq®, no changes to the product information were considered necessary by the CHMP. Measures were put in place to further minimize the risk of PCV virus or PCV DNA entering the manufacturing process of RotaTeq®.

The identification of an adventitious agent in a vaccine using new massively parallel sequencing-based techniques emphasized the potential role of these technologies in vaccine and reagent characterization. There have been workshops in recent years exploring advances in new technologies and testing methodologies. Manufacturers, regulators, and academic researchers have contributed to this research and discussion. While there are obstacles to routinely employing these assays, regulators continue to explore how information from new methodologies can be used to support regulatory decisions. In November 2013, the FDA co-sponsored a public international workshop to discuss the capabilities of the next-generation sequencing technologies, which may be used for the purpose of screening or testing for adventitious agents.

FDA approved revised prescribing information and patient labeling for both Rotarix® and RotaTeq® vaccines to include information about findings related to PCV and posted information on their website for the public.

5.6. Vaccine supply implications

Since the marketing authorizations of the rotavirus vaccines were not suspended, the European authorities’ decision did not impact the vaccine supply in the EU. However, it should be noted that whereas the rotavirus vaccines were available in all EU Member States, only Belgium, Luxembourg, Austria and Finland and some of the federal states of Germany had introduced the rotavirus vaccination in their pediatric immunization program.

Because two vaccines were approved for marketing in the USA, the recommendation for temporary suspension of Rotarix® use did not significantly affect supply.

5.7. Public transparency and communication with other organizations

A journal referee who reviewed the Victoria et al. manuscript advised the publisher and authors to notify the manufacturer of their findings in advance of publication, because of the potential public health implications [personal communication]. On 9 February 2010, in advance of publication, Delwart notified GSK of their findings. The firm notified EMA and FDA of its confirmatory findings on March 15, 2010, and WHO on March 16, 2010.

On 22 March 2010, the EMA issued a press release related to the unexpected presence of PCV DNA in Rotarix®, which was made available on the website of the European Agency. This first statement was followed by several web publications including press releases related to the CHMP (re)assessment of the benefit/risk balance. Questions and Answers on the review of Rotarix® and RotaTeq®, the monthly highlights of the CHMP meetings where the rotavirus item was featured in the agenda, the assessment report for the so-called Article 5 (3) procedure related to the detection of adventitious viral agents in live attenuated vaccines, the final assessment reports published in September 2010 and January 2011 for Rotarix® and RotaTeq® respectively, and eventually the updates of the Summary of Product Characteristics of both vaccines.
On 22 March 2010, FDA provided information to the public through a press release and posting of information on the FDA website regarding the finding of PCV-1 DNA in Rotarix®, and the recommendation that clinicians and public health professionals temporarily suspend use of Rotarix® in the U.S. while the finding was investigated. FDA informed the public of the finding of PCV-1 and PCV-2 DNA fragments in RotaTeq® on 6 May 2010. FDA presented the findings at the VRBPAC meeting on 7 May 2010. Based on a thorough assessment of all available data and advice of the VRBPAC, FDA updated its recommendations on Rotarix® on 14 May 2010 stating it is appropriate for clinicians and healthcare professionals to resume use of Rotarix® and continue use of RotaTeq®.

Information on the rotavirus vaccines was posted publicly on the FDA website for use by health care providers, public health professionals, parents, and caregivers. The website was updated in a timely manner as additional information became available.

On 22 March 2010, WHO published a statement on its website indicating its preliminary position that there should be no change to the use of Rotarix® [49]. On 26 March 2010, the GACVS issued a statement on its website [47]. On 21 April 2010, WHO’s SAGE issued a preliminary statement on the issue that was published on the SAGE website [48]. On 28 May 2010, the full report of the SAGE meeting of 21 April was published on the SAGE website [50]. On 3 June 2010, WHO published a list of questions and answers relating to PCV in rotavirus vaccines [51]. On 23 July 2010 in the WHO Weekly Epidemiological Record, WHO published a summary of the GAVCS meeting of 16–17 June 2010 [52]. Given the absence of any known risk, SAGE strongly recommended the continued use of Rotarix® by immunization programmes, in particular in those parts of the world with elevated mortality associated with rotaviruses among children aged less than 5 years of age.

5.8. Public health and other issues

The overall assessment of potential risks to public health due to PCV contamination of rotavirus vaccines was that any risk was outweighed by the benefit of the vaccines. That conclusion was based on epidemiologic data, in vitro studies on viral replication, human transplant data, clinical trial databases, and post-marketing databases.

There was no evidence for a pathogenic role of PCV in humans even though PCV has been found in human stool samples. It is not known whether PCV found in human stool represent virus that has replicated in the human gut or virus that has simply passed through the system upon ingestion of contaminated porcine food products. Currently there is no evidence to support PCV replication in the human gut, but the possibility cannot be excluded.

