Biosafety and Regulatory Requirements for Vaccines

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Abstract

Biosafety is an important requirement for all laboratories, vaccine production units and aquaculture facilities. Its relevance for framing regulatory protocols under different categories of biosafety has to be emphasized from time to time. Regular monitoring and documentation following Standard Operating Protocols should be within the framework of well established guidelines to maintain uniformity in the functioning of these institutions. Biosafety requirements with respect to living and genetically modified organisms should be formulated keeping in view the impact on preservation of the indigenous populations and is indeed a matter of great concern to biologists, researchers, operating personnel and fish handlers. Imparting skills through training and orientation programmes is the way forward in keeping abreast with the latest developments in biosafety protocols.

Keywords

Biosafety levels · Regulatory requirements · Risk groups · Vaccines · Vaccination

1 Biosafety in Aquaculture

Biosafety refers to the safe handling of infectious microorganisms, living modified organisms, hazardous biological materials and their containment to safeguard the environment and human health. In light of the fact that most aquatic biological diversity still resides in natural populations; all biotechnologies that have the potential to improve fish production may have an adverse impact on wild aquatic

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resources [1]. In aquaculture sector, there are several pilot projects and research programmes in many parts of the world that are developing commercially important vaccines. The scope of the current biosafety protocols should include vaccines and disease-resistant strains as well and their impact on the natural biodiversity including the wild relatives of domesticated aquatic species.

These biosafety protocols, or similar regulations, should eventually strive to protect these resources while allowing for the development of aquaculture and international trade [2]. Regulatory guidelines should be clearly defined for the use of vaccines in cultured fish populations in order to preserve the natural biodiversity and to preserve certain populations facing extinction [1]. At present, European Union, USA, Canada and Norway have issued guidelines about regulatory requirements for veterinary vaccines that include those for fish as well [3–6]. In India, fish vaccines are still not included in the veterinary supplement 2018 of Indian Pharmacopoeia. However, the Aquaculture Authority is in the process of drafting a specific legislation for disease control within aquaculture facilities in the Compendium on Aquatic Medicines and Animal Health Management [7]. Hence, this chapter will focus on biosafety in relation to regulatory requirements for vaccines.

2 Some Common Terms

Biosafety In The American Heritage Medical Dictionary, biosafety is defined as 'A set of measures or activity undertaken to ensure the safe handling of biohazardous materials, such as pathogens, biological contaminants and genetically modified organisms, especially to prevent their accidental spread beyond a laboratory or research facility' [8]. As per the Segen's Medical Dictionary, it is defined as 'Any activity intended to safeguard a population from the untoward effects of potentially infectious biological materials or infectious agents, and minimize their environmental impact' [9].

Therefore, guidelines laid down by national level organizations are implemented through institutional bodies that periodically review biosafety norms practiced in the laboratory or production settings to prevent large-scale loss of biological integrity that may otherwise affect the environment and human health [10].

Veterinary Biological Substances or derivatives/mixture of animal origin such as helminth, protozoa or microorganism, or any substance of synthetic origin that is manufactured, sold or represented for use in restoring, correcting or modifying organic functions in animals, or products for use in the diagnosis, treatment, mitigation or prevention of a disease, disorder and symptoms, thereof in animals. These veterinary biologicals include vaccines, bacterins, bacterin-toxoids and diagnostic kits [11].

Production Outline This can be defined as a detailed description of processes followed while producing a veterinary biologic, and diluents, if any, followed by the tests used to establish its purity, safety, potency and efficacy, and the results of all

such tests including the methods and procedures to be employed in handling, storing, administering and testing a veterinary biological [11].

Safety Safety for a veterinary biological can be defined as the freedom from properties or agents causing local or systemic reactions when the biological is used as recommended [11].

Purity Purity is defined as the quality of a biological prepared to a final form that is relatively free of extraneous microorganisms and material, as determined by established test methods and approved in the production outline [11].

Potency Potency is defined as a measure of the relative strength of a biological that correlates to its immunogenicity/efficacy when tested by established methods as documented in the production outline [11].

Efficacy Efficacy is defined as a measure of the specific protective response to the biological when used at the recommended dose [11].

