Infectious Coryza: Overview of the Disease and New Diagnostic Options

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INTRODUCTION

Infectious coryza is an acute respiratory disease of chickens. The clinical syndrome has been recognized since the 1930s (5). The disease occurs worldwide and causes economic losses due to an increased number of culls and a marked (10% to more than 40%) drop in egg production, particularly on multi-age farms. Early workers identified the causative agent as "*Haemophilus gallinarum*," an organism that required both X (hemin) and V (NAD) factors for growth in vitro. However, from the 1960s to the 1980s, all isolates of the disease producing-agent have been shown to require only V factor and have been termed *Haemophilus paragallinarum* (5). As discussed in more detail below, V-factor-independent *H. paragallinarum* isolates have been encountered in the Republic of South Africa since 1989 (26). Thus, the causative agent of infectious coryza is regarded as *Haemophilus paragallinarum*, an organism that can be either V-factor dependent or independent.

This review covers information that has emerged in recent years and that emphasizes the complex nature of infectious coryza outbreaks in developing countries, where other disease agents and/or stress factors are important complicating factors. As well, the literature indicating that the phenotypic and serological nature of *H. paragallinarum* has changed to some degree in some geographical regions is covered. The impact of these changes on control and prevention measures is critically reviewed. In addition, the impact on some of these emerging issues of a new-generation diagnostic test based on the PCR technique is reviewed. Since there are several texts on the disease, the causative agent, and vaccines (2, 5, 8), this review covers only recent developments in detail.

CLINICAL DISEASE

Infectious coryza may occur in growing chickens and layers. The most common clinical signs are nasal discharge, facial swelling, lacrimation, anorexia, and diarrhea. Decreased feed and water consumption retards growth in young stock and reduces egg production in laying flocks (5). The potential impact of coryza on meat chickens has been emphasized by reports on economically important outbreaks in two states of the United States (17, 19).

Unusual clinical signs have been reported in the Americas. In both North and South America, outbreaks of coryza in which chickens have shown clinical signs more typical of a swollen-head-like syndrome have been reported (17, 30).

The vastly different nature of infectious coryza when complicated by other pathogens and stress factors has been demonstrated by reports from countries such as Argentina, India, Morocco, and Thailand. Unique clinical presentations such as arthritis and septicemia, presumably complicated by the presence of the other pathogens detected, such as *Mycoplasma gallisepticum*, *M. synoviae*, *Pasteurella* spp., *Salmonella* spp., and infectious bronchitis virus, have been found in broiler and layer flocks in Argentina (30). The isolation of *H. paragallinarum* from nonrespiratory sites such as the liver, kidney, and tarsus was reported for the first time in these outbreaks (30). In the Kurnool district of India, infectious coryza has been reported as the second most important bacterial disease associated with mortality after salmonellosis (32). A study in Morocco reported on 10 coryza outbreaks that were associated with drops in egg production of 14 to 41% and mortalities of 0.7 to 10% (36). A study of village chickens in Thailand has reported that infectious coryza was the most common cause of death in chickens less than 2 months old and those over 6 months old (36). Only in village chickens between 2 and 6 months old did other diseases, specifically Newcastle disease and pasteurellosis, cause more deaths than coryza (36). Overall, these reports emphasize that the clinical signs and economic impact of the complicated coryza infections seen in developing countries can be markedly different from those in the uncomplicated infections typically seen in developed countries.

"EMERGING" OR VARIANT SEROVARS

As a brief background to the issue of "emerging" or variant serovars, it is important to understand that two different but

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related serotyping schemes for *H. paragallinarum* have been mainly used—the Page (29) and the Kume (23) schemes.

The Page scheme was initially developed by using a plate or slide agglutination test to recognize the three serovars, A, B, and C (29). However, the use of hemagglutination-inhibition (HI) technology has been shown to be a much better method for identifying the Page serovar of field isolates of *H. paragallinarum* (3). It is widely accepted that the three Page serovars represent distinct "immunovars," since inactivated vaccines based on any one Page serovar provide no protection against the other two Page serovars (5). It is generally accepted that cross-protection occurs within a Page serovar (5).

