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## Biography

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# Influenza B viruses: underestimated and overlooked

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**Abstract.** Influenza B viruses circulate globally every year causing respiratory disease with significant clinical and socio-economic impacts. IBV are considered exclusive human pathogens with no established animal reservoirs, which suggests with concerted effort it may be possible to eradicate this virus from human circulation. However, this requires a deeper understanding of IBV virology and immunology and the design of vaccines that induce universal immunity to antigenic variants of IBV.

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## Introduction

Influenza A and B viruses (IAV and IBV) circulate annually causing seasonal epidemics around the world. Influenza viruses are single-stranded negative sense RNA viruses with segmented genomes belonging to the family of *Orthomyxoviridae*[1]. They replicate is the respiratory tract and cause influenza disease which can vary from asymptomatic and mild upper respiratory tract disease to severe lower respiratory tract disease and in some cases fatal disease[1]. Although IAV exists in a wide range of animal hosts, IBV does not have an established animal reservoir[2]. The potential of antigenically novel IAV viruses to 'jump' from animals into humans and cause severe disease, and is some instances global pandemics, has placed IAV in the spotlight. The lack of an established animal reservoir, and therefore lack of pandemic potential, for IBV has left this type of influenza virus considerably underestimated and overlooked. However, IBV has substantial health and socio-economic impacts annually. Additionally, the lack of an animal reservoir means that it may be possible to eradicate this virus from human circulation with highly effective, broadly protective vaccines and broad population coverage. To achieve that, a thorough understanding of IBV virology and immunology is needed.

## The underestimated impact of IBV infections

Seasonal epidemics caused by IAV and IBV result in 3–5 million cases of severe disease and 290 000–650 000 deaths annually[1]. IBV accounts for on average 23% of the annual influenza burden[3] but can comprise up to ~80% of infections in some countries in selected years[4]. It is estimated that IBV infections result in 7.9 million lower respiratory tract infections and 1.4 million hospitalisations annually[5]. Although the clinical severity of IBV was initially thought to be lower than that of IAV, recent studies have contested this notion, with hospitalisation and mortality rates in adults being similar for IAV and IBV[6,7]. Importantly, IBV incidence is higher in children, in which IBV can cause severe systemic complications and frequent hospitalisation and death, with up to 52% of influenza-related paediatric deaths being attributed to IBV[8–10]. Additionally, in children under the age of 16 years, IBV can have higher mortality rates than IAV and a significant rate of ICU admission[11]. Fatal infections of IBV in children are associated with secondary bacterial pneumonia as well as cardiac injury[12]. Lastly, IBV infections account on average for 37% of influenza-associated healthcare costs, with projected costs in the US of US$0.96–2.6 billion annually[13]. Overall, IBV has significant clinical and socioeconomic impacts. This impact could be minimised with highly effective vaccines and intervention strategies.

## Known and unknowns of the IBV life cycle

IBV replicates in epithelial cells of the respiratory tract. The virus uses its surface glycoprotein haemagglutinin (HA) for attachment to sialic acid receptors on the cell surface and subsequent membrane fusion in endosomes. This results in the release of eight viral ribonucleoprotein (vRNP) complexes, which replicate in the nucleus of the cell. Viral RNA replication combined with protein expression are followed by assembly and budding of newly formed virions from the cell surface. The viral surface glycoprotein neuraminidase (NA) releases virions from attached sialic acid receptors on the cell surface[14]. During IBV infection, the viral non-structural 1 (NS1) protein of IBV has a critical role in counteracting immune recognition by innate receptors such as RIG-I as well as interferon-stimulated genes such as protein kinase R (PKR) and ISG-15, which are potent inhibitors of IBV[14,15]. Interestingly, the NS gene of IBV exhibits the highest rate of selection pressure among the genes of IBV[16]. Given its critical role in counteracting innate immune responses, understanding the evolution of the IBV NS1 protein in humans would be of great interest.

Although the life cycle of IAV and IBV is in many ways similar, it is pertinent to note that the two types of influenza viruses encode different sets of accessory proteins (Figure 1). Specifically, IBV lacks expression of immunomodulatory virulence factors PB1-F2 and PA-X found in IAV. Conversely, IBV encodes a unique open reading frame (ORF) called NB, that overlaps with the NA ORF[15]. NB is a small transmembrane protein that is heavily glycosylated and is incorporated in the IBV virion. Despite the high conservation of NB in IBV, NB expression is dispensable for virus viability and replication *in vitro*[17,18] and its role in viral replication is unclear[18]. Dissecting the role and