3 Committees Involved in Biosafety

In India, vaccines and other recombinant products are regulated by the 'Rules for the manufacture, use/import/export and storage of hazardous microorganisms/Genetically Modified Organisms (GMO's) or cells, 1989' (Rules, 1989) notified by the Ministry of Environment and Forests (MoEF), GOI under the Environment Act (1986) [10]. These rules are implemented by Ministry of Environment and Forests (MoEF), Department of Biotechnology (DBT), Ministry of Science and Technology (MoST) and state governments through six competent authorities notified under the Rules, as follows:

1. Recombinant DNA Advisory Committee (RDAC)
2. Institutional Biosafety Committee (IBSC)
3. Review Committee on Genetic Manipulation (RCGM)
4. Genetic Engineering Appraisal Committee (GEAC)
5. State Biotechnology Co-ordination Committee (SBCC)
6. District Level Committee (DLC)

Functions of each committee RDAC is mainly an advisory body whereas IBSC, RCGM and GEAC have a regulatory function. SBCC and DLC are responsible for monitoring the activities related to GMO's in the state/district levels, respectively. RDAC, RCGM and GEAC are constituted at the central level by DBT and MoEF.

Role of IBSC An IBSC should be constituted by organizations engaged in research, handling and production activities related to GMO's and is a statutory committee that operates as a liaisoning body between DBT and the institution and should

co-ordinate with SBCC and DLC wherever necessary. Therefore, it has a pivotal role within an organization for implementation of a biosafety regulatory framework.

4 Classification of Biosafety Levels and Risk Groups

In this section, the major characteristics of the four biosafety levels, definition of 'risk group' with examples, and how risk groups are used in conjunction with risk assessment to set biosafety levels, are discussed. A biosafety level is the assignment of agent based on risk assessment, depending on the type of agent and the conditions of use requiring professional judgment, especially in the case of unknown pathogens. Hence, the biosafety levels are classified into four groups based on the risk assessment [10].

1. **Biosafety Level 1 (BSL 1):** This type of facility is suitable for work involving well-characterized agents not known to consistently cause disease in immuno-competent adult humans. They pose a minimal potential hazard to laboratory personnel and the environment. The laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open benchtops using standard microbiological practices. Special containment equipment or facility design is not required. However, laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science [10].
2. **Biosafety Level 2 (BSL 2):** It builds upon BSL 1 and has additional safety features over the basic set up. It is suitable for work involving agents that pose moderate hazards to personnel and the environment. The laboratory personnel should have undergone specific training in handling pathogenic agents and be supervised by scientists competent in handling infectious agents and associated procedures. There is restricted access to the laboratory when work is being conducted in the facility. Procedures involving or creating infectious aerosols or splashes are conducted in biological safety cabinets (BSCs) [10].
3. **Biosafety Level 3 (BSL 3):** It is applicable to clinical, diagnostic, teaching, research or production facilities where work is performed with indigenous or exotic agents that may cause serious or potentially lethal diseases through inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents. Such work should be supervised by scientists competent in handling infectious agents and associated procedures. The work should be conducted in Class III biosafety cabinets placed in rooms with airlock systems with HEPA filters, to prevent the infectious agents escaping from the facility by placing air showers at the entrance and airlocks thereafter at each stage inside the facility. Personnel should wear additional protective gear such as N95 masks, goggles, protective gowns for respiratory and personal protection as determined by risk assessment. As discussed above, a BSL 3 laboratory has special engineering design features, primarily among them being a directional airflow maintained by air pressure and interlocking doors [10].

4. **Biosafety Level 4 (BSL 4):** Such a facility is required for work with dangerous and exotic pathogens that pose a high individual risk of life threatening diseases, aerosol transmission or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL 4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level or re-designate the level. The laboratory staff should have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment and laboratory design characteristics. All laboratory staff and supervisors must be made competent in handling agents and procedures requiring BSL 4 containment. Access to the laboratory for designated personnel is controlled by the laboratory supervisor in accordance with the institutional policies. In this type of facility, there are two separate entities that provide absolute separation of the worker from the infectious agents, namely, the suit and the biosafety cabinet. Hence there are two distinct laboratories, namely, suit laboratory and cabinet laboratory [10].
5. According to the biosafety levels, the infectious agents are divided into four major risk groups [10]:
   (a) **Risk Group 1:** These agents do not pose any individual and community risk. It defines a microorganism that is unlikely to cause human or animal disease.
   (b) **Risk Group 2:** These agents pose a moderate individual risk and low community risk. It defines a pathogen that causes human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. The agent may cause serious infection in a particular species but can be controlled by effective treatments, and preventive measures are available in order to limit the risk of spread.
   (c) **Risk Group 3:** These agents pose a high individual risk but low community risk. It defines a pathogen that usually causes serious human or animal disease but does not ordinarily spread to other species. Effective treatment and preventive measures are available to control their spread.
   (d) **Risk Group 4:** These agents pose high individual and community risk. It can be defined as a pathogen causing serious human or animal disease readily transmitted from one individual to another. Effective treatment and preventive measures are usually not available for their control or limit their spread.