The Kume serotyping scheme was originally based on hemagglutination-inhibition tests that recognized seven serovars organized into three serogroups termed I, II, and III (23). Subsequent publications have reported the existence of two further serovars and the recognition that the three Kume serogroups correspond to the three Page serovars (4). Hence, the reorganised Kume scheme now recognizes three serogroups (termed A, B, and C) which correspond to the Page serovars, with four serovars being recognized within both Kume serogroups A and C (4). It is important to understand that the Kume serotyping scheme is a complex and technically demanding system (5) and that no laboratory around the world appears to currently perform full Kume serotyping. While the definitive cross-protection experiments for all four serovars within both Kume serogroups A and C have not yet been performed, the accepted dogma is that serovars within a Kume serogroup are cross-protective (5).

These accepted dogmas on cross-protection within Page serovars and Kume serogroups have been challenged recently by the emergence of "variant" or unusual serovars. In both Argentina and Brazil, around 40% of the Page serovar A isolates examined to date are not recognized by a monoclonal antibody specific for this serovar (6, 34). These monoclonal antibody-negative isolates, which have not been recorded anywhere else in the world, have not been examined by the Kume scheme. It has been speculated that these "variant" Page serovar A isolates may be sufficiently different from typical serovar A vaccine strains that vaccine failures may occur (34).

There is evidence that Argentinian serovar B isolates are quite genetically distinct from all other *H. paragallinarum* isolates, regardless of serovar (10). This has led to speculation that the unique nature of these Argentinian serovar B isolates may mean that commercial vaccines based on "typical" serovar B isolates from North America or Europe may not provide protection (35).

There is some evidence to support this speculation about antigenic diversity in Page serovar B. Bivalent vaccines based on Page serovars A and C provide protection against Page serovar B strain Spross but not against two South African isolates of Page serovar B (39). Furthermore, there is only partial cross-protection within various strains of Page serovar B (39). While the Kume serotyping scheme recognises only one serovar, B-1 (4), this should not be regarded as evidence of antigenic homogeneity. Rather, it is a reflection that only a small number of Page serovar B isolates have been examined by the Kume serotyping scheme. It is highly likely that further serovars would be recognized within Kume serogroup B if a collection of such isolates were studied.

There has been evidence of a dramatic shift in the incidence of serovars of *H. paragallinarum* in South Africa in recent years. Bragg et al. (11) have reported on the serovars of *H. paragallinarum* during the 1970s, 1980s, and 1990s. Using a partial Kume serotyping scheme, they reported that Kume serovar C-3 has emerged as the dominant serovar in recent times. The incidence of Kume serovar C-3 has increased from 30% in the 1970s to over 70% in the early 1990s (11). This emergence of Kume serovar C-3 has occurred at a time when infectious coryza has remained an important and widespread disease, despite the extensive use of commercial vaccines (11). Bragg et al. (11) have suggested that the apparent failure of the commercial vaccines in South Africa (none of which contain Kume serovar C-3) has occurred because the dominant serovar in the field is Kume serovar C-3. They have speculated that the Kume serovar C-3 isolates are so antigenically distinct from the other Kume C serovars (C-1 and C-2) included in commercial vaccines that cross-protection is limited (11).

Overall, there have been a number of reports suggesting that serologically "variant" *H. paragallinarum* isolations may be causing vaccine failures. However, there have been no reports based on definitive evidence from vaccination challenge trials to support these suggestions. There is a need for such work, including work examining the level of cross-protection within Kume serogroups A and C.

