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Review Article—

Veterinary Autogenous Vaccines for Poultry in Europe—Many Ways to Crack an Egg

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SUMMARY. In the past decade, European animal farming has increasingly used autogenous vaccines for the prevention of nonnotifiable diseases. In Europe, these vaccines are exclusively inactivated bacterial and viral vaccines, with a set of specific regulations that differentiate them from conventional vaccines. The highest number of applications most likely occurs in poultry, as these animal species are farmed in the highest numbers compared with other types of food-producing animals. In 2019, autogenous vaccines came within the scope of harmonized European regulation for the first time, although many important aspects are still missing and need to be further developed. Consequently, several important legal provisions remain in national legislations and can vary tremendously between different member states of the European Union. The inclusion of autogenous vaccines in the management of certain diseases of poultry is justified by the nonavailability of licensed vaccines and the evolution and diversity of antigens in the field that are not covered by licensed vaccines. In addition, these vaccines aid in reducing the use of antibiotics. The methods for isolating and typing pathogenic isolates to obtain relevant antigens are pathogen specific and require a careful approach based on clinical evidence. Manufacturing processes are optimized according to regulatory standards, and they represent the most critical factor influencing the quality of autogenous vaccines and their placement on the market. This review presents the important requirements for manufacturing autogenous vaccines for poultry in addition to the relevant regulatory considerations. The results from a survey of several European Union member states regarding specific provisions within their national legislations are also presented.

RESUMEN. Vacunas veterinarias autógenas para la avicultura en Europa: "Varias formas de romper un huevo".

En la última década, la ganadería europea ha utilizado cada vez más vacunas autógenas para la prevención de enfermedades no declarables. En Europa, estas vacunas son exclusivamente vacunas inactivadas bacterianas y virales, con un conjunto de regulaciones específicas que las diferencian de las vacunas convencionales. El mayor número de aplicaciones probablemente se produce en la avicultura, ya que estas especies animales se crían en mayor número en comparación con otros tipos de animales productores de alimentos. En el año 2019, las vacunas autógenas entraron por primera vez en el ámbito de aplicación de una regulación europea armonizada, aunque todavía faltan muchos aspectos importantes y es necesario desarrollarlos más. En consecuencia, varias disposiciones legales importantes permanecen en las legislaciones nacionales y pueden variar enormemente entre los diferentes estados miembros de la Unión Europea. La inclusión de vacunas autógenas en el manejo de determinadas enfermedades avícolas se justifica por la falta de disponibilidad de vacunas autorizadas y la evolución y diversidad de antígenos en el campo que no están cubiertos por vacunas con licencia. Además, estas vacunas ayudan a reducir el uso de antibióticos. Los métodos para aislar y tipificar aislados patógenos para obtener antígenos relevantes son específicos de cada patógeno y requieren un enfoque cuidadoso basado en evidencia clínica. Los procesos de fabricación se optimizan de acuerdo con las normas reglamentarias y representan el factor más crítico que influye en la calidad de las vacunas autógenas y su comercialización. Esta revisión presenta los requisitos importantes para la fabricación de vacunas autógenas en la avicultura, además de las consideraciones regulatorias relevantes. También se presentan los resultados de una encuesta realizada en varios estados miembros de la Unión Europea sobre disposiciones específicas dentro de sus legislaciones nacionales.

Key words: vaccine, autogenous, legislation, poultry, manufacture

Abbreviations: APEC = avian pathogenic *Escherichia coli*; ARV = avian reoviruses; AV = autogenous vaccine; AVVs = autogenous veterinary vaccines; CMDv = Coordination Group for Mutual Recognition and Decentralised Procedure—Veterinary; EU = European Union; EMAV = European Manufacturers of Autogenous Vaccines & Sera; FAdV = fowl adenoviruses; GMP = good manufacturing practice; HPAI = highly pathogenic avian influenza; ORT = *Ornithobacterium rhinotracheale*

Vaccination is one of the greatest discoveries in medicine. It has helped to control many infectious diseases in humans and animals, and it serves as a cornerstone of public health systems worldwide. Changes in European poultry husbandry from cages to alternative housing systems over the last two decades have triggered the recurrence of long-known diseases, mostly caused by bacteria and parasites, that are rarely observed in caged animals (1,2). Consequently, the changes have created the need for intensive use of medication and research for new and improved preventive methods. In combination with enhanced biosecurity, farming, and husbandry practices, vaccines are regarded as one of the most important prophylactic instruments for reducing the usage of antimicrobials and safeguarding the well-being and productivity of livestock (3). Many types of vaccines are in use nowadays for these purposes. These vaccines generally fall under two categories: inactivated vaccines (whole bacterin, subunit, or toxoid vaccines) and live vaccines [live attenuated or live virulent, recombinant vector vaccines] (4,5).