Infection studies on human cell lines with PCV-1 and PCV-2 demonstrated that the viruses were able to initiate their DNA replication, but infection of human cells with PCV was not productive, because infectious viral particles are not released [53]. This is a likely reason why PCV-1 cannot induce antibodies in species other than pigs, although results published so far on antibody induction are inconclusive. In addition, tests in two high-risk human groups showed no evidence of PCV-2 antibodies [54].

The majority of pork meat (70% in the USA) contains porcine circoviruses (which are also regularly found in human stool). These findings illustrate that it is most unlikely that there is a serious safety risk for individuals who were vaccinated orally with Rotarix®. The EMA found that while PCV-1 is commonly found in certain meat and other food products, it is not known to cause disease in either humans or animals [55].

In transplantation medicine the risk of potential PCV infections has been assessed, and no risk for human transplant recipients of porcine tissue could be detected. There is also no evidence of possible recombination of potential human circoviruses with porcine circoviruses, as human circoviruses are unknown [56].

There is a large safety database for Rotarix®, with about 100,000 children having received the vaccine during clinical trials, and about 112 million doses distributed worldwide as of 2011.

No safety signal has been identified in humans vaccinated with Rotarix® that could be assessed as being related to a potential PCV-1 infection. It is probable that all Rotarix® batches ever produced were contaminated with PCV-1, since both the MCB and WCB were shown to be contaminated. Nevertheless, despite this possible large-scale contamination of Rotarix® in the past, there is no evidence from the extensive historical data available that this possible PCV-1 contamination has caused any risk for vaccinated infants or that it would compromise the vaccine’s efficacy. This database has been assessed several times and no relevant safety signal has been found.

Based on these data, it was concluded that the target population was not exposed to any known risk.

Irradiated trypsin and synthetic amino acids are being used to prepare new master cell banks and in the production of the rotavirus vaccines. The use of new molecular biology methodologies capable of screening for many different genetic materials in a short period of time raises the possibility that additional findings on starting materials used to produce biologicals can be expected. Practical risk assessments will be needed in each instance.

Consideration should be given to the best methodologies for performing such risk assessments in the most objective and expedient manner possible.

5.9. Overall outcome

The careful assessment of risk associated with PCV-1, as well as the DNA from PCV-1 and PCV-2, in rotavirus vaccines led to the conclusion that vaccines had not been exposed to any identifiable risk. Many raw material producers are treating starting materials such as trypsin to inactivate potential contaminating microbial agents to minimize the risk of a live microbial contaminant, and this practice should be encouraged.

5.10. Lessons learned

Clinical trial databases and post-marketing databases can be very valuable sources of safety information when assessing a new potential risk that is known to have been present during product development.

Benefit/risk assessment is a key factor in decision-making, and the outcome of that assessment may change as more data become available.

The discovery of a previously undetected contaminant in a licensed biological medicinal product was not entirely novel and occurs typically through the application of new analytical technologies often with improved sensitivities or capabilities not previously available. A thorough scientific investigation involved both manufacturers and independent public sector laboratories acting in a collaborative and interactive manner. These studies involved full characterization of the nature of the contaminant, the extent of the contamination, its origin, its infectious nature, along with a thorough re-examination of all available clinical data on the use of the vaccines. The scientifically-based regulatory approach that was used in this case should be applied to future incidents, if they occur.

The use of hi-tech analytical methods may deliver test results of medicinal products that reveal new impurities present at very low concentrations and/or hard to detect contaminations that were not picked up previously because of limitations of the analytical instruments/methods, including sensitivity and specificity.
Some developing country regulators found it difficult to reconcile the different initial regulatory decisions/actions taken by FDA and EMA. This had the potential to undermine public health in the very countries with the highest disease burden for which the populations have the greatest benefit vs. risk. However, the final regulatory actions by FDA and EMA were the same. The only difference was that FDA recommended a “pause” in use of Rotarix® in the U.S. for a short period of time while the issue was investigated further.

It is critical for NRAs to clearly communicate the risk/benefit basis for any regulatory decisions that are made. Such transparency would allow other NRAs to take those factors as well as others that are specific for their populations into consideration in their own decision-making process. WHO can facilitate information sharing in the interest of better understanding of the rationale for national regulatory actions including public health impact.

6. General discussion

These four case studies cover a broad range of possible contaminants of viral vaccines. It is interesting to note that there is a common thread running through all four case studies: in each case, the initial response was to determine whether the finding posed an unacceptable risk to public health in light of the proven or expected benefits of the vaccine in question, and whether the signal really was indicative of a live infectious virus contaminant with serious adverse effects on recipients. In all four cases, and after consideration of scientific advice, the vaccines concerned were not removed from the market, or were only temporarily suspended, since the benefits of immunization were believed to be much more beneficial than the risk of any potential adverse effects. After further evaluation, that initial assessment proved to be correct.

