Infectious Coryza

Zeinab M. S. Amin Girh[1], Nagwa S. Rabie[1] and Mona S. Zaki[2]

[1]Department of Poultry Diseases, National Research Centre, Dokki, Giza, Egypt
[2]Hydrobiology Department, National Research Centre, Dokki, Giza Egypt

Abstract: Infectious coryza is a well-recognized and commonly encountered upper respiratory tract disease of chickens that is caused by the bacterium Haemophilus paragallinarum. In developing countries, coryza is commonly complicated by the presence of a range of other infections, resulting in severe disease and significant economic losses. Unusual forms of the disease, involving arthritis and septicemia, again associated with the presence of other pathogens, have been found in South America. Newly recognized bacteria such as Ornithobacterium rhinotracheale and phenotypic variant forms of both H. paragallinarum and close relatives (variant in that they no longer require V-factor for growth in vitro) have increased the difficulty associated with diagnosing the disease. Definitive evidence to confirm or deny the role of these "variants" in vaccine failures is currently not available. A new DNA-based diagnostic technique, involving PCR, has been recently described and will greatly assist in the diagnosis of infectious coryza. 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.
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1. Introduction:

Infectious Coryza is an acute respiratory disease in chickens caused by gram-negative bacteria, *Avibacterium paragallinarum* (Blackall *et al*., 2005). The disease causes high morbidity but low mortality. The clinical signs of the upper respiratory tract include nasal discharge, facial edema, swollen wattles, and conjunctivitis (Chukiatsiri and Chansiripornchai, 2007). The signs become evident within 24-72 hr after contact with other infected chickens. The chickens may also have diarrhea as well as decreased feed and water consumption, which result in a decrease in growth performance of the chickens, lower egg production in layers, and complications with other pathogens (Blackall and Soriano-Vargas, 2013). In case of no complication, the infected birds should be recovered within few weeks. These drawbacks can then lead to economic loss (Chukiatsiri *et al*., 2010). Infectious coryza is an acute respiratory disease of chickens. The clinical syndrome has been recognized since the 1930s (Blackall etal., 1997). 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* (Blackall et al., 1997).

A. paragallinarum has been classified by 2 schemes, the Page and Kume schemes. The Page scheme divides *A. paragallinarum* into 3 major serovars: A, B, and C, by the plate agglutination test (Page, 1962). The Kume scheme divides the bacteria into 3 major serogroups: I, II, and III by the Hemagglutinin Inhibition (HI) test (Kume *et al*., 1983). However, Page serovars can be classified by the HI test and correlated with the Kume serogroup. As a result, Page serovars A, B, and C match the modified Kume serogroups I, II, and III, respectively. Currently, 9 Kume serovars have been classified: A-1, A-2, A-3, A-4, B-1, C-1, C-2, C-3, and C-4 (Kume *et al*., 1983; Blackall *et al*., 1990). Importantly, 3 Page serovars are distinct from each other as the antibodies from each serovar are unable to protect chickens from 2 other serovars, while they can provide protection against the serovars within the same group. For example, bivalent vaccine, which contains *A. paragallinarum* serovar A and C, is unable to protect serovar B-1 infected chickens but can protect the chickens with A-1, A-2, A-3, A-4, C-1, C-2, C-3, and C-4 infection (Soriano *et al*., 2004).

Chicken infected with IC manifested the clinical signs of nasal discharge, conjunctivitis and swelling of the sinuses, face and wattles. *A. paragallinarum*

causes catarrhal infla-mmation of the upper respiratory tract mainly in the sinuses and nasal passage (Blackall, 1999). The epidem-miology of IC is very complex. The outbreak of IC is common in farms that keep birds of various ages together. Over crowding, cold weather and co-infection with pathogenic microorganisms (*Pasteurella multocida*, *Pesudomonas aeruginosa* and chronic respiratory disease) determine the incidence of the IC in the poultry farm (Blackall, 1999; Reid and Blackall, 1984; Giurov, 1984). The mortality of chicken due to IC varies from 1 to 15% and it can be increased if birds are co-infected with other microorganisms (Sarhiland et al., 2003).