NAD-INDEPENDENT *H. PARAGALLINARUM*

In 1989, isolates of an apparently new bacterium (causing a clinical disease identical to infectious coryza) were obtained from South African chickens (20). While these isolates did not require V-factor, they were shown by DNA techniques to be typical *H. paragallinarum* (26). The vast majority of the NAD-independent isolates are Page serovar A (12, 25), although a recent report has shown that some isolates are Page serovar C (12). A representative collection of the Page serovar A NAD-dependent *H. paragallinarum* isolates have been shown to share a unique DNA fingerprint, suggesting that they are clonal in nature and may have arisen from a point source (25).

The emergence of NAD-independent *H. paragallinarum* has had a significant impact in South Africa. In the Kwazulu-Natal region of South Africa, NAD-independent *H. paragallinarum* isolates are now more common than classic *H. paragallinarum*. As an example, the ratio of classic *H. paragallinarum* to NAD-independent *H. paragallinarum* isolates has gone from 1:1.4 in 1989 to 1:9.8 in 1993 (20).

Horner et al. (20) have also suggested that the NAD-independent isolates may cause air sacculitis more commonly than the classic *H. paragallinarum* isolates do. Furthermore, there has been speculation that the NAD-independent isolates may be sufficiently different to cause failures with vaccines based on traditional NAD-dependent *H. paragallinarum* (12, 20). Definitive cross-protection trials are needed to determine if this is the case.

DIFFERENTIAL DIAGNOSIS: ROLE OF VARIANT BACTERIA

In recent years, a number of new or "variant" bacteria have been recognized as being present in poultry that have made it more difficult to confidently diagnose infectious coryza.

In the early 1990s, a new bacterium was isolated from South African broilers showing mild respiratory problems and poor growth. It was not until 1994 that the organism was classified as *Ornithobacterium rhinotracheale* (37). The organism is present in Europe (1) and the United States (16, 28). While there is still some dispute, there is evidence that *O. rhinotracheale* can cause growth retardation after intra-airsac administration and growth retardation, air sacculitis, and pneumonia after aerosol administration in both chickens and turkeys (38). For the purpose of this review, the disease associated with *O. rhinotracheale* will be termed ornithobacterosis. A recent molecular study

TABLE 1. Distinguishing properties of the avian haemophili and *O. rhinotracheale*[a]

Property	*O. rhinotracheale*	*H. paragallinarum*	*P. avium*	*P. volantium*	*Pasteurella* sp. taxon A
Catalase	−	−	+	+	+
ODC[b]	−	−	−	V	−
β-Galactosidase	+	+	−	+	V
Acid from:					
Arabinose	−	−	−	−	+
Galactose	+	−	+	+	+
Maltose	+	+	−	+	V
Mannitol	−	+	−	+	V
Sorbitol	−	V	−	V	−
Sucrose	−	V	+	+	+
Trehalose	−	−	+	+	+

[a] All species are gram-negative rods. *H. paragallinarum*, *P. volantium*, *P. avium*, and *Pasteurella* sp. strain A are variable in their requirement for V factor for growth in vitro. *O. rhinotracheale* does not require V factor. +, positive (>90%); −, negative (>90%); V, variable reaction.
[b] ODC, ornithine decarboxylase.

has suggested that isolates of *O. rhinotracheale* from commercial poultry are a small group of closely related clones, indicating that possibly this organism was only recently introduced from wild bird populations (1).

The generally accepted clinical picture associated with both infectious coryza and ornithobacterosis indicates that most authorities believe that two diseases should not present similar clinical signs. However, a recent study from South Africa (12) reported that a total of 40 *O. rhinotracheale* isolates were obtained from the sinuses of chickens showing clinical signs similar to those associated with infectious coryza. Hence, laboratories need to be prepared to consider infectious coryza and ornithobacterosis in the differential diagnosis of chickens with mild upper respiratory tract disease.

In most countries, differentiation between *H. paragallinarum* and *O. rhinotracheale* is not difficult because classic *H. paragallinarum* shows NAD dependency while *O. rhinotracheale* is independent of any requirement for NAD. However, in areas where NAD-independent *H. paragallinarum* is known to exist (and this is limited to South Africa at the moment), differentiation of *O. rhinotracheale* and NAD-independent *H. paragallinarum* requires that carbohydrate fermentation patterns be determined. Diagnostic microbiologists must be aware of the biochemical properties of *H. paragallinarum* and be prepared to recognize *H. paragallinarum* on the basis of biochemical properties, even though the isolate may be NAD independent.

