

CHAPTER 2.8.2.

ATROPHIC RHINITIS OF SWINE

SUMMARY

Atrophic rhinitis is an infectious disease of swine characterised by serous to mucopurulent nasal discharge, shortening or twisting of the snout, atrophy of the turbinate (conchal) bones and reduced productivity. It may occur enzootically or more sporadically, depending on a variety of factors including herd immunity. The most severe progressive form is caused by infection with toxigenic strains of Pasteurella multocida alone or in combination with Bordetella bronchiseptica. Infections with B. bronchiseptica alone can cause a mild to moderate form with nonprogressive turbinate bone atrophy. Turbinate atrophy may only be obvious at slaughter or may be detected in the live animal by use of radiography or tomography. Environmental and management factors also contribute to the severity and incidence of this disease. A large proportion of apparently normal pig herds may be infected with B. bronchiseptica or nontoxigenic P. multocida and show a mild degree or low prevalence of turbinate atrophy.

Identification of the agents: *The diagnosis of atrophic rhinitis depends on clinical and post-mortem observations in affected swine assisted by the recovery and characterisation of P. multocida and B. bronchiseptica. The isolation of both organisms is often complicated by the more profuse growth of other bacteria. Isolation rates are improved by preservation of the nasal or tonsillar swab at 4–8°C in a non-nutritive transport medium and by using a selective culture medium.*

Pasteurella multocida and B. bronchiseptica can be identified by traditional biochemical tests. Pasteurella multocida isolates may be further characterised by their capsular and somatic antigens. Capsular type D is most prevalent in many areas of the world, but in some regions type A predominates. Capsular antigens may be distinguished serologically by indirect haemagglutination or immunofluorescence, chemically by flocculation in acriflavine, or by susceptibility to hyaluronidase. Somatic antigen types can be distinguished by a gel diffusion precipitation test, with type 3 found most frequently in swine. Toxigenicity of P. multocida isolates can be demonstrated by testing for cytotoxicity in cultured cells. A commercially available toxin-specific enzyme-linked immunosorbent assay (ELISA) is now widely used in some areas of the world to differentiate toxigenic from nontoxigenic isolates. In addition, it is suitable for detection of toxin production by bacteria from primary culture plates without the need for prior isolation and identification of individual colonies.

Assays based on the use of DNA probes or polymerase chain reaction (PCR) provide rapid, sensitive and highly specific detection of B. bronchiseptica and both toxigenic and nontoxigenic P. multocida for those laboratories with the capability to perform them. A multiplex PCR useful for capsular typing of P. multocida has also been described.

Serological tests: *Detection of antibodies to P. multocida and B. bronchiseptica is of little value as nontoxigenic strains of P. multocida share cross-reactive antigens with toxigenic strains and B. bronchiseptica can be isolated from many swine herds. A test based on detection of antibodies to the P. multocida toxin is commercially available but its usefulness is limited as not all infected swine develop such antibodies. Widespread vaccination with P. multocida toxoid induces antibodies of vaccinal origin, complicating interpretation of results.*

Requirements for vaccines and diagnostic biologicals: *Several vaccines are available commercially that contain bacterins of B. bronchiseptica and a mixture of toxigenic and/or nontoxigenic strains of P. multocida, or a toxoid derived from P. multocida or from a recombinant Escherichia coli.*

A. INTRODUCTION

Atrophic rhinitis is an infectious disease of swine characterised by stunted development or deformation of the nasal turbinate (conchal bone) and septum.

1. Causal pathogens

Two forms of atrophic rhinitis have been recognised, depending on the causal agent(s) (De Jong, 2006):

- i) A severe progressive form caused by toxigenic isolates of *Pasteurella multocida*, most commonly capsular types D or A, alone or in combination with *Bordetella bronchiseptica*.
- ii) A less severe, nonprogressive form with mild to moderate turbinate atrophy, often without significant snout changes, caused by *B. bronchiseptica*.