Another observation is that the response to the initial findings in each case benefited from knowledge gained from past experiences. It is important to make adjustments based on lessons learned from experience. These events highlighted that in order to respond effectively, it is essential to have access to expert scientific advice, good communication, public transparency, international interactions and effective global coordination.

The manner in which the international scientific community dealt with the RT issue should serve as a model for dealing with similar issues in the future because of the excellent coordination and collaboration during the scientific investigation. In responding to the RT case, there was a global consensus on what regulatory actions were appropriate, which facilitated clear communication of the issues.

There was good collaboration in addressing the PCV issue also, but the PCV incident highlighted the complexity of being able to arrive at a global consensus when local/regional considerations are taken into account. Different NRAs/NCLs may have different benefit/risk considerations for their country based on vaccine supply, disease prevalence and severity, and their specific epidemiological situation, among other factors. All of these factors must be taken into consideration when making a decision.

The potential impact of regulatory decisions on public health should be discussed with public health officials. The importance of transparency in the decision-making process is clear. The PCV case also highlighted the same issue that was identified by the bacteriophage case: the quality of reagents such as sera and trypsin used in vaccine production and in the preparation of important biological starting materials, such as cell banks and viral seeds, must be well-controlled in order to have a final product that is free from contamination. Indeed, concern about the quality of starting materials has recurred periodically (e.g., RT in eggs, bovine spongiform encephalopathy in bovines). Such risks of microbial contamination can be minimized by limiting the use of animal-derived materials in manufacturing, applying good manufacturing practices, and conducting stringent testing and control of raw materials, manufacturing intermediates, and final product.

The issue of the safety of vaccines for global use is an area where WHO Collaborating Centers and other expert advisors to WHO may provide advice on cell substrates viral safety upon request from WHO. This expert advice, based on state-of-the-art technologies developed by manufacturers, NRAs/NCLs, and academic institutions, would serve as valuable support to WHO to facilitate responding in a timely manner to unexpected findings of adventitious agents in vaccines.

The final general observation is the key role that WHO has played in the coordination of a global response to the finding of an adventitious agent (or signal of an agent). Importantly, the role of WHO in the global coordination of actions to be taken and communication among NRAs of various countries and regulatory regions has been and continues to be pivotal in such situations, as it was, for example, in the case of the discovery of RT activity in chicken-cell-derived measles vaccine. This is especially the case for licensed vaccines that may be in use in many countries globally. WHO coordinates efforts directed towards achieving international consensus, but NRAs ultimately make decisions based on benefit-risk assessment for their own populations. That may result in different decisions in different countries or regions. In order to avoid confusion, it is important that NRAs clearly communicate the rationale and basis for their decision. In the interest of global public health, any signal of an adventitious agent in a marketed vaccine should be reported promptly to WHO. In particular, the NRAs and manufacturers of prequalified vaccines have a responsibility to immediately inform WHO and to take appropriate follow-up actions.

Since the finding of SV40 in polio vaccines, there have been many advances in science and technology as well as routine use of more efficient methods for communication and exchange of information. All of these changes have led to an increasingly comprehensive and transparent response to the finding of an adventitious agent (or signal) in a marketed vaccine. Manufacturers and regulators have investigated new technologies, and when new methods have proved to be superior to existing ones, updated practices should be introduced.

In responding to these events, a coordinated global effort, facilitated by WHO, has served to protect public health. Based on these experiences, WHO is coordinating the development of a document that describes the scientific principles to consider in the regulatory risk evaluation on finding an adventitious agent, or a signal of an agent, in a marketed vaccine [1]. That document is meant to provide guidance to regulators regarding the principles of risk evaluation when evidence for a potential adventitious agent is detected in a marketed vaccine. Among the most important lessons from the past is the desirability of transparency and open communication. When all parties with a vested interest in the outcome of a regulatory risk evaluation are aware of and understand the bases on which decisions are made, the probability of miscommunication and error is minimized. WHO has a critical role to play in coordinating at the global level public communication of regulatory decision-making.

Regulatory risk evaluation is a dynamic process both in terms of how it has evolved over the past 60 years and in the way in which information is accumulated and evaluated in any given instance. Much has been learned since the discovery of SV40 as a contaminant of polio vaccines in the 1960s, and it is hoped that the lessons of past instances of finding an adventitious agent in vaccines will provide useful guidance for the future. A central element of the regulatory risk evaluation process is that the assessment needs to
be updated each time a new significant data emerge; thus, it is an iterative process. Nevertheless, it is often the case that there is a need for immediate decisions at an early phase of the evaluation when many of the answers to questions will not be available. This presents particularly challenging situations for all interested and affected parties. Among the most important lessons from the past is that decision-making should be based on sound science, and the positive impact that transparency and open communication can have on public confidence in decision-making, and ultimately on public health.

References

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