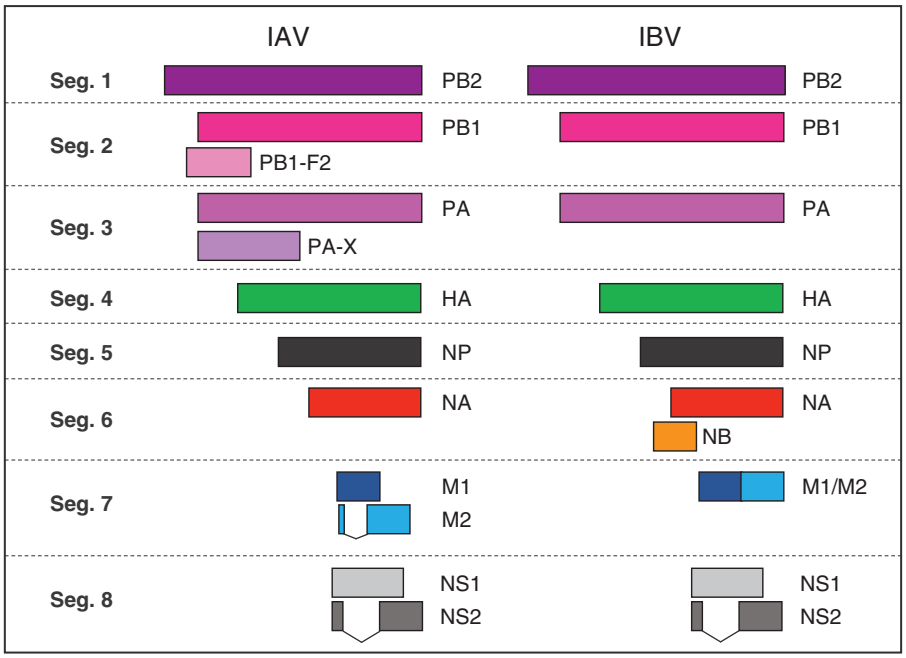


Figure 1. Comparison of genomes of IAV and IBV. Each segment is depicted with the encoded open reading frames for IAV and IBV. The 8 gene segments and known open reading frames (ORFs) of IAV and IBV are shown. Segment 2 and 3 of IAV encode overlapping ORFs PB1-F2 and PA-X respectively, which are not found in IBV. Conversely, IBV encodes an overlapping ORF on segment 6 (NB), not found in IAV. Segment 7 for both viruses encodes two ORFs, which in IAV are expressed by alternative splicing, while in IBV an alternative stop-start codon mechanism is utilised.

function of NB in the life cycle of IBV would assist the understanding of the IBV life cycle.

## Host species tropism of IBV

Another important difference between IAV and IBV is host species tropism. Although IAV can be found in many animal species, IBV is considered exclusively a human pathogen. However, it is important to note that natural infections of animals with IBV have been reported for a variety of species (Table 1) and some have been recapitulated experimentally. However, most of these infections have occurred in animals in proximity with humans (domestic and farm animals or animals in zoos/research centres) and likely represent isolated reverse zoonosis events. A notable exception is the

Table 1. Infection of animals with IBV.