2. Description of the disease

Initial clinical signs include sneezing, snuffling and eye discharge with resultant dark tear-staining and subsequent nasal discharge, which can vary from serous to mucopurulent; in some cases pigs may show epistaxis. Atrophy of the nasal turbinate and septal deviation may lead to shortening or twisting of the snout and, in severe cases, difficulty in eating. Increased severity is associated with overstocking and poor management, housing and environmental conditions. Reduced productivity is generally associated with moderate to severe atrophic rhinitis, although the precise relationship between infection with these bacteria and reduced weight gains has not been thoroughly elucidated.

Bordetella bronchiseptica or toxigenic *P. multocida* may be present in a herd without clinical evidence of disease, especially when other respiratory pathogens are absent and environmental and management conditions are optimal. Such carrier herds pose a risk of transmitting these agents to other herds in which progression to severe disease may occur. *Bordetella bronchiseptica* and toxigenic *P. multocida* are commonly found in many domesticated and wild animal species that could potentially transmit the bacteria to swine herds.

3. Zoonotic risk and biosafety requirements

Infection of humans by *B. bronchiseptica* is rare and occurs most often in immunocompromised persons exposed to infected or vaccinated pets; transmission from swine to humans has not been reported. *Pasteurella multocida* can be a serious human pathogen but most zoonotic infections are associated with exposure to pets or wild animals. *Pasteurella multocida* has also been frequently isolated from healthy human carriers working in swine production sites or living nearby and has additionally been associated with chronic or acute respiratory disease in such individuals (Donnio *et al.*, 1999; Marois *et al.*, 2009). Transmission occurs primarily through bites or scratches and wound contamination from infected material, but may also result from inhalation of aerosols. Appropriate precautions should be observed by persons having contact with swine infected with *P. multocida*, particularly those who may be immunocompromised. Containment level 2 practices are recommended for handling clinical specimens and cultures of *B. bronchiseptica* and *P. multocida*.

4. Differential diagnosis

Porcine cytomegalovirus also causes rhinitis in young pigs but does not progress to turbinate atrophy or nasal distortion. Asymmetric bone development resulting from habitual biting or chewing of stalls or drinkers may lead to a noticeable misalignment of the jaw in some pigs. Careful inspection can distinguish this condition from shortness or deviation of the snout characteristic of atrophic rhinitis.

B. DIAGNOSTIC TECHNIQUES

The diagnosis of atrophic rhinitis depends on clinical, pathological and microbiological investigations, with the latter being particularly important for herds infected subclinically. It is generally accepted that a herd in which toxigenic *P. multocida* is present be defined as affected with progressive atrophic rhinitis, whether or not clinical signs of the disease are evident (Pedersen *et al.*, 1988). Control in many countries has, therefore, centred on detection of infection, even in subclinically infected animals considered to be potential carriers.

1. Pathological diagnostic criteria

Turbinate atrophy may only be seen at slaughter when snout sections at the level of the first/second premolar tooth are examined. Subjective assessment of turbinate atrophy is convenient and often useful for monitoring herds (De Jong, 2006), but objective scales of measurement (Gatlin *et al.*, 1996) are best suited for studies requiring data analysis. Radiography (Done, 1976) and tomography (Magyar *et al.*, 2003) can provide objective observations from live animals; tomography reveals not only severe lesions but more subtle changes that may not be apparent by radiography. However, these techniques are of limited use due to the equipment and expertise required. Diagnosis is assisted by detection of characteristic histopathological features including fibrous replacement of the bony plates of the ventral conchae with varying degrees of inflammatory and reparative changes.