One type of vaccines gaining popularity are autogenous vaccines (6). In Europe, autogenous veterinary vaccines (AVVs) are widely used as inactivated bacterial and viral vaccines in farmed animals, with a set of specific regulations that differentiates them from conventional vaccines (7). These legal provisions for AVVs have only

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recently been entailed in European legislation. Still, many important aspects remain to be developed for a fully harmonized procedure at the European Union (EU) level. These aspects will cover manufacture, quality control, placement on the market, import, export, supply, use, and pharmacovigilance (8). Until then, such provisions are part of national legislations that vary substantially between different member states. AVVs are used only when vaccines with marketing authorization are not available in the EU, or when registered vaccines cannot be supplied (9). Notably, AVVs do not require registration and are exempt from marketing authorizations (10). Despite being used only on an exceptional basis, AVVs currently constitute an integral part of health management in farmed animals, and they serve as a valuable complement to the registered vaccines in the poultry market (11). In light of their increased use over the past decade and the transition from national to standardized European legislation, this review aims to gather the most up-to-date and crucial information available on legal, practical, and scientific knowledge of AVVs for poultry. It also aims to provide an overview of their clinical relevance to veterinary poultry specialists, farmers, and the general public to clarify ambiguities on the EU level. Furthermore, critical manufacturing procedures related to the main components of autogenous vaccines are discussed. For the purpose of gaining information on current national regulations, a questionnaire was sent to the responsible authorities. Data from the limited number of responses received are summarized in the present review.

HISTORY OF USE OF VETERINARY AUTOGENOUS VACCINES FOR POULTRY IN EUROPE

Today's AVVs are inactivated vaccines. The first reports on the inactivation of infectious agents and their use for immunization date back to 1886 (12). Formaldehyde, the most important chemical agent in manufacturing AVVs, became established for use in producing toxoids in the 1920s (13). Over time, vaccine manufacturing became increasingly more regulated by national and international authorities. The first good manufacturing practice (GMP) guidelines for pharmaceutical products were published in 1962. In Germany, the licensing of veterinary vaccines became part of the drug legislation during the 1970s, and the EU organized a major review and relicensing activity during the 1990s. Although the standards for manufacturing, testing, and licensing registered veterinary vaccines were incrementally increased and updated (14), the options for manufacturing and using autogenous vaccines were maintained (15). According to Hera and Bures (16), the Central European countries, the Czech Republic, Hungary, and Slovakia may have the longest tradition of using autogenous vaccines. In the early 1990s, a large-scale study combining a live *Salmonella* Typhimurium vaccine with autogenous *Salmonella* Enteritidis oil emulsion vaccines was reported in Germany (17). The vaccines were shown to be safe, with transient swelling at the site of injection as the only adverse effect, and they showed considerable efficacy in protecting vaccinated broiler parents and their progeny during test infections. The protection of progeny by maternal antibodies was better when the inactivated autogenous vaccines were used twice in comparison with a single application.

DEFINITION AND REGULATORY CONSIDERATIONS

The concept of AVVs is based on strain or antigen specificity related to a targeted treatment of a restricted number of animals from a particular herd (18). The official definition of AVVs is provided by Regulation (EU) 2019/6 in Article 2 (3), which describes them as "inactivated immunological veterinary medicinal products which are manufactured from pathogens and antigens obtained from an animal or animals in an epidemiological unit and used for the treatment of that animal or those animals in the same epidemiological unit or for the treatment of an animal or animals in a unit having a confirmed epidemiological link" (8). A few synonyms for these vaccines, such as autovaccines, autologous vaccines, tailor-made vaccines, and farm- or herd-specific vaccines, are occasionally encountered in the literature. AVVs are applied in emergency situations on a case-by-case basis to fill the existing gaps in licensed vaccines, meaning they must be available within a few weeks (10). Therefore, the manufacture and release of batches are accomplished in a very short time, usually only 3–8 wk, depending on whether the pathogen is already available at the site and on its growth characteristics.

Legal considerations for AVVs for poultry encompass their production quality, distribution (export/import), surveillance, and use in the field. They are, however, not poultry specific but rather shared with other animal species such as pigs, cattle and small ruminants, fish, and pet and zoo animals. Within poultry species, AVVs are used in laying hens, broiler and laying breeders, turkeys, and waterfowl (19).

Previous European legislation merely defined AVVs, focusing entirely on registered veterinary medicinal products. Nevertheless, within their description, some basic rules (inactivated and only for use in the same flock and in the same geographical location from which the isolate originates) for the use of AVVs were laid down, albeit without legal obligations. During this time, the legal provisions were specific for each member state and great variability existed between national legislations, ranging from the absence of any measures governing the manufacture and use of AVVs in some countries to the presence of very demanding measures in others (16). A periodic overview of relevant differences was published by the Coordination Group for Mutual Recognition and Decentralised Procedures—Veterinary (CMDv) in 2004 and 2017, with the conclusion that no fundamental changes had been implemented, but the use of AVVs had increased over time, resulting in more experience gained in the member states (7). Another independent survey was also undertaken and reported by Austrian authors in 2013, summarizing the requirements for the manufacture, current production levels, import, and conditions of use of AVVs in EU member states (10).

The divergent statutory provisions can greatly impede trade of AVVs and their placement on the market, because their production moved from mainly national by governmental diagnostic or research centers to international manufacturers that export them within the EU and beyond (20). A long-sought harmonized procedure, Regulation (EU) 2019/6, came into force on January 28, 2022, laying down legal provisions and consistency for inactivated autogenous vaccines across Europe (8). The most important requirement in this new legislation is the adherence to GMP principles during the manufacture of AVVs, although the detailed guidelines adjusted to these products have yet to be fully elaborated. This task has been taken up by the European Medicines Agency, with consultancy of the International Alliance for Biological Standardization in Europe, and the European Manufacturers of Autogenous Vaccines & Sera (EMAV), who jointly organize meetings among competent authorities, manufacturers, users, and other stakeholders to coordinate scientific evaluation, experience, and expertise to define new standards for the manufacture and the continuing availability of AVVs (8,21). EMAV was formed in 2019 by leading European producers of AVVs to promote their interest to relevant stakeholders, protect the product, ensure its high-level

quality, and to inform the public on science-based evidence on the positive impact of AVVs on animal health and welfare, among other activities. Their recently published proposal for an EU-GMP-Annex aiming to foster the harmonization process within the official EU legislation is available at http://www.emav.be (21).