Another group of "variant" organisms that can cause difficulty in correctly diagnosing infectious coryza are the organisms once known as "*Haemophilus avium*," nonpathogenic avian *Haemophilus* strains that were formally recognized in the 1970s (18). DNA hybridization studies have shown that "*H. avium*" consists of three DNA homology groups, and these three new species being placed in the genus *Pasteurella* as *P. volantium*, *P. avium*, and *Pasteurella* sp. taxon A (27). Until recently, all isolates of these three taxa obtained from chickens were NAD dependent. However, Bragg et al. in South Africa have described NAD-independent isolates of all three taxa (12). A further complication is that these aberrant or "variant" organisms were obtained from chickens showing clinical signs similar to those of infectious coryza (12). However, these organisms are generally accepted as not causing any disease, suggesting that it is important to ensure that accepted pathogens such as *H. paragallinarum* and *O. rhinotracheale* are not missed while nonpathogens are isolated and identified.

Hence, chickens with clinical signs suggestive of infectious coryza may yield the following organisms: *H. paragallinarum*,

both NAD dependent and NAD independent; *O. rhinotracheale*, NAD independent; *P. volantium*, both NAD dependent and NAD independent; *P. avium*, both NAD dependent and NAD independent; and *Pasteurella* sp. taxon A, both NAD dependent and NAD independent.

Of these five bacteria, only *H. paragallinarum* is universally recognized as a pathogen. Of the other four, three (the *Pasteurella* species) are generally accepted to be commensal organisms that do not cause disease in chickens; there is no consensus on the pathogenic potential of *O. rhinotracheale*.

The correct and confident identification of bacteria isolated from chickens showing mild clinical signs of upper respiratory tract disease is an absolute requirement for the development, application, and monitoring of sustainable prevention and control programs. A wrong diagnosis may result in the inappropriate use or even misuse of antibiotics or the incorrect adoption of a vaccination program that targets a disease that is not involved. Clearly, diagnostic laboratories dealing with chicken respiratory diseases now face a demanding task in isolating and correctly identifying the bacteria associated with these diseases.

DIAGNOSTIC OPTIONS

Traditional Phenotypic Identification

The traditional definitive method for the diagnosis of infectious coryza requires the isolation of the suspect bacterium and then an extensive biochemical characterization to confirm the identity of the isolate (5). This is a challenging set of requirements. *H. paragallinarum* is a fastidious, slow-growing organism. Hence, it is often overgrown by other, faster-growing commensals. Biochemical characterization requires the availability of specialized, expensive media that can support the growth of NAD-dependent bacteria; such media are often beyond the resources of diagnostic laboratories, particularly those in the developing countries where coryza remains a pressing problem. The emergence of NAD-independent *H. paragallinarum* as well as *O. rhinotracheale* and the NAD-independent isolates of *P. avium*, *P. volantium*, and *Pasteurella* sp. taxon A has greatly added to the complexity of the situation.

Table 1 lists the phenotypic tests that can be performed that allow the differentiation of *H. paragallinarum* from the *Pasteurella* organisms that can be found in chickens, as well as *O. rhinotracheale*. Details of the methods for performing these tests have been recently published (8).

Molecular Identification

There has been a recent significant improvement in the tools available to aid in the diagnosis of infectious coryza. A PCR test that is specific for *H. paragallinarum* has been developed (14). This test is rapid (results are available within 6 h compared with days for conventional techniques) and recognizes all *H. paragallinarum* isolates tested (14). Over 40 *H. paragallinarum* isolates were positive in the test, including the NAD-independent *H. paragallinarum* from South Africa and the variant Page serovar A isolates and the unusual Page serovar B isolates from Argentina (14). In addition, this PCR, termed the HP-2 PCR, has given negative reactions with many closely related bacteria. In particular, the NAD-dependent forms of *P. volantium*, *P. avium*, and *Pasteurella* spp. as well as *O. rhinotracheale* give a negative reaction in this PCR (24). This PCR was developed by a random-cloning method, and there is no knowledge of the role, if any, of the target, which has a size of 0.5 kb (14).