2. Identification of the agents

2.1. *In vitro* culture

As *P. multocida* preferentially colonises the tonsil, tonsillar swabs or biopsies will provide the highest isolation rates (Ackermann *et al.*, 1994). Nasal swabs are preferred for isolation of *B. bronchiseptica*. When sampling the tonsil is not practical, nasal swabs suffice for isolation of both organisms. Swabs with flexible shafts should be used; sample collection in young pigs is facilitated by the use of mini-tipped swabs. A single swab is used to sample both sides of the nasal cavity and should then be placed in a non-nutritive transport medium (e.g. phosphate buffered saline) and kept at 4–8°C during transit to avoid overgrowth by other faster-growing bacteria. Transit time should not exceed 24 hours.

Although both *P. multocida* and *B. bronchiseptica* grow readily on blood agar, a selective medium is preferred as overgrowth of other bacteria that are present in higher numbers often interferes with their detection. An additional difficulty related to *B. bronchiseptica* is that this organism grows more slowly than most other bacteria present in clinical samples. Various formulations of media containing antibiotics have been used for isolation of *P. multocida*, but comparison of studies in the literature indicates that the highest isolation rates are obtained with modified Knight medium (bovine blood agar containing 5 µg/ml clindamycin, 0.75 µg/ml gentamicin) (Lariviere *et al.*, 1993) or KPMD (bovine blood agar containing 3.75 U/ml bacitracin, 5 µg/ml clindamycin, 0.75 µg/ml gentamicin, and 2.25 µg/ml amphotericin B) (Ackermann *et al.*, 1994). MacConkey agar with 1% glucose and 20 µg/ml furaltadone is used by many laboratories for selective growth of *B. bronchiseptica* from nasal swabs, but a modified Smith-Baskerville medium (a peptone agar formulation containing 20 µg/ml penicillin, 20 µg/ml furaltadone, and 0.5 µg/ml gentamicin) appears superior, especially when the number of *B. bronchiseptica* present is low (Lariviere *et al.*, 1993; Smith & Baskerville, 1979). A further improvement in isolation rate was reported using blood agar containing 40 µg/ml cephalexin (Lariviere *et al.*, 1993). A selective blood agar medium for simultaneous isolation of both *P. multocida* and *B. bronchiseptica*, containing 5 mg/litre clindamycin-HCl, 0.75 mg/litre gentamicin sulphate, 2.5 mg/litre K-tellurite, 5 mg/litre amphotericin-B and 15 mg/litre bacitracin, has also been described (De Jong & Borst, 1985). However, it should be noted that K-tellurite has sometimes been found to be inhibitory to the growth of type D *P. multocida* (Lariviere *et al.*, 1993).

2.2. Biochemical characteristics

Pasteurella multocida is a Gram-negative, bipolar, pleomorphic rod and forms nonhaemolytic, greyish colonies on blood agar with a characteristic, 'sweetish' odour. It fails to grow on MacConkey agar but yields positive oxidase and catalase reactions and produces indole.

Bordetella bronchiseptica is also a Gram-negative rod, forming convex colonies 1–2 mm in size, usually haemolytic, on blood agar or Bordet-Gengou medium after 48 hours of growth. It is nonfermentative, but positive for oxidase, catalase, citrate and urea and grows in 6.5% NaCl.

Agglutination tests using specific antisera have been described for confirming the identity of presumptive *B. bronchiseptica* isolates but appropriate sera are not widely available for use.

2.2.1. Capsular typing of *P. multocida*

Capsular typing of *P. multocida* is useful for epidemiological purposes, as *P. multocida* often has a mucoid capsule. Serotyping by indirect haemagglutination has traditionally been used (Carter, 1955) but only a few laboratories throughout the world make and maintain the antisera required. However, simpler chemical methods can usually distinguish most swine isolates. Those producing a type D capsule form a heavy flocculate in 1/1000 aqueous acriflavine (Carter & Subronto, 1973), while capsular type A strains can be identified by inhibition of growth in the

presence of hyaluronidase (Carter & Rundell, 1975). A small proportion of swine isolates are noncapsulated.