Although there are well-established and centralized GMP requirements in the manufacture of licensed veterinary immunologicals at the EU level (14), the aforementioned parties are in agreement that such requirements should be specifically adapted for the unique manufacturing conditions of AVVs and should be included as a stand-alone document without interfering with the written guidelines for licensed vaccines (8). In general, the document should reflect the most important aspects that guarantee avoidance of risks related to manufacture, namely, the quality of starting materials and their compliance with standards of the European Pharmacopoeia (Ph. Eur.), premises and personnel, manufacturing processes and inactivation kinetics, quality control (according to Ph. Eur. standards), and pharmacovigilance. Some manufacturers are still referring to the CMDv recommendations published in 2017 (7). Key differences regarding GMP production between commercial and autogenous vaccines should be carefully considered. For example, batch sizes vary widely for AVVs, and many small-sized batches are manufactured, rendering the product as nonindustrial (8). Further, many different antigens may be handled by nonautomated procedures in the same room, and the spatial separation of antigens would not be economically feasible in the case of AVVs (7).

It is clear from the preceding information that several important aspects of the streamlined legislation addressing AVVs are still missing and remain within national laws that must be adhered to for the time being. Thus, the questionnaire sent to competent authorities for the purpose of the current review was an attempt to collect information on availability, use, and quality standards of AVVs for poultry. Competent national authorities from 17 member states responded to the questionnaire, namely, Austria, Bulgaria, the Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Liechtenstein, the Netherlands (Kingdom of the Netherlands), Poland, Romania, Slovakia, Slovenia, and Spain. In the case of Liechtenstein, the authorities stated that the legal provisions are closely related to Switzerland in the custom treaty, although there was no timely response from the latter authorities to the questionnaire. A response from Spain did not include answers but rather information on coming national legislation to replace the Royal Decree 109/1995, which is in force in combination with the Regulation (EU) 2019/6. The latter regulation is similar to that of the Netherlands, whose authorities announced upcoming Dutch regulation requiring inactivation of pathogen isolates from the same epidemiological unit, GMP requirement for the manufacturers, and so forth. The Danish authorities emphasized that the info provided for the questionnaire reflects the current situation in their country as per March 2023, and the regulation and use of AVVs are subject to constant changes; thus the situation may change in foreseeable future. Overall, AVVs for poultry are used in all the mentioned countries except Romania, whereas information on use was inconclusive in the case of Bulgaria because the authorities stated that there is no national legislation in force and thus no related information. Except for Denmark and Germany, none of the countries could provide the number of batches produced or doses of AVVs used at farms, claiming that no such data exist. A complete overview of questions and responses is given in Table 1, and the list of currently existing national legislation in the EU and European Economic Area, including the United Kingdom, is given in Table 2.

Regarding AVV manufacturers, previous reviews focusing on regulatory aspects of AVVs in the EU provide some additional information, with broad similarities among the different member states. Overall, AVVs must be produced in licensed facilities that hold a specific manufacturing authorization obtained upon demonstrating the appropriate expertise, reliability, and technical laboratory equipment. The name of the applicant, the site of production, and the organisms and antigens that are handled at site must be stated in the application (8,10). A control system in the form of inspection of manufacturers by local authorities responsible for GMP is required in most of the member states where AVVs are produced (7).

Some other regulatory considerations (e.g., specifications on antigens) that are not mentioned in this section, but are relevant for separate headings, may be encountered further in the text.

INACTIVATION

Different methods to inactivate infectious agents exist (22,23). Chemical inactivation is the most important method for AVVs, with formaldehyde being the most important inactivating agent. Ph. Eur. monographs provide guidance and regulatory reassurance on the procedure of inactivation and acceptable levels of residual inactivating agent, including testing and inactivation of formaldehyde (European Pharmacopoeia 11.0 2.4.18). Testing and validation of the inactivation procedures are commonly requested from regulators, as the risk of incomplete inactivation is deemed high and critical (European Pharmacopoeia 11.0 0062). This risk is particularly true for autogenous vaccines, which are always produced from nonattenuated, fully virulent field strains. Although potentially a false-negative test for inactivation of the treated antigen is likely to be detected later during the mandatory sterility testing of the final product for most bacterial antigens, this does not apply for viral vaccines.

Chemical inactivation has downsides such as toxicity, residues, and the destruction of immunogenicity through chemical alteration of the antigen structure (23,24). Inactivation of West Nile virus by two commonly applied chemicals, formaldehyde and β-propiolactone, showed no difference with regard to neutralizing antibody titer in mice (25). In another study, vaccination of chickens with inactivated highly pathogenic avian influenza (HPAI) virus using both chemical agents resulted in similar antibody titers and protection against the challenge (26) based on measurement of both antibody and cellular immune responses in chickens. Moreover, inactivation of low pathogenic avian influenza virus with β-propiolactone resulted in increased antibody levels and a more pronounced cell-mediated response in comparison with formaldehyde and gamma radiation (27). The effect of different inactivation methods on the outcome of immunization with bacterins of *Escherichia coli* have been summarized by Ghunaim *et al.* (28).