5 Biosafety Requirements for Vaccines

Most of the pathogens of fish can be classified in risk groups 1 and 2. Few may belong to risk group 3. However, handling of any microorganism, virus or recombinants should be carried out following biosafety protocols during handling and preparation of vaccines that can be divided into four phases [3].

Some of the common steps in vaccine preparation [5, 12, 13] include:

- Isolation and identification of the causative agent and preparation of master seed stock and working seed
- Culturing of the microorganism itself or the target antigen in some other way using biotechnological tools
- In case of inactivated vaccines, inactivation steps using chemical or physical agents to kill the microorganism
- Confirmatory tests to prove that the vaccine is free of extraneous agents.
- Confirmation that the vaccine is safe for the target species using safety studies following approved protocols
- Confirmation that the vaccine is effective in preventing and reducing the disease in the target species of fish
- Adequate sterility and quality control checks, including vaccine formulation in appropriate diluents, carrier with or without adjuvant and in a package to facilitate storage

In order to meet all the regulatory requirements for safety, quality and efficacy, the development process needs to generate a complete dossier to satisfy the accessors and in case of rDNA vaccines, the dossier should go through RCGM [10].

Three 'R's in the approaches are recommended in the production and quality control of vaccines [3] and that include Replacement, Reduction and Refinement.

Replacement: Includes methods which permit a given purpose to be achieved without conducting experiments or other scientific procedures on animals.

Reduction: Includes methods for obtaining comparable levels of information from the use of fewer animals in scientific procedures, or for obtaining more information from the same number of animals.

Refinement: Includes methods which alleviate or minimise potential pain, suffering or distress, and which enhance animal well-being during the safety and efficacy studies [3].

6 Autogenous Vaccines and Regulatory Issues

Autogenous vaccines are recommended for important diseases of sporadic nature that do not have commercially available vaccines in place. These vaccines are custom-made and prepared from a pathogen isolated during a sporadic outbreak or a specific epidemic as compared to commercially available vaccines that are prepared from standardized cultures. Some companies can provide autogenous vaccines for certain diseases. Hence regulations governing the use of autogenous vaccines vary among countries. In USA, autogenous vaccines are prepared from culture of microorganisms that have been inactivated or are non-toxic. Further, the product should be prepared for use only under the direction of a veterinarian and under a veterinarian-client relationship or such products may be prepared for use under the direction of a person with appropriate expertise in specialized situations such as aquaculture, if approved by USDA [4, 14]. Autogenous vaccines should be

prepared using seed organisms isolated from sick or dead fish, to be used in the population of origin or adjacent populations after due permissions [15]. Other regulatory requirements include information on the designated facility, specifying the precautions that will be taken to prevent contamination of licensed products [5, 13].

7 Regulatory Requirements for Licensing of Vaccines

These requirements include full information on the original organism used as master seed, details of various aspects of in-process controls to the final batch release tests, quality control test data, results of the batch-safety test performed on all the batches of the vaccine produced for sale. The batch-safety test should have been carried out on a target species for the first five to ten batches of vaccine produced [5, 13]. These quality control checks are designed to ensure batch-to-batch consistency in the production of vaccines that begin as cultivation of a living microorganism including slight variations in how the microorganism is grown, replicated and standardized to obtain the final product making each vaccine a unique development [3, 5, 13].

At present, there is no regulatory authority for fish vaccines in India, and standard guidelines for fish vaccines are yet to be established [7] and incorporated in the Indian Pharmacopoeia. Unlike USA, Canada and Norway, where strict guidelines for product licenses and permits are in place, the fish vaccines are classified as veterinary biologicals, and licenses are issued by Veterinary Biologics and Biotechnology Section (VBBS) [5].