2.2.2. Acriflavine test procedure for capsular type D *P. multocida*

- i) Inoculate a tube containing 3 ml of brain–heart infusion broth, using a freshly grown bovine blood agar culture, for each *P. multocida* isolate to be tested. Include a known type D strain and a known type A strain as positive and negative controls.
- ii) Incubate inoculated tubes at 37°C for 18–24 hours.
- iii) Pellet bacteria by centrifugation and remove 2.5 ml of the supernatant.
- iv) Add 0.5 ml of a 1/1000 aqueous solution of acriflavine neutral. Acriflavine solution should be freshly prepared each week and stored at 4°C, protected from light.
- v) Mix to resuspend the bacterial pellet and incubate the tube at room temperature, without shaking.
- vi) Observe at 5 minutes for the presence of a heavy flocculent precipitate.

2.2.3. Hyaluronidase test procedure for capsular type A *P. multocida*

- i) Prepare fresh bovine blood agar cultures of the isolates to be tested. Include a known type A strain and a known type D strain as positive and negative controls.
- ii) Inoculate each strain to be examined on a separate trypticase soy blood agar plate with 5% sheep blood or 6% bovine blood by streaking several parallel lines of growth, approximately 3–5 mm apart, across the diameter of the plate. For maximum production of hyaluronic acid it is important that the plates be fresh and not dehydrated.
- iii) Heavily streak a hyaluronidase-producing strain of *Staphylococcus aureus* at right angles to the lines of *P. multocida* growth.
- iv) Incubate the plates at 37°C, in a humidified atmosphere, and observe periodically for up to 24 hours. Type A strains will exhibit a marked inhibition of growth in the region adjacent to the growth lines of *S. aureus*.

2.2.4. Somatic antigen typing of *P. multocida*

Differences in the cell wall lipopolysaccharide among *P. multocida* strains provide the basis for somatic antigen typing. Sixteen types can be distinguished by a gel diffusion precipitation test (Heddlestone *et al.*, 1972), with type 3 found most frequently in swine. Although the required antisera are not widely available, many reference laboratories and some diagnostic laboratories offer somatic antigen typing.

2.2.5. Detection of the *P. multocida* toxin

Diagnosis of progressive atrophic rhinitis depends upon characterisation of *P. multocida* isolates as toxigenic. The heat-labile toxin of *P. multocida* produces dermonecrosis in guinea pigs and is lethal in mice following intraperitoneal injection. Toxigenicity can also be demonstrated *in vitro* by testing for cytopathic effects on monolayers of embryonic bovine lung (EBL) cells (Rutter & Luther, 1984), African green monkey kidney (Vero) cells (Pennings & Storm, 1984) or bovine turbinate cells (Eamens *et al.*, 1988). The bacteria are grown in brain–heart infusion broth incubated at 37°C for 24 hours and then pelleted by centrifugation. The supernatant is sterilised by filtration and titrated in monolayer cultures prepared in microtitre plates. Following incubation at 37°C for 2–3 days, the monolayers are stained with crystal violet and examined microscopically to detect cytopathic effects. A rapid cell culture test, in which the suspect colonies are grown on an agar overlay of EBL cells (Chanter *et al.*, 1986), permits more efficient analysis of large numbers of isolates.

An enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies can detect the toxin in mixtures of bacteria recovered from primary isolation media (Foged, 1992). This is an important advantage as swine may be colonised simultaneously with a mixture of toxigenic and nontoxigenic strains (Ackermann *et al.*, 1994; De Jong, 2006). Cell culture methods would require every colony of *P. multocida* in the sample to be tested, which is clearly impractical, to achieve the same level of sensitivity as the ELISA.

This ELISA is commercially available throughout Europe and in a few other selected regions of the world¹ (though not in the United States of America) and has been widely adopted in many areas as the preferred test for identification of carriers and for control of progressive atrophic rhinitis. Though highly specific, a positive result without previous history of disease or suspicious signs should be thoroughly investigated to recover toxigenic isolates from the animals sampled.