Improved technologies based on chemical inactivation with hydrogen peroxide may be applicable for manufacturing AAVs in the future (24,29). Physical inactivation with ultrasound (30), radiation (31), pulsed lasers (32), or light-based strategies (33) bear potential, but would need to be available as small-scale, robust, and affordable technical platforms for application in commercial AAV production.

APPLICATION AND FORMULATION OF AUTOGENOUS VACCINES: THE CHALLENGE OF CHOOSING THE RIGHT ADJUVANT

AVVs produced and distributed in Europe are inactivated vaccines containing whole inactivated viral or bacterial cells (21). They consist of two main components, an antigen and an adjuvant, each requiring

Table 1. Review of responses from competent national authorities in regard to allowance for use, national manufacture, import, type of antigens (bacterial, viral, or both), restrictions on number and combination of antigens, restrictions on use in relation to antigen (prerequisites, type of pathogen. . .), requirements for on-farm-safety test for each used batch, review of relevant documents, and the release of imported batches by the responsible authority for poultry veterinary autogenous vaccines.

EU member state	Use of AVs[A]	Manufacture	Import	AV type	Restrictions on number and combination of antigens	Restrictions use of AVs (e.g., prerequisites, pathogens..)	OFS[B]	Review of documentation (RC,[C] CoA[D])	Release of imported batches by authorities
Austria	Yes	Yes	Yes	B[E] + V[F]	No	No	No	No, but an accompanying letter is mandatory	Yes
Bulgaria	n/a[G]	No	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Denmark	Yes	No	Yes	B	No, but the case-by-case evaluation of AV in scope is in place	No	Yes	Yes	Yes
Czech Republic	Yes	No	Yes	B + V	No	No	Yes	No	No
Finland	Yes	No	No	B + V	No legal requirements but the number and combination of antigens should be justified	Yes, the antigens cannot be prepared from pathogens interfering with national control programs (e.g., NDV[H])	No	No	Yes
France	Yes	Yes	Yes	B	No	No	No	No	No
Germany	Yes	Yes	Yes	B + V	No	No	No	No	No
Italy	Yes	Yes	No	B + V	No	No, apart from requirements for inactivation as per (EU) 2019/6	No	No	No
Latvia	Yes	No	Yes	B + V	No	only inactivated and Nontoxic pathogens	Yes	Yes	No
Netherlands	Yes	Yes	Yes						
Poland	Yes	Yes	n/a	B + V	n/a	n/a	No	n/a	n/a
Slovakia	Yes (chickens, turkeys)	No	Yes	B	No	Yes (e.g., no import from third countries, 6-mo shelf life)	Yes	Yes	No
Sweden	Yes	No	No	B + V	The number of antigens that can be used in an AV is assessed and approved on a case-by-case basis	No	No	Yes	The assessment and approval for use is done on a case-by-case basis as a special permit.

[A]AV = autogenous vaccines.
[B]OFS = on-farm safety test.
[C]RC = release certificate.
[D]CoA = certificate of analysis.
[E]B = bacterial.
[F]V = viral.
[G]Data not available.
[H]NDV = Newcastle disease virus.

Table 2. Existing national legislations in member states of the EU, the EEA (The European Economic Area), and United Kingdom, according to the feedback to the questionnaire submitted to competent authorities and the knowledge of authors in case of additional countries.

EU member state	National legislation
Austria	Bestandsspezifische Imfstoffe Betriebsordnung (BIBO) 2010.
Czech Republic	Law n 378/2007 Coll. On medicinal products and amendments to certain related law; Guideline of the Institute for State Control of Veterinary Biopreparations and Medicines: Requirements for the manufacture, control, prescribing and use of veterinary autogenous vaccines in Czech Republic USKVBL/INS—02/2021.
Denmark	The Danish Medicines Agency issues a special national permit to import and dispense autogenous vaccines; this permit is currently issued to Statens Serum Institut; there is a requirement for GMP according to the Danish executive order nr. 1358 of 18 December 2012, as amended.
Finland	Medicines Act 295/1987 Section 21g.[A]
France	Autogenous vaccines may be used in case the vaccine with marketing authorization is not able to solve the situation on the field (route of administration not appropriate, shortage...). In France, an authorization is given for each manufacturer which includes the list of adjuvants and the list of the couple target species/pathogens authorized. Any derogation should be handled individually.
Germany	Animal Vaccines Act of 2006 (TierImpfVO 2006).
Hungary	Decree No. 94/2012. (VIII. 30.) VM (MRD).
Italy	Legislative decree of the Ministry of Health, March 17th, 1994, n. 287.
Latvia	Cabinet Regulation No 326 "Distribution and control of veterinary medicinal products" Section 9.8. "Requirements for distribution of inactivated autogenous vaccines."
Slovakia	Act No. 362/2011 Coll. on Medicinal Products and Medical Devices—especially "Special conditions for the production, prescription, control and use of veterinary autogenous vaccines"—§12c, §12d.
Spain	Royal Decree 109/1995.[B]
Sweden	An autogenous vaccine can be approved as a special permit to in accordance with Swedish Medicines Law and provisions.
UK	The Veterinary Medicines Regulations 2013 SI 2033.

[A]This act gives the Finnish Food Safety Authority (nowadays Finnish Food Authority) the right, in the case of a serious animal disease epidemic, to grant an import permit or a permit for use for an immunological veterinary medicinal product that does not have marketing authorization if a suitable product is not otherwise available or if the epidemic situation otherwise so requires. The Finnish Medicines Agency must be notified of the granted permit without delay.