A. paragallinarum has spread worldwide. Some countries, including Thailand, China, Taiwan, the United States, Mexico, Germany, and South Africa, have reported all 3 serovars of *A. paragallinarum*. Australia and Japan reported only serovar A and C but no serovar B (Soriano *et al*., 2004; Chukiatsiri *et al*., 2012). Prevention and control of Infectious Coryza can achieve through strict biosecurity, antimicrobial application (Noonkhokhetkong *et al*. 2013) and relevant vaccination, as commercial inactivated bacterin in aluminum hydroxide gel or mineral oil vaccines against Infectious Coryza are available. For example, the bivalent vaccines such as *A. paragallinarum* serovars A and C, trivalent vaccines containing serovars A, B, and C, and tetravalent vaccines containing serovars A, B, C, and B variant (Soriano *et al*., 2004; Fernandez *et al*., 2005; Christensen *et al*., 2009). Suitable vaccines should be matched with reported serovars as there is no guaranteed cross protection between different serovars. Although, the HI test is used for classified serovars of *A. paragallinarum*, the HI titer does not represent the level of host immunity. Hence, a challenge study is still the best method to evaluate the protection efficacy of Infectious Coryza vaccines (García *et al*., 2008).

Epidemiology

The disease occurrence is worldwide. Early workers identified the causative organism as Haemophilus gallinarum, an organism that required both as Hemin factor X and NAD factor V for growth in vitro. However from 1960s to 1980s, all isolates of the disease producing agents have been shown to require only V factor and have been termed H. paragallinarum V factor independent isolates of H. paragallinarum have been encountered in the Republic of South Africa since 1989. Thus the causative agent of this disease is regarded as H. paragallinarum, an organism that can be either V-factor dependant or independent (Blackallet al., 1997). The potential impact of coryza on meat chicken has been emphasized by reports on economically important outbreaks in two states of the United States (Droual et al., 1990). Unusual clinical signs have been reported in the America. 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 (Sandoval et al.,1994). In Alabama, an Infectious coryza outbreak in broilers, which was not complicated by any other disease agent, caused a condemnation rate of 69.8 percent virtually all due to air saculitis (Horner, et al., 1995). The enormously different nature of infectious coryza 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 presence of pathogens detected, such as Mycoplasma gallisepticum, M. synoviae, Pasteurellla spp. Salmonella spp. and infectious bronchitis virus, have been found in broiler and layer flocks in Argentina. The isolation of H. paragallinarum from non respiratory sites such as liver, kidney and tarsus was reported for the first time in these outbreaks (Sandoval et al., 1994). A study in Morocco reported on 10 coryza outbreaks that were associated with drop in egg production of 14 to 41% and mortalities of 0.7 to 10% (Thitisak et al., 1988). A study of village chicken in Thailand has reported that infectious coryza was the most common cause of death in chicks less than 2 months old and those over six months old (Thitisak et al., 1988). It has been estimated that over the three year period the disease caused losses of about 100 million yuan (app $ US 16.5 million at 1996 exchange rate) in China (Chen et al., 1993). In the US it is most prevalent in California and the southeastern US. In New England AIC has occurred in Connecticut in the 80's, but has not been diagnosed in Maine during the last 20 years. In Indonesia, the isolation of H paragallinarum has been reported in 1975, 1978 and 1987. Unfortunately, none of the isolates from these studies were maintained and, therefore, there is no information of the serovars to which they belonged. Akhtar et al. (2001).

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 (Blackall et al.,1997). The potential impact of coryza on meat chickens has been emphasized by reports on economically important outbreaks in two states of the United States (Droual et al.,1990). 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 (Chen et al.,1998). 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 (Odor et al.,1997). The isolation of *H. paragallinarum* from nonrespiratory sites such as the liver, kidney, and tarsus was reported for the first time in these outbreaks (Odor et al.,1997). In the Kurnool district of India, infectious coryza has been reported as the second most important bacterial disease associated with mortality after salmonellosis (Sandoval et al.,1994). 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% (Terzoio et al.,1993). 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 (Terzolo et al.,1997). Only in village chickens between 2 and 6 months old did other diseases, specifically Newcastle disease and pasteurellosis, cause more deaths than coryza (Terzolo et al.,1997).