| Animal[A] | Location, year | Detection methods | Frequency of animals positive (%) | Comments | Reference |
|---|---|---|---|---|---|
| Natural infections | | | | | |
| Harbor seals *(Phoca viulina)* and gray seals *(Halichoreus grypus)* | The Netherlands, 1995–1999 | HAI and ELISA to HA, NA and NP | 8/391 (2) | – 580 samples prior to 1995 were seronegative<br>– 1 RT-PCR$^+$ throat swab in 1999<br>– B/Seal/The Netherlands/1/99 virus isolated | 19 |
| Harbor seals *(Phoca viulina)* and gray seals *(Halichoreus grypus)* | The Netherlands, 2002–2012 | HAI | 10/625 (1.6) | – Seropositive samples only detected in 2010 (9/21) and 2011 (1/150) | 20 |
| Caspian seals *(Phoca capsica)* | Caspian Sea, 1997–2000 | ELISA with whole virus | 5/77 (6) | 3 of seropositive animals in 2000 were <1 year old, suggestive of recent introduction of IBV | 21 |
| South American fur seals *(Arctocephalus australis)* | Uruguay, Sep. 2004 | HAI | 25/37 (67.6) | – Seropositive cut-off set at HAI >80 for 1993 strain<br>– Lower seropositivity rates for 1999 and 2001 strains | 22 |
| Horse | Japan, 1977 | HAI | 16/504 (3.2) | – Seronegative animals in the study: cattle ($n$ = 812), dogs ($n$ = 158), cats ($n$ = 52), mink ($n$ = 62), rats ($n$ = 33), chickens ($n$ = 389), ducks ($n$ = 10), pigeons ($n$ = 250), wild birds ($n$ = 55) | 23 |
| Swine | Japan, 1968–1977 | HAI | 1/1030 (0.1) | | |
| Pigs | USA, 2010–2012 | HAI, verified by NT | 41/560 (7.3) | – 3 RT-PCR$^+$ nasal swabs<br>– Limited region of virus sequenced | 24 |
| Pigs | Great Britain, Oct. 1991–Feb. 1992 | HAI, verified by NT and immunoblot | 8/2000 (0.4) | Seropositive samples spread across England and Wales | 25 |
| Chimpanzees | The Netherlands, 1986, 1992, 1998, 2000 | Magnetic bead-based assay, verified by immunoblot | 80/305 (26.2) | Housed in biomedical research Centre | 26 |
| Gorillas | Not specified, reported in 2014 | | 45/77 (58.4) | Zoo animals | |
| Orangutans | Indonesia, 1994–1998 | | 135/179 (75.4) | House in animal rehabilitation center | |
| Dogs | Taiwan, June–July 1971 | Virus isolation from nasal swabs | 1/372 (0.3) | No virus isolated from cats ($n$ = 28) | 27 |
| Dogs | Japan, Jan. 2009–Feb. 2010 | NT, verified by immunoblot | 6/366 (1.6) | Samples from indoor domestic dogs, no illness reported | 28 |
| Horses | Canada, 1960–1963 | Complement fixation assay | Numbers not reported (30) | Animals from farms | 29 |
| Guinea pigs | Ecuador | ELISA with whole virus, recombinant HA and NP, verified by immunoblot | 28/40 (70) | Animals raised as livestock | 30 |
| Birds | Not specified, reported in 1980 | Not specified | Numbers not reported (4.1) | Full text study not available | 31 |
| Ruminants | Not specified, reported in 1984 | Not specified | Not specified | Full text study not available | 32 |
| **Animal** | **Inoculation** | **Disease** | **Transmission** | **Comments** | **Reference** |
| Experimental infections[B] | | | | | |
| Pigs | Intranasal and intratracheal | ILI and lung lesions | Yes (contact) | – Limited transmission | 24 |
| Guinea pigs | Intranasal | Histopathological changes in nasal tissue | Yes (contact and aerosol/ droplet) | – Replication in upper respiratory tract<br>– High efficiency of transmission | 33 |
| Cynomolgus macaques *(Macaca fascicularis)* | Intranasal and intratracheal | – Fever, loss of appetite/ weight loss<br>– No sneezing or coughing<br>– Lung lesions | Not assessed | – Replication in upper and lower respiratory tract<br>– Inflammatory cytokine detected in upper respiratory tract | 34 |

[A]Species indicated where available. [B]Other than mice and ferrets. HAI, hemagglutination inhibition assay; ELISA, enzyme-linked immunosorbent assay; HA, hemagglutinin; NA, neuraminidase; NP, nucleoprotein; NT, neutralisation assay.

presence of IBV in seals that has been detected across species of seals and geographical sites between 1995 and 2012[19–22]. The single virus isolated from a seal in 1999 had high homology to a human IBV isolate. However, it is not known whether the presence of IBV in seals represents a single introduction from humans and subsequent spread amongst seals between 1995 and 2012 or multiple distinct reverse zoonosis events in that period. Overall, while a variety of mammals are susceptible to natural IBV infection, there is no evidence of established animal reservoirs in any species.

Understanding the factors that contribute to the exclusivity of IBV in humans is of great importance. The host species restriction of avian IAV in birds, and the requirement for significant adaption for efficient replication and transmission in humans, occurs at many stages of the life cycle, including HA mediated attachment and entry as well the activity of the influenza virus replication machinery[35]. Interestingly, it was recently reported that the IBV HA exhibits optimal activity in the pH and temperature conditions of the human upper respiratory tract, more so than IAV strains tested in that study, indicating significant host adaptation in the human host environment[36]. Additionally, IBV can interact with mammalian (human and murine) but not avian homologues of host proteins required to support viral replication[37,38], providing a potential mechanistic basis for the lack of IBV in avian species. The IBV NS1 protein can counteract the effects of the antiviral protein ISG-15 in a species-specific manner, by interacting with human and non-human primate ISG-15 but not with canine or murine homologues[39]. Overall, these studies demonstrate considerable adaptation of IBV to mammalian and often specifically human hosts, which may restrict the ability of IBV to efficiently replicate in other species. It is important to note the recent discovery of IBV-like viruses in lower vertebrates[40]. These viruses show similar genome architecture to human IBVs[40] and encode functional homologues of HA and NA but are not recognised by human serum samples[41]. Understanding the virology and host-restriction of these viruses could provide novel insights into IBV evolution and host species tropism.