[B]A new decree is being developed and drafted to replace the existing one.

careful consideration of scientific knowledge, know-how of the manufacturer, and veterinary advisory in order to blend them into a high-quality functional product. Similar to licensed inactivated vaccines, AVVs are usually administered via intramuscular injection in the breast or leg muscle or via subcutaneous injection in the neck (34,35). While even a small amount of antigen in live vaccines may trigger a bird's immune response by replication, inactivated vaccines require larger antigen quantities. Furthermore, inactivated vaccines have to be combined with an adjuvant to enhance the immune response in the short term and to generate a long-lasting protective immunity in the bird (36).

In general, the suitable adjuvant for inactivated autogenous vaccines for poultry must meet a number of requirements: it must be approved for the use in food-producing animals; be cost effective, stable, and easy to process and inject; and induce few adverse effects (37,38). When selecting an adjuvant, the risk of extensive tissue reactions must always be weighed against the need for an effective immune response (39). In poultry, aluminum hydroxide precipitate gels and mineral oils are commonly used as adjuvants (36). Aluminum salts strongly stimulate the cellular immune response but are rapidly cleared from the injection site and induce rather short-term immune responses (40). Based on the mild inflammatory potential, aluminum hydroxide may be preferably combined with highly immunogenic antigens such as *Pasteurella multocida* or *Avibacterium paragallinarum* (36). Aluminum hydroxide adsorption of bacterial endotoxins might further contribute to reducing the adverse effects (41). Mineral oils, however, provide a strong depot effect at the injection site and elicit an inflammatory response. Hence, they are combined preferably with less reactive antigens (37). The ratio of antigen and adjuvant may vary for each individual vaccine depending on viscosity and tissue reactivity of the adjuvant, as well as the immunogenicity of the respective antigen(s) (36). Oil-based adjuvants are known to be highly effective, and water-in-oil emulsions are most frequently used in poultry (37). An emulsion is a mixture of two liquid components that are insoluble in each other. One liquid (the dispersed phase) is dispersed in the other (the continuous phase) in the form of small droplets to produce a homologous liquid. Emulsion stability may be achieved by adding surface-active agents to reduce the interface tension (42). In a water-in-oil emulsion, the dispersed phase is water, while the continuous phase is oil. The aqueous phase usually contains the antigen, and mineral oil is most frequently used to enhance the immune response in poultry (36). Local reactions are generally accepted in favor of strong, long-term immunity, especially in turkeys, layers, and broiler breeders (42).

Research has been performed, mainly by adjuvant companies, to provide efficacious, stable, and safe oil adjuvants (36). Traditional adjuvants, such as Freund's complete or incomplete adjuvant and Tween/Span combinations have been repeatedly shown to induce strong adverse reactions (39,43–45). In contrast, modern commercial mineral oil adjuvants elicit fewer tissue reactions and higher antibody titers (26,46). Consequently, the antigen load or the vaccine dose may be reduced, thereby improving cost effectiveness and vaccine efficacy (43). Nonmineral oils have also been considered as potential alternatives to mineral oils based on their lower potential to induce local reactions, but they are generally considered less efficient than mineral oils (26,37). In addition to safety and efficacy, destabilization by culture media components or unfavorable storage conditions are accounted for during the development of new commercial adjuvants (47). The development of improved adjuvants is of crucial importance because they can also decisively improve the quality of autogenous vaccines.

Local reactions are mainly reflected by injection site injuries that occasionally occur as a result of focal inflammatory myositis, cellulitis,

and myositis of the neck region (48). In a study by Mitrivic *et al.* (49), white mineral oils were indicated as the sole irritating component of fowl cholera bacterin vaccine in chickens following subcutaneous injection. Interestingly, the authors did not observe any reactions caused by subcutaneous injection of a cultured, live but nonadjuvanted *P. multocida* (49), whereas in a study in rabbits, a nonadjuvanted *P. multocida* bacterin caused irritation when injected subcutaneously (50). Breast injection of oil-adjuvanted vaccines in chickens induces long-lived residual lesions of varying degree, which was shown exemplarily after the injection of a *Mycoplasma gallisepticum* bacterin (45). Similar results were also obtained in turkeys, in which varying degrees of irritation from mineral-oil–adjuvanted fowl cholera bacterins were observed (51). In this regard, the age of birds or route of injection did not seem to be an influential factor in causing irritation, but the reactions were less severe after 15 days postinfection in comparison with 5 days (50). Mineral oils are composed of mineral oil hydrocarbons that are categorized into two main groups: mineral oil saturated hydrocarbons and mineral oil aromatic hydrocarbons (52). Mineral oil or liquid paraffin is defined in European Pharmacopoeia 11.0 0240 as a purified mixture of liquid saturated hydrocarbons obtained from petroleum, and according to the European Food Safety Authority, it is a potentially toxic component with adverse health effects (53). The use of oils that are devoid of these components may eventually solve the issue of undesired residual mineral oil hydrocarbons. The need for adjuvants also has safety implications for the user of inactivated autogenous vaccines. Wherever needles and animals are handled concurrently, the risk of self-injection is high. Mineral oils may cause severe reactions when administered accidentally to human tissues, with effects ranging from swelling, pain, and reduced function to total loss of a finger or the whole hand (36). Accordingly, precautions regarding the handling of AVVs and accidental self-injection should be included on labels and in package leaflets (21).