2.3. Molecular methods

Colony morphology and biochemical testing remain the basis for identification of toxigenic *P. multocida* and *B. bronchiseptica* in many laboratories. However, a number of assays based on the use of DNA probes (Kamps *et al.*, 1990; Register *et al.*, 1998) or polymerase chain reaction (PCR) (Kamp *et al.*, 1996; Lichtensteiger *et al.*, 1996; Nagai *et al.*, 1994; Register & DeJong, 2006) for detection of toxigenic *P. multocida* and/or *B. bronchiseptica* from swine provide faster, more specific and more sensitive diagnostic tools. Diagnostic laboratories are increasingly using PCR for identification of these agents as the equipment and expertise become more readily available. Proper in-house validation with known controls as well as standardised, ongoing quality control measures are essential (see Chapter 1.1.6 *Principles and methods of validation of diagnostic assays for infectious diseases*).

A multiplex PCR assay for capsular typing of *P. multocida* (Townsend *et al.*, 2001) appears to provide more reliable results than phenotypic methods and is frequently used in suitably equipped diagnostic laboratories.

Various DNA fingerprinting techniques, including restriction endonuclease analysis (REA), ribotyping, pulsed-field gel electrophoresis, and PCR-based methods have been evaluated by numerous groups for the purpose of differentiating *P. multocida* isolates. Few direct comparisons between methods have been carried out using strains from pigs with atrophic rhinitis, but REA currently appears to be the method of choice for epidemiologic investigations as it provides a high level of discrimination without the need for specialised equipment or reagents (Djordjevic *et al.*, 1998; Gardner *et al.*, 1994; Harel *et al.*, 1990).

3. Serological tests

At present there are no satisfactory serological tests that can be relied on to detect those animals infected with toxigenic *P. multocida* and capable of developing or spreading the disease. Detection of antibodies to *P. multocida* is not helpful, as nontoxigenic strains share many cross-reacting antigens with toxigenic strains. An ELISA for detection of antibodies to the *P. multocida* toxin has been described (Foged, 1992) that is commercially available in Europe and a few other areas of the world (see footnote 1). However, many animals infected with toxigenic *P. multocida* fail to produce antibodies to the toxin, and widespread use of toxoid-containing vaccines limits the diagnostic value of this ELISA to herds with no history of vaccination or to detection of vaccine response in vaccinated herds.

Infection with *B. bronchiseptica* can be detected serologically by agglutination testing with formalin-treated bacteria or with a more sensitive ELISA (Venier *et al.*, 1984). Unless monitoring the status of a negative herd, *B. bronchiseptica* serology may be of little value as the organism is present in many apparently healthy pig herds.

C. REQUIREMENTS FOR VACCINES

1. Background

There are several commercially available vaccines that contain whole-cell bacterins of *B. bronchiseptica* combined with a toxigenic *P. multocida* bacterin and/or a *P. multocida* toxoid. The toxigenic *P. multocida* bacterin component is most often capsular type D but some vaccines additionally include a type A strain, which may be toxigenic or nontoxigenic. Live, attenuated *B. bronchiseptica* vaccines are also available. Vaccines containing only *B. bronchiseptica* are not suitable for control of progressive atrophic rhinitis, but may be of benefit in herds with the nonprogressive form. *Pasteurella multocida* and *B. bronchiseptica* vaccines appear to reduce the level of colonisation by these bacteria, but do not eliminate them or prevent infection. Most commercially available vaccines contain either an oil adjuvant or aluminium hydroxide gel.