When used directly on sinus swabs obtained from artificially infected chickens in pen trials performed in Australia, the HP-2 PCR was equivalent to culture in accuracy but was much more rapid (14).

While the HP-2 PCR was originally developed in Australia, it has now been successfully transferred to China. In comparing traditional culture and the HP-2 PCR in China, it has been shown that the PCR outperforms traditional culture when used on routine diagnostic submissions (13). The PCR test and traditional culture were used in parallel to investigate suspected infectious coryza outbreaks on eight commercial farms in China. The provisional diagnosis of infectious coryza was based on field diagnosis. Live chickens or chicken heads were then shipped from the field to the Beijing laboratory. Sinus swabs were collected and were examined directly by PCR as well as being cultured for *H. paragallinarum*. The HP-2 PCR detected 15 of 39 chickens as positive, with these 15 birds coming from six of eight farms, while culture detected only 8 of the 39 chickens as positive, with these birds coming from only four of the eight farms (13). On the two farms that had chickens that were positive by PCR but negative by culture, the chickens showed typical clinical signs, thereby providing further evidence that the culture results were false-negatives. The submitted chickens from the two farms that were negative by both culture and PCR did not show typical clinical signs of infectious coryza when received at the central laboratory (13). The problems of poor samples, delayed transport, and low-quality (but expensive) media mean that culture will have a higher failure rate in developing countries than in developed countries.

Recent work has shown the robust nature of the HP-2 PCR. In further work performed in China, it has been shown that samples can be stored for up to 180 days at 4 or −20°C and the majority of known positive samples will remain positive in the PCR. In contrast, culture failed to detect *H. paragallinarum* after 3 days of storage at 4 or −20°C (15).

Overall, the HP-2 PCR represents a significant step forward in diagnosing infectious coryza. While PCR technology initially appears complex and expensive, the validation and evaluation work of the HP-2 PCR in China is demonstrating that it can be used and can give significantly better results than traditional culture in developing countries.

Serology

A range of tests have been described for the detection of antibodies to *H. paragallinarum* in chickens (5). Despite this range of tests, only HI tests are in widespread use. While a range of HI tests have been described, three main forms of HI tests have been recently recognized: termed simple, extracted, and treated HI tests (8). Full details of how to perform these tests are available elsewhere (8). In this section, the advantages and disadvantages of the three HI tests are briefly and critically reviewed.

The simple HI test is based on whole bacterial cells of Page serovar A *H. paragallinarum* and fresh chicken erythrocytes (21). Although simple to perform, this HI test can detect antibodies only to serovar A. It has been widely used to detect antibodies in infected as well as vaccinated chickens (5).

The extracted HI test is based on KSCN-extracted and sonicated cells of *H. paragallinarum* and glutaraldehyde-fixed chicken erythrocytes (31). This extracted HI test has been validated mainly by using Page serovar C organisms. The test is capable of detecting a serovar-specific antibody response in Page serovar C-vaccinated chickens (31). A major weakness of this assay is that the majority of chickens infected with serovar C remain seronegative (40).

The treated HI test is based on hyaluronidase-treated whole bacterial cells of *H. paragallinarum* and formaldehyde-fixed chicken erythrocytes (41). The extracted HI test has not been widely used or evaluated. It has been used to detect antibodies to Page serovars A, B, and C in vaccinated chickens, with only serovar A- and C-vaccinated chickens yielding high titers (39). It has also been used to screen chicken sera in Indonesia for antibodies arising from infection with serovars A and C (33).