In a few EU member states, pharmacovigilance systems related to AVVs are in place and are mostly related to recording data on quality defects and adverse effects (7). On-farm safety tests are currently practiced in the Czech Republic, Denmark, Hungary, Latvia, Slovakia, and the United Kingdom (Table 2) as a part of the batch release procedures. This commonly means injection of a small number of birds on a farm, with a 15-day follow-up period to screen for adverse events and local tissue reactions (54). In the case of adverse reactions of considerable severity, the results are reported to the competent local authority and the vaccine batch is rejected.

Combining the antigen with the most suitable adjuvant may significantly improve the efficacy of autogenous vaccines (8). The efficacy of vaccines with best-fitting adjuvants for different inactivated antigens are usually evaluated in the literature by either measuring the immune response or the protection of birds in experimental challenge models following immunization. For instance, oil adjuvants were found to induce an enhanced antibody response and to lower the viral load in the tracheas of broilers injected with low pathogenic avian influenza H9N2 autogenous vaccine, highlighting the importance of updating the viral antigens with a more recent isolate (55). Furthermore, oil-based adjuvants were found to be the most immunogenic in combination with inactivated HPAI antigen in comparison with carbohydrate, mineral nanoparticle, or seaweed-derived adjuvants (26). Where available, data on the best-fitting adjuvant for relevant antigens used in AVVs for poultry are mentioned in separate headings further in this review.

POTENCY, DOSAGE, AND EFFICACY OF AUTOGENOUS VACCINES AGAINST SELECTED AVIAN PATHOGENS

Inactivated autogenous vaccines are highly important to ensure poultry health because licensed vaccines are not available for all relevant pathogens or may lack efficacy against new emerging strains (21). Consequently, in contrast to licensed vaccines that undergo a lengthy and strenuous marketing procedure, safety and efficacy studies are not mandatory for AVVs. Potential resulting risks are controlled by rules for production and marketing, which include restriction to inactivated vaccines and use only within an epidemiological unit (8,21). In practice, the efficacy of AVVs is measured empirically by recording the clinical outcomes and flock performance data. However, published data on potency, efficacy, and safety of autogenous vaccines are very limited. In the following section, data available for different important avian pathogens are summarized (Supplemental Table S1). AVVs for poultry may include a combination of relevant strains of one or more pathogens. Careful consideration is recommended when choosing the relevant isolate(s) for each individual case. Isolates should be well characterized, and only pure seed material should be used (21). In general, interference of different inactivated antigens is less likely in comparison with live vaccines (36).

HOMOLOGOUS PROTECTION—HOW TO SELECT THE RIGHT STRAINS FROM CLINICAL CASES

Although licensed inactivated vaccines contain well-characterized seed strains that are tested in animal models, the seed material for AVVs needs to be selected under time pressure from individual clinical cases. Overall, the aim is to supply a relevant immunogenic antigen to protect animals in the flock diagnosed with an infectious disease or to protect the offspring of a breeder flock against neonatal infections through maternal antibodies in a timely manner. Realizing this aim requires a close interaction of the ability of poultry practitioner to select representative animals for analysis, diagnostic skills to isolate pathogens and adequately type them in specialized laboratories, and a vaccine manufacturer to produce high quality immunogenic antigen rapidly (56). Decisions are made on a case-by-case basis to select the relevant clinical isolates to become part of the vaccine. Modern strain typing methods are indispensable for the identification of antigens within different serotypes of the same species (57), virulence markers (58), and other relevant traits. Such methods are established for a variety of poultry pathogens, and they can be based on various methods, including molecular biology, biochemistry, and biophysical methods such as MALDI-TOF mass spectrometry. The latter bacterial typing technique is proving useful as a rapid confirmation of the taxa involved in clinical outbreaks affecting poultry when classic bacteriology may provide unconvincing results (59–61), providing further aid in urgent situations in which AVVs need to be placed. Moreover, the practical need to update strains in AVVs permanently is reflected in some official regulations setting maximum periods for strains to be used in AVVs (e.g., Switzerland and the Czech Republic).

FOWL ADENOVIRUS

Fowl adenoviruses (FAdVs) are genetically very diverse group of avian viruses, with five different species, including various genotypes and different serotypes, having been described (62). Virulence may vary among different serotypes and even between strains of the same serotype (63). Moreover, two or more strains are often isolated simultaneously from one bird. FAdVs are widespread among chicken flocks

independent of the vaccination status of the breeders, and FAdVs should always be considered in multifactorial disease (62,64). Additionally, cross-protection between different serotypes is limited (65). Development of an efficacious commercial vaccine against FAdVs is therefore challenging, and no licensed product is available in Europe. Consequently, autogenous vaccines are frequently used to prevent disease outbreaks and reduce economic losses (62). Because vertical transmission plays a crucial role and young broilers are primarily affected, the focus is mainly on vaccination of breeders and protection of the offspring through maternal antibodies (62). In contrast to inclusion body hepatitis and hepatitis hydropericardium syndrome, maternal antibodies seem to be less protective in preventing adenoviral gizzard erosion (66). Although various concerns related to safety, efficacy, and applicability of autogenous vaccines have been raised (64), these vaccines have been shown to protect chickens and their offspring effectively in different studies. After the first reports of FAdV-associated disease outbreaks, the disease was effectively controlled by implementing a vaccination procedure with an inactivated autogenous vaccine prepared from infected livers of affected birds (67,68). Thereafter, sufficient protection against homologous challenge could also be shown when the virus was isolated and grown on chicken embryo liver cells (69). It was also shown that birds may be at least partly protected against heterologous challenge after vaccination (70) and a combination of different serotypes may further enhance protection of progeny against different challenge strains (69). These results indicate that selection of the correct vaccine strains is decisive for the efficacy of vaccination. Despite the fact that adenoviruses generally induce a strong humoral response (64), sufficient protection against FAdV challenge was also observed in birds without detectable neutralizing antibody titers, suggesting that the cell-mediated immunity may also be involved in protection against FAdVs (71). Moreover, in addition to isolate selection, the choice of the adjuvant may have an impact on the efficacy of an autogenous FAdV vaccine (72). Consequently, the success of autogenous vaccines as a prevention strategy in affected flocks requires careful consideration of the composition of the autogenous vaccine, and it depends on continuous health monitoring, serological monitoring, and identification of circulating strains (64).