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 (Horner et al.,1995). While these isolates did not require V-factor, they were shown by DNA techniques to be typical *H. paragallinarum* (Mouahid et al.,1992). The vast majority of the NAD-independent isolates are Page serovar A (Miflin et al.,1995), although a recent report has shown that some isolates are Page serovar C (Chen et al.,1998). 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 (Miflin et al.,1999). 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. (Horner et al.,1995) 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* (Chen et al.,1998).

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* (Thitisak et al.,1988). The organism is present in Europe (Amonsin et al.,1997) and the United States (Odor et al.,1997). 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 (Vandamme et al.,1994). For the purpose of this review, the disease associated with *O. rhinotracheale* will be termed ornithobacterosis. A recent molecular study 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 (Amonsin et al.,1997). 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 (Chen et al.,1998). 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 (Miflin et al.,1999). 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 (Mutters et al.,1985). 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 (Chen et al., 1998).

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 (Biackall et al.,1997). 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 (Blackall and Yammaoto,1998).

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 (Chen et al.,1998). This test is rapid (results are available within 6 h compared with days for conventional techniques) and recognizes all *H. paragallinarum* isolates tested (Chen et al.,1998). 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 (Chen et al.,1998). 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 (Miflin et al.,1995). 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 (Chen et al.,1998).

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 (Chen et al.,1998). 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 (Chen et al.,1996). 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 (Chen et al.,1996). 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 (Chen et al.,1996).

Serology

A range of tests have been described for the detection of antibodies to *H. paragallinarum* in chickens (Blackall et al.,1997). 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 (Blackall and Yammaoto,1998). Full details of how to perform these tests are available elsewhere (Blackall and Yammaoto,1998). 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 (Iritani et al.,1977). 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 (Blackall et al.,1997). The extracted HI test is based on KSCN-extracted and sonicated cells of *H. paragallinarum* and glutaraldehyde-fixed chicken erythrocytes (Sawata et al.,1982). 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 (Sawata et al.,1982). A major weakness of this assay is that the majority of chickens infected with serovar C remain seronegative (Yamaguchi et al.,1988). The treated HI test is based on hyaluronidase-treated whole bacterial cells of *H. paragallinarum* and formaldehyde-fixed chicken erythrocytes (Yamaguchi et al.,1989). 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 (yamaguchi et al.,1991). It has also been used to screen chicken sera in Indonesia for antibodies arising from infection with serovars A and C (Takagi et al.,1991). Vaccinated chickens with titers of 1:5 or greater in the simple or extracted HI tests are

protected against subsequent challenge (Sawata et al.,1982). 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) (Zhang et al.,1999). 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 (Blackall et al.,1991), around 40% of Page serovar A isolates examined to date from Argentina and Brazil do not react (Zhang et al.,1999). As well, isolates of Kume serovar C-4, which has been found only in Australia and consists of just 13 isolates (Blackall et al.,1990), 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 (Zhang et al.,1999). 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 (Iritani et al.,1977) is suitable for either infections or vaccinations associated with serovar A, the extracted or treated HI tests (Sawata et al.,1982) are suitable for vaccinations associated with serovar C, and the treated HI test (yamaguchi et al.,1989).

Impact: Economic

IC occurs in all ages of growing chickens and is of economic importance due to an increased number of culls and a marked reduction (10-40%) in egg production (Blackall et al., 1997). IC is often regarded as a disease that has its greatest impact in intensively raised chickens. In Indonesia, the disease was formerly not considered to be widespread in village chickens. However the isolation of *A. paragallinarum* in village chickens has indicated that the disease can be present in less intensive production systems (Poernomo et al., 2000). In China, it has been estimated that over a three year period, 1986-1988, the disease caused cases of about 1000 million Yen (approximately US $18 million) at the 1992 exchange rate (Chen et al., 1993).