## Antigenic diversity and immune responses to IBV

Two antigenically and genetically distinct lineages of IBV co-circulate globally. These lineages, named B/Yamagata/16/1988-like (or B/Yamagata) and B/Victoria/2/1987-like (or B/Victoria), are estimated to have diverged in the 1970s[42]. While B/Victoria viruses were dominant in the late 1980s in most countries, B/Yamagata viruses dominated in the 1990s, during which B/Victoria viruses were virtually absent globally, except for a 1996/1997 outbreak in Asia[42]. B/Victoria viruses re-emerged in 2001 and the two lineages have co-circulated since[42,43].

Both IBV lineages undergo gradual antigenic drift by accumulating escape mutations in the head domain of the HA protein – the major antigenic target of protective antibodies[14]. Mutations are primarily focused on sites surrounding the receptor binding site of the HA and overlap with sites of antibody recognition. Interestingly, since 2015 the B/Yamagata HA has not acquired any mutations in those sites. Instead, it has acquired 7 mutations on the NA protein[16], although the effects of these mutations in antigenic evolution and immune escape are unclear. In contrast, since 2015 the B/Victoria viruses have undergone significant diversification of their HA gene, including the recurrent but independent emergence of viruses with 2–3 amino acid deletions in one of the antigenic sites[16]. These deletions significantly alter the antigenicity of those domains and have necessitated the inclusion of these strains in the influenza vaccine[44]. Intriguingly, similar amino acid deletions have been previously detected in IBV strain from 1940–1988[16], an observation that warrants further investigation as it indicates this might be a common escape mechanism of IBV.

Although such mutations can escape antibody recognition, conserved domains of the HA protein can be recognised by broadly cross-reactive antibodies[45,46]. These can target highly conserved sites of the HA head as well as the HA stem domain and cross-react with both IBV lineages[46]. Cross-recognition of the two lineages can also occur by cytotoxic T cells, which can recognise and kill virally infected cells, providing an additional level of immune protection[47]. The repeated isolation of multiple broadly cross-reactive antibodies in different studies indicates that such antibody responses may not be uncommon, although their prevalence and abundance in serum samples is unknown. Nonetheless, their discovery indicates that universal immunity across both lineages of IBV is feasible. Antibodies to the IBV NA also show broad cross-reactivity across both lineages and can mediate protection from challenge[48]. Understanding how such broadly cross-reactive immune responses to HA and NA are generated through infections and vaccination during the human lifespan will assist in the design of broadly cross-protective vaccines.

## Vaccination strategies against IBV

Influenza vaccines primarily comprise unadjuvanted inactivated split virions or recombinant proteins that induce antibodies towards the HA and vaccine composition needs to be updated annually to accommodate for the emergence of escape mutants. A live attenuated influenza vaccine (LAIV) is also approved in some countries.

Traditionally, a trivalent influenza vaccine (TIV) has been used that includes two IAV strains along with one IBV strain from the lineage predicted to dominate the upcoming influenza season. However, due to the frequent mismatch of the predicted and the circulating IBV lineage[8], in 2012 the WHO recommended where possible the use of a quadrivalent vaccine (QIV) that includes one IBV strain from each lineage. Despite this, the average vaccine effectiveness for IBV is only 54%[49] and the advantages of the QIV formulation remain contested[50]. An alternative to annual administration of a strain-specific vaccine would be the design of a universal vaccine that induces broadly cross-reactive immunity and does not require annual reformulation. This can be achieved by rationally designing vaccines that focus the immune response to highly conserved sites of the IBV HA and NA proteins, although such vaccines are only in pre-clinical development. Overall, despite the introduction of QIV, current vaccination strategies against IBV only provide modest and partial protection and further research is needed to improve vaccine effectiveness. The development of more effective IBV vaccines will assist efforts to eliminate IBV from human circulation.

## Future directions

Despite the consistent seasonal circulation globally and the significant health and socio-economic impacts of IBV, initial misconceptions of relatively lower clinical severity have left IBV underestimated and overlooked. As a result, there is only limited focus on the control of IBV infections. Significant advances in the last decade have demonstrated the potential for universal immunity across both lineages of IBV. The lack of animal reservoir and subsequently pandemic potential, once a reason for neglecting IBV, is now considered its Achilles' heel and could allow for the high-level suppression or even elimination of this virus. However, this can only be achieved by global concerted efforts to understand the antigenic evolution of IBV, the generation of broadly cross-reactive immunity and the rational design of universal vaccines.

## Conflicts of interest

The authors declare no conflicts of interest.

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