The *P. multocida* toxin is the single most important protective antigen with respect to progressive atrophic rhinitis. Vaccines based on a *P. multocida* toxoid offer specific protection against the action of the toxin, which, by itself,

¹ For availability/purchase information contact DakoCytomation Denmark A/S, Produktionsvej 42, DK-2600 Glostrup, Denmark; <http://www.dakocytomation.com>.

can be used to reproduce all of the major signs of this disease (see Foged, 1992 for review). The level of toxin produced by *P. multocida* is relatively low and the toxin-specific antibody response induced by bacterin-only vaccines may not be optimal. Purified toxoid (inactivated by formaldehyde) is more immunogenic than crude toxoid and the immunogenicity of the inactivated form is not affected by mixture with a *B. bronchiseptica* bacterin. However, the difficulty and expense of large-scale purification from cultures of *P. multocida* prevent routine incorporation of native, chemically inactivated toxoid into vaccines. Recombinant vaccines containing subunit toxin proteins or genetically detoxified derivatives of the full-length toxin are highly efficacious and less costly to produce (Hsuan *et al.*, 2009; Pejsak *et al.*, 1994). A DNA vaccine encoding a full-length but enzymatically inactive toxoid was shown to be highly immunogenic in pigs but has so far not been evaluated for efficacy against challenge (Register *et al.*, 2007).

Bordetella bronchiseptica produces a variety of toxins and adhesins that are potential virulence factors in swine. Only one, the outer membrane protein pertactin, has been shown to protect against disease in pigs (Kobisch & Novotny, 1990). Despite this fact, a dermonecrotic toxin produced by *B. bronchiseptica*, unique from the toxin produced by *P. multocida*, has traditionally been regarded as the primary virulence factor and protective immunogen in swine (De Jong, 2006). Several studies strongly implicate the toxin as a virulence factor and it undoubtedly plays a role in pathogenesis and, perhaps, in protection. However, the role of pertactin and several additional virulence factors in protective immunity is most likely equal to, or perhaps exceeds, that of the toxin.

Bordetella bronchiseptica is subject to phenotypic variation under certain growth conditions (e.g. temperatures below 37°C or the presence of chemical modulators such as MgSO₄ or nicotinic acid), in which production of most virulence factors is reversibly turned off. Spontaneously occurring mutants, permanently unable to produce most virulence factors, also arise with a low frequency during culture. Careful attention to colony morphology, on an area of the plate with well-separated colonies, is essential to retain cultures in the phase I (also known as Bvg⁺), or virulent, mode. Phase I colonies are small (1–2 mm in diameter), domed, and haemolytic on blood agar. Loss of haemolysis and the appearance of larger, flat colonies indicate conversion to the avirulent form. Whenever possible, cultures should be propagated using single haemolytic colonies to minimise the slow accumulation of avirulent clones within the culture.

2. Outline of production and minimum requirements for conventional vaccines

2.1. Characteristics of the seed

2.1.1. Biological characteristics

The seed-lot system should be employed for the bacterial strains used to prepare whole-cell bacterins, as well as for the strains from which purified antigens are derived.

In the case of whole-cell bacterins, the origin and history of both the *P. multocida* and *B. bronchiseptica* strains should be described and the full characterisation of the master seeds should be laid down in a master seed batch protocol. *Bordetella bronchiseptica* used for vaccine production should be a phase I virulent culture and the *P. multocida* isolates used should be toxigenic.

Working seeds used for vaccine production should be derived from the master seed and checked for all relevant properties as described in the master seed batch protocol.

2.1.2. Quality criteria

Both the master seed and the working seed must be pure cultures, free from bacterial, mycotic, mycoplasmal and viral contamination.

Identity of the bacterial species and the production of relevant antigens should be confirmed.