Disease Treatment

Various sulfonamides and antibiotics have been used to treat IC, usually in feed or drinking water. Birds usually respond to treatment but relapses may occur when treatment is discontinued. Many drugs and antibiotics have been used, including streptomycin, erythromycin, sulfodimethoxine, tylosin tartrate and spectinomycin (Charlton et al., 2000). Drug combinations found effective for the treatment of IC include sulfachloropyridazine/trimethoprim (Poernomo and Ronohardjo, 1987), and sulfamethoxazol/trimethoprim (Takahashi et al., 1990; Poernomo et al., 1997). It should be noted that sulfa drugs may cause a temporary drop in egg production and overdoses may be toxic. Similarly, streptomycin causes severe stress in chickens, which can last for 24 hours (Bains, 1979). Erythromycin and oxytetracycline are two commonly used antibiotics (Blackall et al., 1997). Other antibiotics found effective in the treatment of IC include norfloxacin (Lublin et al., 1993), enrofloxacin, ciprofloxacin, ampicillin (Prabhakar et al., 1998), and gentamycin (Muhammad et al., 1998). Some strains of *A. paragallinarum* are resistant to various antibiotics, including cloxacillin, erythromycin, ampicillin, lincomycin (Prasad et al., 1999), neomycin, cotrimoxazol, amikacin, and cephalexin (Prabhakar et al., 1998). Strains of *A. paragallinarum* resistant to various antibiotics have not been found to carry plasmids (Blackall, 1988).

Prevention and Control

Farm-level Control

Recovered carrier birds are the main source of infection, so practices such as buying breeding males or started chicks from unknown sources should be discouraged. Only day-old chicks should be secured for replacement purposes unless the source is known to be free of IC. Isolation rearing and the housing away from old stock are desirable practices. To eliminate the agents from a farm, it is necessary to depopulate the infected or recovered flock (s), because birds in such flocks remain reservoirs of infection. After the cleaning and disinfecting of equipment and houses, the premises should be allowed to remain vacant for 2-3 weeks before restocking with clean birds (Blackall et al., 1997), raised, in so far as is possible, in quarantine (Charlton et al., 2000). It is important to avoid the introduction of infected chickens to the farm and if this occurs then the early recognition of disease and institution of appropriate treatment is vital. Good husbandry and management procedures prevent spread of disease; isolation of age groups of chickens on an all-in, all-out basis (Bain, 1979). It is necessary to depopulate flocks that have experienced the disease, because recovered birds

remain reservoirs of infection. The method of eradication depends upon circumstances on the farm, the size of the flock, facilities, and purpose of the flock. The infected birds may be marketed at once and the premises cleaned before new chicks are brought onto the farm. Another more popular method is to treat the affected flock and keep it isolated until new stock has been raised in isolation as replacements. After the infected or recovered birds are marketed, the house should be cleaned and disinfected before housing clean stock. As the organism may survive in exudates for several days at low temperatures, it would be advisable to allow the cleaned house to remain vacant for about 1 week, particularly during the cooler periods of the year (Yamamoto, 1984). **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), Both killed adjuvant associated and live vaccines are available against the disease. As mentioned elsewhere, that no convincing, specific protective antigen against multiple serovars of this organism have been identified so far, an issue of failure of cross protective mechanism still exists with this disease. Previously workers across globe have tried killed vaccines based on egg yolk culture or tissue culture grown antigens and found certain degree of cross protection when compared with killed vaccines based on broth grown antigen. Interestingly, the most suitable protective antigen was found out to be Haemagglutinin (HA) of the polysaccharides capsule of this bacterium and was putatively considered as immuno- stimulating.52 In another study, people evaluated appreciable, good homologous protection following the usage of broth based antigens.53,54 The inactivating agent also seems to be affecting the efficacy of the killed vaccine and was observed at several trials and apparently shown thimerosal as a best agent over formalin. When considered adjuvants, Aluminium hydroxide had shown better efficacy and less adverse reaction to the site of injections over mineral oil based adjuvants. Usually, vaccination with two doses of aluminium hydroxide killed infectious coryza vaccine at three weeks apart extends a long term immune protection, which lasts for around 30-40weeks after vaccination. Vaccination with live vaccines containing avirulent *Avibacterium paragallinarum* insinuate very closelyto natural exposure, where it is believed that the cross serovar protection would be higher as compared to killed vaccines, apart from easy, natural route of administration. The work by (Blackall et al.,1994)55 proved similar observation, having better cross protection against different virulent serovars following utilization of live attenuated strains of *Avibacterium paragallinarum.* Similarly chemically mutated strains of bacteria were also created and significant level of protection was noted on experimental trials.55 Despite of this development, killed vaccines are predominantly in use globally, probably because of fear about genetic transmutation of live strains of bacterium in to more pathogenic serovars. In young chicks, the vaccination against this disease is mainly carried out at the age of 6-8weeks of age and also before the age of egg laying.

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