Vaccinated chickens with titers of 1:5 or greater in the simple or extracted HI tests are protected against subsequent challenge (31). There is not enough knowledge or experience yet to draw any sound conclusions on whether there is a correlation between titer and protection for the treated HI test.

A recently described serological test is a monoclonal antibody-based blocking enzyme-linked immunosorbent assay (ELISA) (43). While having shown very good specificity and acceptable levels of sensitivity, this test has several drawbacks. Since there are only monoclonal antibodies for Page serovars A and C, the assay can detect antibodies only to these two serovars. The monoclonal antibodies that form the heart of the assays are not commercially available, limiting access to the assays. Finally, some isolates of *H. paragallinarum* do not react with the monoclonal antibodies, and thus infections associated with these isolates cannot be detected by these ELISAs. While around 49 Japanese serovar A isolates and over 20 serovar A isolates from other countries react with the serovar A monoclonal antibody (7, 9, 42), around 40% of Page serovar A isolates examined to date from Argentina and Brazil do not react (6, 34). As well, isolates of Kume serovar C-4, which has been found only in Australia and consists of just 13 isolates (4), do not react with the serovar C monoclonal antibody. This ELISA has not been widely evaluated, and there is no knowledge about any correlation between ELISA titer and protection. The reduced sensitivity of the ELISA for serovar C infections indicates that the test would have to be used as a flock test only (43).

Overall, the serological test of choice for coryza varies with the serovar and the intended use, i.e., to detect vaccination or infection responses. The simple HI test (21) is suitable for either infections or vaccinations associated with serovar A, the extracted or treated HI tests (31, 41) are suitable for vaccinations associated with serovar C, and the treated HI test (41) is suitable for infections associated with serovar C. There has been so little work performed on serological assays for infections or vaccinations associated with serovar B that it is not possible to recommend any test.

The limitations outlined above for the HI tests mean that

there is still a need for robust, well-characterized serological assays. The monoclonal antibody-based ELISAs have shown the potential of this format to diagnose infectious coryza, and future research on this type of approach, including the development of new panels of monoclonal antibodies, particularly to Page serovar B, may help overcome some of the problems of the current ELISAs.

Vaccines

Commercial vaccines for infectious coryza, typically based on killed *H. paragallinarum*, are widely available around the world. An extensive review of the literature on inactivated infectious coryza vaccines has been recently published (2). For this reason, only two aspects of infectious coryza vaccines are covered in this review.

Until recently, most of these vaccines contained only Page serovars A and C. This concept of a bivalent vaccine was based on the belief that Page serovar B was not a true serovar and that serovar A and C based vaccines provided cross-protection. However, because it has now been conclusively shown that Page serovar B is distinct, commercial trivalent vaccines are now available from the major international vaccine companies (22).

An emerging issue in vaccines is the comparison between "local" and "international" vaccines. The major global vaccine companies tend to base their vaccines on standard, internationally recognized strains. These international vaccines are sold around the world on the basis that local variation is not sufficient to justify adding or removing strains. Recently, a number of research groups, including Bragg et al. in South Africa (11) and Terzolo et al. in Argentina (35), have suggested that such international vaccines are not providing protection against the local variants of *H. paragallinarum*. There is a need for definitive cross-protection trials to determine if "international" vaccines are indeed failing to provide protection against local variants.

CONCLUSIONS

This review has covered literature evidence stressing that infectious coryza, while often seen as a simple, mild upper respiratory disease of chickens in developed countries, can be a much more complicated disease in developing countries. There have been significant increases in our ability to accurately diagnose infectious coryza, particularly the validation of the HP-2 PCR. In other areas, such as the study of "variant" organisms that show aberrant growth factor requirements and emerging "variant" serovars, we are now more aware of how little knowledge we have. There is a need for definitive work to investigate the role of the NAD-independent forms of *H. paragallinarum* in possible vaccine failures. Similarly, there is a need for definitive work on whether serologically "variant" *H. paragallinarum* isolates are associated with vaccine failures.

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