AVIAN REOVIRUS

Newly hatched birds are very susceptible to infection with avian reoviruses (ARV), thus vaccination of breeders is a common strategy to provide protection of the offspring via maternal antibodies (73). However, ARV infection may also be controlled in the offspring without detectable antibody titers, suggesting an additional level of protection provided by cellular immunity (74). This possibility is further supported by the short half-life of maternal antibodies in the offspring (4.7 days post hatch) (75). Many variants of ARV with broad genetic and antigenic diversity have been described, which complicates vaccination and may be the reason for reports on vaccine failure (76,77). Even though licensed vaccines against ARV are available for chickens in Europe, the emergence of new variant ARV strains limits their efficacy in the field (78). Commercial vaccines for turkeys are not available, but the use of autogenous vaccines in turkey breeders is common and highly successful in preventing viral arthritis (73). In contrast to heterologous protection, homologous protection is adequate (79), which underlines the need to choose a representative strain from an affected flock to provide sufficient protection by vaccination and to adapt the autogenous vaccine based on detailed follow-up investigations on circulating strains (73,78). Combining different genotypes of ARV in a polyvalent autogenous vaccine may provide broader protection against circulating field strains than the use of a single isolate (80).

ESCHERICHIA COLI (COLIBACILLOSIS)

Vaccination is one of the pillars of *E. coli* control and is recently becoming even more important as disease control by antimicrobials is limited because of increasing antimicrobial resistance and stricter regulations on antibiotic use (81). As *E. coli* strains have high genetic and antigenic diversity, licensed vaccines are often ineffective in individual disease outbreaks (28). Owing to the attention regarding treatment and prevention of *E. coli* infections, research in recent years has also focused on the applicability and efficacy of autogenous vaccines. It has long been recognized that inactivated autogenous vaccines are effectively preventing disease after homologous challenge (82,83). As a first step to obtain antigens of clinical importance, the route of isolation has proven to be crucial, focusing on bone marrow, where septicemic isolates commonly reside (84). Combining the available typing methods is useful to narrow down the numerous isolates into high-risk clonal groups, which might further aid in antigen selection for vaccine production (58). Protection of newly hatched chicks may only be achieved via high maternal antibody titers (85). High maternal antibody titers were shown to provide full protection of the progeny up to 14 days posthatch, and polyvalent vaccination may further provide polyvalent protection. However, no protection against heterologous challenge could be observed (85). Recent studies further support these observations (30,86,87). The vaccine strain has a decisive impact on the efficacy of vaccination, but the choice of the adjuvant and the inactivation method should be well thought out (30,86,88). Possible factors further influencing the efficacy of breeder vaccination with an autogenous vaccine against *E. coli* include inoculation dose and route, hen age, and the number of strains used in the vaccine (89). In laying hens, the use of an autogenous vaccine in affected flocks may help to reduce morbidity and mortality and lessen the impact on egg production. Furthermore, it may provide an efficient protective measure without the disadvantages of egg contamination and the associated withdrawal period required after antibiotic treatment (81). Interesting insights on the importance of clinical models of infections to assess vaccine efficacy in the case of avian pathogenic *E. coli* (APEC) were reported by researchers in Denmark. The group studied AVV efficacy through challenge infections of field-vaccinated broiler breeder hens. Although similar animals and vaccine were used, efficacy was shown only when using an aerosol challenge model closely resembling the natural route of infection in comparison with an invasive challenge model that included injection into the salpinx following laparotomy of hens under general anesthesia. These results highlight the complexity of modeling field infections in standardized experimental settings, particularly in multifactorial bacterial disease (90,91). All cited studies assessing the autogenous model of immunization and challenge against APEC included oil-adjuvanted bacterins.

PASTEURELLA MULTOCIDA (FOWL CHOLERA)

Both commercial (live and inactivated) and autogenous (inactivated) vaccines are frequently used to reduce the economic losses associated with fowl cholera (92). The first report on vaccination using an attenuated vaccine dates back to 1881, when Louis Pasteur successfully immunized chickens against fowl cholera (93). Effective immunity against homologous challenge may also be established using an

inactivated vaccine, but cross-protection between different serovars is limited by differences in the lipopolysaccharide outer core structure (94,95). Vaccination alone may not satisfactorily prevent fowl cholera, as stress and environmental conditions may largely influence vaccine efficacy (96,97,98). Vaccination breakthroughs may appear after years and require comprehensive follow-up investigations to identify the reasons and possible consequences (98). Serovars present in an outbreak flock may persist or change over time (99). Regular serotyping and characterization of isolates present in a flock are essential, as best protection will be achieved when all relevant strains are present in an autogenous vaccine against fowl cholera (99).