2.2. Method of manufacture

2.2.1. Procedure

Precise details of standards for the production of effective commercial vaccines are not available, but they are known to contain 10¹⁰ cells of formalin-killed *B. bronchiseptica* and 10 µg of *P. multocida* toxoid per dose. *Bordetella bronchiseptica* should be confirmed to be a phase I culture and, for *P. multocida*, it should be confirmed that the culture contains sufficient levels of toxin. A defined number of passages should be used to give the production culture. *Bordetella bronchiseptica* cells, and either *P. multocida* cells and/or toxin, are inactivated, detoxified and formulated with an adjuvant. As the toxin of *P. multocida* has an intracellular location and is released on cell lysis during the stationary phase, the culture supernatant should be harvested approximately 48 hours after the end of the exponential phase of growth.

2.2.2. Requirements for media

All bacterial strains should be propagated in media that support efficient growth and allow optimal expression of the antigens that are relevant for the induction of protective antibodies.

2.2.3. In-process controls

Seed and production cultures are inoculated on blood agar plates and incubated. No nonspecific colonies should grow on these plates.

Cultures are inactivated with formaldehyde. Tests are performed to check the effectiveness of the inactivation process and to test for residual formaldehyde.

Quantification of antigens is carried out by performing a total cell count using a bacterial counting chamber for enumerating whole cells or an antigenic mass determination for defined antigens, e.g. *P. multocida* toxin, by quantitative enzyme immunoassay.

2.2.4. Final product batch tests

Every batch of vaccine should be tested for sterility according to standard methods (see Chapter 1.1.9 *Tests of biological materials for sterility and freedom from contamination*) described in the European Pharmacopoeia or the United States Code of Federal Regulations.

Every batch of vaccine should be tested for safety in the target animal, by giving a double dose by the recommended route of vaccination, and a second, single dose 2 weeks later. No abnormal local or systemic reactions should occur. When a preservative is used, the concentration should be measured for each batch. It must not exceed the maximum permitted level.

Every batch of vaccine should be tested for potency using a validated serological test that correlates with the protection obtained in the efficacy experiment, as described under Section C.2.3.2. The potency test is not necessarily carried out in the target animal – mice or rabbits can be used. In these latter cases, correlation has to be shown with protective antibody levels in the target animal.

2.3. Requirements for authorisation

2.3.1. Safety requirements

Although inactivation of the bacterial cultures by a validated method is a standard procedure, both bacterial species produce dermonecrotic toxins; detoxification of these toxins should be confirmed when toxoids are used as vaccine components. Standard safety tests for inactivated vaccines should be carried out (see Chapter 1.1.8 *Principles of veterinary vaccine production*).

When an oil emulsion is used as the adjuvant, accidental injection of the operator can cause a severe local reaction. Medical attention should be sought immediately, treating the wound as a grease-gun injury.

2.3.2. Efficacy requirements

Normally the vaccine is applied during the late stage of pregnancy, so that progeny will be protected by the uptake of colostral antibodies. The efficacy of a trial vaccine should be measured by vaccinating groups of pregnant sows. Their progeny should be challenged by virulent cultures of *B. bronchiseptica* and toxin-producing *P. multocida*. Significant protection should be obtained against the clinical signs of the progressive form of atrophic rhinitis, i.e. turbinate atrophy. The clinical signs induced in the controls and vaccinates may be compared according to the scoring system of Done (1976).

When the vaccine may be applied irrespective of the stage of pregnancy, duration of immunity should be at least 6 months, so that booster vaccinations twice a year should maintain effective antibody levels.

2.3.3. Stability

Every batch of vaccine should be subjected to an accelerated shelf-life test, which has been correlated with real-time shelf-life testing.

3. Vaccines based on biotechnology

A number of vaccines incorporating enzymatically inert *P. multocida* toxin subunits or whole toxin rendered inactive through mutation have been described and evaluated under experimental conditions. The recombinant proteins are generally expressed in *Escherichia coli* and purified prior to use. At present, only a few vaccines that include recombinant toxin or toxin subunits are commercially available; they may or may not include a *P. multocida* bacterin. Evidence so far suggests such vaccines elicit levels of toxin-specific antibody equal to or greater than those obtained with vaccines containing native, chemically inactivated toxoid.

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