As per Canadian regulatory guidelines [11], veterinary biologicals can be classified based on the qualitative risk assessment as follows:

1. **Class I (low risk):** includes inactivated viral/bacterial vaccines (conventional or rDNA), viral/bacterial subunit vaccines (conventional or rDNA), cytokines and monoclonal antibody (hybridoma) products and modified live conventional or gene deleted vaccines.
2. **Class II (high risk):** includes vaccines using live vector to carry recombinant derived foreign genes and live organisms modified by introduction of foreign DNA.
3. **Unknown Risk:** Plasmid DNA vaccines are a novel group of vaccines that are presently undergoing evaluation by VBBS for risk classification. The novel biology of plasmid DNA vaccines poses several regulatory challenges, particularly in the evaluation of safety and potency. Safety concerns focus on the potential for integration, tumour formation, replication activity and germ line transmission.

For most vaccines, a Biosafety level 3 containment facility having biosafety cabinets and other primary devices are required for all activities to be carried out without creating aerosols. Any spillage is considered as hazardous and disposed as per biosafety norms. Protective clothing, access control through directed airflow

using airlock systems provide sterility as well as containment of the potential pathogen.

8 Operator Safety During Injection Vaccination of Fish

There are a number of reports of adverse health effects of vaccinators after accidental self-injection of fish vaccine during injection of fish [16]. A reaction is considered serious if it led to absence from work for more than 10 min. Most of the self-injections occurred exclusively on fingers and hands. The reactions to injuries can be differentiated into four types:

1. The most common reaction is mild localized pain, oedema and sometimes infiltration induced by superficial stabs from the tip of the needle of syringes.
2. Depending on the dose and type of vaccine injected, reactions may spread to whole hand or parts of the underarm. In some cases, fever with lymphangitis and swollen axillary glands was reported. In cases where there was accidental injection of vaccine containing aluminium hydroxide and mineral oil as adjuvant, the affected finger had to be amputated.
3. Injectors also experience lymphatic reactions along with influenza-like syndrome with fatigue, dizziness, headache, fever and muscle ache lasting 2–12 h.
4. Perhaps, the most severe manifestation is the form of anaphylactic reaction. Within minutes after self-injection, symptoms like tachycardia, breathing difficulties, nausea and loss of consciousness appear [16].

Overall, the risk of self-injection seemed to be associated with lack of experience and awareness, as experienced vaccinators seldom self-inject themselves compared to seasonal inexperienced workers. Medical treatment includes mainly non-steroid anti-inflammatory drugs indicated for some days and, in case of anaphylactic reactions, adrenaline is recommended. Local doctors should be acquainted with the characteristics of such injuries or local reactions and the treatment for the same for timely intervention whenever necessary [16].

Devices to protect the fingers against self-injections have been developed. A double bow is attached to the tip of the syringe on both sides of the needle, allowing the fish to be supported during the injection, and at the same time shielding the tip of the needle [12]. This has been extensively used in Norwegian aquaculture [17] that includes risk assessment and risk mitigation measures, whereby the number of reports of self-injection drastically reduced. However, although use of automatic injection devices and immersion or oral administration of vaccines are being adopted, self-injection still remains a potential hazard for inadequately equipped and untrained aquaculture workers especially in unorganized sectors [12, 16].

9 Conclusion

In the Indian context, biosafety and regulatory requirements for fish vaccines are still in the nascent stage. So far, no Standard Operating Protocols (SOP) or product information regarding bacterial or viral vaccines of fish are available in the Indian Pharmacopoeia. Hence, the biosafety requirements for veterinary vaccines are to be used as a guideline at present, as most countries classify the fish vaccines as veterinary biologicals. What is more important is that the safeguards to be used in the injectable fish vaccines are overlooked, leaving much to be desired as far as biosafety and regulatory issues are concerned. However, research on recombinant vaccines is being undertaken worldwide, as these vaccines can be administered by immersion technique or oral routes. Nevertheless, the evaluation of their biosafety is under consideration by international bodies as these vaccinated fish will enter the human food chain. Moreover, protection of the wild aquatic species also comes under the scope of biosafety in aquaculture. These facts further reiterate the important role of biosafety and regulatory bodies in fish vaccines and aquaculture. Therefore, we must recognize that in the formulation of biosafety policy and regulations for living modified organisms, the characteristics of the organisms and of potentially accessible environments are more important considerations than the processes used to produce those organisms.

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