RIEMERELLA ANATIPESTIFER

Riemerella anatipestifer is an important bacterial pathogen of water fowl, but it also affects other domestic and wild birds (92). Several serotypes have been described, and one or more serotypes may be isolated from disease outbreaks. The causative serotype, host age, and environmental factors are decisive for the severity of the disease (92). The use of autogenous inactivated vaccines against *R. anatipestifer* has been an essential preventive measure for years now. In general, the safety and efficacy of an autogenous vaccine against *R. anatipestifer* may be excellent if certain rules are followed (100). Effective homologous protection against experimental challenge after a single vaccination has been reported (101). Booster injections may further enhance immunity and reduce mortality (102). Use of an inactivated autogenous vaccine may significantly reduce mortality and pathologic lesions (103), but particular attention should be paid to strain selection to ensure the broadest possible protection against all circulating serotypes (102). The choice of the adjuvant may also be decisive for vaccine efficacy. Mineral-oil emulsions significantly reduced the number of lesions, whereas aluminum hydroxide-adjuvanted vaccines had no significant effect (100). Furthermore, the use of oil adjuvants was shown to provide a longer duration of immunity, which outweighs the risk of tissue reactions in most cases (102). Maternal antibodies were found in eggs from vaccinated ducks up to 3 mo postvaccination and titers were considered sufficient for at least 2 mo postvaccination. A booster injection might further elevate these antibody titers in the progeny, where a rather rapid decrease in maternal antibody titers was observed between 3 and 10 days pos hatch (104).

ORNITHOBACTERIUM RHINOTRACHEALE

Ornithobacterium rhinotracheale (ORT) is a respiratory disease agent that is responsible for economic losses in the poultry industry worldwide (105). Autogenous vaccines may help to reduce condemnation rates, mortality, and the use of antibiotics and to improve viability and performance in affected flocks efficiently (106,107). Thus, action plans may be implemented on affected farms to reduce the economic impact of ORT infections significantly. These action plans should be developed in close cooperation between farmers, veterinarians, and other entities to ensure consideration of all relevant measures: biosecurity, vaccination program, and regular updates of the vaccine based on continuous monitoring (106). As for all other bacterial and viral autogenous vaccines, the clinical relevance of the strains selected for the vaccine is crucial for its efficacy and homologous protection is the most efficient (108,109). In cases of mixed infections, it is also possible to include other bacteria, such as *E. coli*, in the autogenous vaccine. The use of combined products is a common practice in the field and does not necessarily limit the safety or efficacy of the vaccine (110). Vaccination of breeders may result in sufficient antibody titers to protect the offspring up to 4 wk posthatch (111). The best serologic response may be obtained by using mineral oil-adjuvanted bacteria (108). Immunization of the progenies may interfere with the presence of maternal antibodies, but the use of a rather strong adjuvant such as mineral oils may still result in sufficient protection (111).

AVIBACTERIUM PARAGALLINARUM (INFECTIOUS CORYZA)

The knowledge of circulating serovars is fundamental for autogenous vaccine efficacy leading to successful health management in flocks affected by infectious coryza. Whereas serovar-specific immunity may be very efficient, cross-protection is limited (112). Further, failure of commercial and autogenous vaccines has been repeatedly reported (113,114). In most cases, vaccine failure may be attributed to a certain serotype circulating in the field, such as serotype B, not being included in the vaccine (115,116). Immunogenicity may vary even between strains within serovar B, further complicating strain selection (117). Antibody titers may be established faster and reach higher levels when mineral oil instead of aluminum hydroxide is used as the adjuvant. However, the choice of the adjuvant is less decisive than the selection of strains that are clinically relevant (118). Different administration routes have been described, but the best results were obtained via intramuscular or subcutaneous injection (119,120).

AUTOGENOUS VACCINES—WHAT IS NEXT?

AVVs are successful products in European poultry, and they have recently obtained an improved regulatory positioning in the EU. This situation should encourage research and development of improved vaccines, and it may bring AVVs to the next technological level. Potential exists along several paths. New adjuvants may result in products that are safer for animals, users, and consumers of poultry products (see section on adjuvants). Mucosal adjuvants may pave ways for the mass application of inactivated antigens and allow for mass vaccination of poultry (88). Improved protocols for the inactivation of bacteria and viruses will be beneficial for antigen quality and more sustainable, as the use of toxic chemicals could be reduced (see section on inactivation).

Whole killed bacteria, as an antigen constituent of autogenous vaccines, contain reactogenic contaminants such as lipopolysaccharide endotoxin, DNA, or RNA (121). The use of purified antigens is safer than crude inactivated microorganisms, but improved safety with reduced reactogenicity can be related to weak immunogenicity (42). The purification protocol developed by Cox *et al.* provides a subunit vaccine based on siderophore receptor proteins of the bacterial cell membrane responsible for iron acquisition as one of the main virulence factors of *E. coli* (122). The vaccine showed efficacy in preventing mortality and peritonitis after APEC challenge infection. This novel technology can be applied under the conditions of autogenous vaccine manufacturing using strains from one epidemiological unit, and it has recently obtained a manufacturing authorization in Germany.

In summary, AVVs are likely to remain as a valuable complement to registered vaccines for the foreseeable future. Many of the EU countries are benefiting from their widespread use and the dual approach along with licensed products currently being integrated in the poultry health management (19). On a global scale, AVVs had a substantial impact valued at over $400 million in 2018, with an estimated compound annual growth rate of 5.5% by 2031 (123).

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